UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOTECNOLOGIA E BIOCIÊNCIAS

André Felipe da Silva

Bioprospecção de fungos produtores de biosurfactantes e desenvolvimento de bioprocessos aplicados à micorremediação de solos contaminados com hidrocarbonetos

Florianópolis
2023

André Felipe da Silva

Bioprospecção de fungos produtores de biosurfactantes e desenvolvimento de bioprocessos aplicados à micorremediação de solos contaminados com hidrocarbonetos

Tese submetida ao Programa de Pós-Graduação em Biotecnologia e Biociências da Universidade Federal de Santa Catarina como requisito parcial para a obtenção do título de Doutor em Biotecnologia e Biociências.

Orientador: Prof. Dr. Diogo Robl
Coorientador: Prof. Dr. Admir José Giachini

Florianópolis
2023

Ficha de identificação da obra elaborada pelo autor,
através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Silva, André Felipe da
Bioprospecção de fungos produtores de biosurfactantes e desenvolvimento de bioprocessos aplicados à micorremediação de solos contaminados com hidrocarbonetos / André Felipe da Silva ; orientador, Diogo Robl, 2023.
248 p.

Tese (doutorado) - Universidade Federal de Santa Catarina, Centro de Ciências Biológicas, Programa de Pós Graduação em Biotecnologia e Biociências, Florianópolis, 2023.

Inclui referências.

1. Biotecnologia e Biociências. 2. biosurfactantes. 3. fungos filamentosos. 4. bioprospecção. 5. solo contaminado. I. Robl, Diogo . II. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em Biotecnologia e Biociências. III. Título.

André Felipe da Silva

Bioprospecção de fungos produtores de biosurfactantes e desenvolvimento de bioprocessos aplicados à micorremediação de solos contaminados com hidrocarbonetos

O presente trabalho em nível de Doutorado foi avaliado e aprovado, em 30 de março de 2023, pela banca examinadora composta pelos seguintes membros:

Profª. Helen Treichel, Drª.
Universidade Federal da Fronteira Sul

Profª. Jaciane Lutz Ienczak, Drª
Universidade Federal de Santa Catarina

Prof. Claudio Roberto Fonseca Sousa Soares, Dr
Universidade Federal de Santa Catarina

Certificamos que esta é a versão original e final do trabalho de conclusão que foi julgado adequado para obtenção do título de Doutor em Biotecnologia e Biociências.

ICP-Edu
Documento assinado digitalmente
Aguinaldo Roberto Pinto
Data: 09/05/2023 18:55:04-0300
CPF: ***.874.718-**
Verifique as assinaturas em https://v.ufsc.br

_____
Prof. Dr. Aguinaldo Roberto Pinto
Coordenador do Programa

ICP-Edu
Documento assinado digitalmente
Diogo Robl
Data: 09/05/2023 16:19:13-0300
CPF: ***.474.209-**
Verifique as assinaturas em https://v.ufsc.br

_____
Prof. Dr. Diogo Robl
Orientador

Florianópolis, 30 de março de 2023

À minha mãe e avó e todos os professores que
contribuíram em meu desenvolvimento intelectual-científico.

AGRADECIMENTOS

À Deus, sob uma concepção de inteligência suprema e causa primária de todas as coisas; e a Espiritualidade pela intuição em minhas tomadas de decisões e proporcionar meu encontro com pessoas que tornaram realidade este trabalho de doutorado.

Aos meus supervisores e co-supervisores pelo suporte durante esta jornada. Ao Prof. Dr. Diogo Robl por seu excepcional intelecto em engenharia de bioprocessos e biotecnologia, e ter contribuído efetivamente em meu crescimento pessoal e profissional. Ao Prof. Dr. Admir José Giachini pela sua sólida expertise em microbiologia, assertividade de ideias, e ter sido o primeiro a me dar a oportunidade de iniciar este trabalho. Ao Prof. Dr. Ibrahim M. Banat (pesquisador sênior em Biosurfactantes) por revisar minhas publicações acadêmicas; e contribuir com sua intelectualidade que somente um longa e brilhante carreira profissional poderia oferecer. Ao Prof. Dr. Rubens Duarte pela sua valiosíssima contribuição durante a execução da pesquisa experimental, bem como pela sua excelente didática no ensino de Microbiologia Industrial e Ambiental.

À Universidade Federal de Santa Catarina por disponibilizar docentes altamente qualificados, e infraestrutura técnica necessária para execução desta pesquisa experimental; e a Universidade Federal do Tocantins pelo incentivo a minha qualificação profissional.

A meus colegas de trabalho do MIP-UFSC. Em particular: Isabela Maria, Sabrina Cavalcante, Gustavo Volpato, Joana Camila, Ana Cláudia, João Monteiro, Carla Biscaino, Breno Oliveira, Renata Silveira, Rosane Kaspary, Marianne Kreusch, e Edenilson Meyer pelo gratuito suporte técnico na execução de experimentos, análises de dados, e/ou simplesmente por proporcionarem momentos de aprendizados e alegrias durante nossa convivência no laboratório.

Aos meus amigos Kaienna Sandy, Isla Leal, Allan Alencar e Rafael Santana pelo suporte emocional que foi essencial durante a execução de minha pesquisa. Em especial, à Elvis pelos inolvidáveis momentos que tivemos profundas conexões mentais especulando reflexões sobre ciência e filosofia, em alguma praia da ilha da magia.

Por último, mas não menos importante, eu agradeço a minha mãe Irene por sempre apoiar ou aceitar todas minhas escolhas. Você foi verdadeiramente especial para que eu concluísse este objetivo em minha vida.

Ensaiando na ciência!

RESUMO

A contaminação ambiental por hidrocarbonetos e metais tóxicos é um problema global que acarreta danos a todos seres vivos. Os fungos são recursos biológicos no desenvolvimento de bioprocessos voltados a remediação ambiental, devido sua capacidade de interagir e/ou produzir metabólitos que favorecem a interação com o contaminante como, por exemplo, os biosurfactantes. Este trabalho objetivou explorar os bioprocessos e bioprodutos fúngicos para remediação ambiental e aplicações industriais, bem como iniciar o desenvolvimento de produção de biosurfactantes por fungos filamentosos. Neste contexto, os avanços técnicos-científicos no desenvolvimento de bioprodutos fúngicos para remediação de hidrocarbonetos de petróleo e metais tóxicos foram revisados, bem como as principais classes de biosurfactantes e o bioprocessamento de biosurfactantes fúngicos desde o isolamento de linhagens até a sua formulação em produtos comerciais. Uma coleção de culturas de 58 fungos isolados de solos contaminados com hidrocarbonetos (REMA/UFSC) foi criada, e linhagens foram identificados como *Fusarium* (n= 40), *Penicillium* (n= 8), *Paecilomyces* (n= 4), *Scolecobasidium* (n= 1), *Scopulariopsis* (n= 1) e *Ilyonectria* (n= 1), usando características morfológicas e/ou sequenciamento da região ITS. Estes isolados e outros de solos contaminados (n = 7), solos antárticos (n= 46) e endofíticos de plantas de mangue (n = 7) foram bioprospectados para produção de biosurfactantes por meio do teste colapso da gota, índice de emulsificação e tensiometria. Estes testes revelaram o potencial de *Aspergillus* sp. SC21P3, *Fusarium* sp. AF99PD, *Thelebolus* sp. SC29P3, *Paecilomyces* sp. AF45D e *Ilyonectria* sp. AF25D para o colapso da gota (>4 mm), exceto as duas últimas para tensiometria. O uso de extrato de levedura (Meio X e Y) ou $NaNO_3$ (Meio N) nas concentrações de 0.5 $g \bullet L^{-1}$ (Meio X) e 5 $g \bullet L^{-1}$ (Meio Y e N) juntamente com glicose (20 $g \bullet L^{-1}$) e óleo de soja (0,5%) influenciou na atividade de superfície dos biosurfactantes, bem como o perfil cinético desta produção. Em 168h, a composição do meio N promoveu as maiores reduções da tensão superficial usando como inóculos as linhagens *Trichoderma* sp. P05R2 (26,9 $mN \bullet m^{-1}$) e RASC1B10122 (42,3 $mN \bullet m^{-1}$), *Pseudogymnoascus* sp. SC23P3 (36,2 $mN \bullet m^{-1}$), *Penicillium* sp. SCUV02.P1(33,1 $mN \bullet m^{-1}$), P10R6 (27 $mN \bullet m^{-1}$), AF38D (40,1 $mN \bullet m^{-1}$) e AF41D (40,8 $mN \bullet m^{-1}$) e *Curvularia* sp. ILRR1A20047 (37,7 $mN \bullet m^{-1}$), enquanto em meio X para *Buergenerula spartinae* (40,6 $mN \bullet m^{-1}$). Os resultados apresentados neste estudo mostram abrangentemente o potencial de fungos em remediação ambiental e/ou uso em bioprocessos industriais; bem como revela que a bioprospecção tem permitido a descobertas de linhagens anteriormente não descritas para produção de biosurfactantes.

Palavras-chave: biosurfactantes; bioprospecção; solo contaminado; fungos filamentosos; hidrocarbonetos.

ABSTRACT

Environmental contamination by hydrocarbons and toxic metals is a global problem that harms all living beings. Fungi are biological resources in bioprocesses development that may be used in environmental remediation due to their ability to interact and/or produce metabolites that favor interacting with the contaminant, e.g., biosurfactants. This work aimed to explore fungal bioprocesses and bioproducts for environmental remediation and industrial applications and begin biosurfactant production development by filamentous fungi. In this context, technical-scientific advances in developing fungal bioproducts for remediation of petroleum hydrocarbons and toxic metals were reviewed, as well as the main classes of biosurfactants and the bioprocessing of fungal biosurfactants from the isolation of strains to their formulation into commercial products. A culture collection of 58 fungi isolated from hydrocarbon-contaminated soils (REMA/UFSC) was created. Strains were identified as *Fusarium* (n= 40), *Penicillium* (n= 8), *Paecilomyces* (n= 4), *Scolecobasidium* (n= 1), *Scopulariopsis* (n= 1) and *Ilyonectria* (n= 1), using morphological characteristics and/or ITS region sequencing. These isolates and other strains from contaminated soils (n = 7), Antarctic soils (n = 46), and mangrove plant endophytes (n = 7) were bioprospecting for biosurfactant production by drop collapse test, emulsification index, and tensiometry. These tests revealed the potential of *Aspergillus* sp. SC21P3, *Fusarium* sp. AF99PD, *Thelebolus* sp. SC29P3, *Paecilomyces* sp. AF45D and *Ilyonectria* sp. AF25D for drop collapse (>4 mm), and except the last two for tensiometry. The use of yeast extract (Medium X and Y) or NaNO3 (Medium N) at concentrations of 0.5 $g \cdot L^{-1}$ (Medium X) and 5 $g \cdot L^{-1}$ (Medium Y and N) together with glucose (20 $g \cdot L^{-1}$) and soybean oil (0.5%) influenced the surface activity of biosurfactants, as well as the kinetic profile of this production. In 168h, the N medium composition promoted the highest surface tension reduction using inoculum *Trichoderma* sp. P05R2 (26.9 $mN \cdot m^{-1}$) and RASC1B10122 (42.3 $mN \cdot m^{-1}$), *Pseudogymnoascus* sp. SC23P3 (36.2 $mN \cdot m^{-1}$), *Penicillium* sp. SCUV02.P1(33.1 $mN \cdot m^{-1}$), P10R6 (27 $mN \cdot m^{-1}$), AF38D (40.1 $mN \cdot m^{-1}$) and AF41D (40.8 $mN \cdot m^{-1}$) and *Curvularia* sp. ILRR1A20047 (37.7 $mN \cdot m^{-1}$), while medium X for *Buergenerula spartinae* (40.6 mN·m-1). The results presented in this study comprehensively show the potential of fungi in environmental remediation and/or use in industrial bioprocesses, as well as reveal that bioprospecting led to the discovery of previously undescribed strains for biosurfactant production.

Keywords: biosurfactant; bioprospecting; contaminated soil; filamentous fungi; hydrocarbons.

RESUMO EXPANDIDO

Introdução

A literatura científica é expansiva sobre o uso de fungos como recursos biológicos para produção de moléculas de interesse industrial e ambiental. Biosurfactantes fúngicos são promissoras moléculas a serem utilizadas para remediação de hidrocarbonetos, uma vez que eles aumentam a solubilidade e disponibilidade do contaminante para remoção e/ou degradação. O isolamento e biopropescção de isolados fúngicos tem permitido a descoberta de novas espécies produtoras de biosurfactantes, e novas classes destas moléculas. A caracterização morfológica e molecular de isolados fúngicos possibilita o uso destas linhagens como inóculos em bioprocessos. O tipo e concentração da fonte nitrogênio pode influenciar a produtividade da produção de biosurfactantes, bem como as propriedades físico-químicas destas moléculas.

Objetivos

Esta tese objetivou investigar a literatura científica sobre desenvolvimento de bioprocessos a partir do uso de fungos para remediação de hidrocarbonetos e metais tóxicos, e para produção de biosurfactantes. Dentre os objetivos específicos, incluíram-se: isolar e caracterizar fungos filamentosos de solos contaminados com hidrocarbonetos; prospectar estes isolados e outros de solos antárticos e endofíticos de plantas de mangue para produção de biosurfactantes; e avaliar a influência desta produção com o uso de extrato de levedura e nitrato de sódio como fonte de nitrogênio.

Metodologia

O primeiro e segundo capítulo desta tese são artigos de revisão que foram delineados, usando o método de pesquisa empírico-indutivo, a partir de uma perspectiva integral em desenvolvimento de bioprocessos/bioprodutos usando células fúngicas para micorremediação e produção de biosurfactantes. O terceiro e quarto capítulo são artigos de pesquisa experimental delineados pelo método de pesquisa hipotético-dedutivo. No terceiro capítulo, fungos filamentosos foram isolados de solos contaminados com hidrocarbonetos, sem ou com a técnica de enriquecimento, usando técnicas clássicas de microbiologia e diluição seriada. Estes fungos foram também caracterizados morfologicamente e molecularmente usando o marcador molecular ITS. No quarto capítulo; estes isolados adicionalmente com outros isolados de solos antárticos e endofíticos de plantas de mangue foram previamente triados para produção de biosurfactantes usando os testes colapso da gota, índice de emulsificação e tensiometria. Alguns destes isolados foram também selecionados e avaliados em relação ao perfil cinético da produção de biosurfactantes, usando três formulações de meios de culturas contendo nitrato de sódio, ou extrato de levedura em diferentes concentrações.

Resultados e discussão

Os resultados obtidos nesta tese possibilitaram a identificação de fungos filamentosos pertencentes aos gêneros *Fusarium*, *Penicillium* e *Paecilomyces,* os quais são geralmente usados como inóculos em bioprocessos para produção de bioprodutos aplicados em remediação ambiental. Espécies dos genêros *Scopulariopsis*, *Scolecobasidium* e *Ilyonectria* foram também identificadas, porém o uso destes gêneros nestes bioprocessos ainda é incipiente. As linhagens *Fusarium* sp. AF99PD, *Paecilomyces* sp. AF45D, *Aspergillus* sp. SC21P3, *Thelebolus* sp.

SC29P3 e *Ilyonectria* sp. AF25D são potencialmente produtoras de biosurfactantes. A formulação do meio de cultura X contendo extrato de levedura (0.5 g•L^{-1}) promoveu a maior redução da tensão superficial do cultivo de *Buergenerula spartinae*; enquanto do meio N contendo nitrato de sódio (5 g•L^{-1}) para as linhagens *Trichoderma* sp. P05R2 e RASC1B10122, *Pseudogymnoascus* sp. SC23P3, *Curvularia* sp. ILRR1A20047 e *Penicillium* sp. SCUV02.P1, P10R6, AF38D e AF41D. Esse estudo é o primeiro relato sobre a produção de biosurfactantes a partir das espécies *B. spartinaea, Thelebolus* sp. e *Ilyonectria* sp.

Considerações finais

É recomendado o uso de outros marcadores moleculares como β-tubulina, calmodulina, fator de alongamento de tradução, e RNA polimerase II- subunidade maior 1 e 2 para melhor especiação dos fungos filamentosos isolados neste estudo. A triagem de linhagens fúngicas revelou a descoberta de espécies anteriormente não descritas para produção de biosurfactantes, as quais são possíveis inóculos a serem utilizados em futuros bioprocessos objetivados na recuperação e aplicação destas moléculas para remediação ambiental.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

CMC	Critical Micellar Concentration
EPS	Exopolysaccharides
SL	Sophorolipid
MEL	Mannosylerythritol lipids
TL	Trehalose lipids
XLs	Xylolipids
CL	Cellobiose lipids
PL	Polyol lipids
LM	Liamocins
PEFA	Polyol fatty acid esters
LPP	Lipopeptides
ITS	Internal Transcribed Spacer
EI24	Emulsification index
MATH	Microbial adhesion to hydrocarbon test
PUM	Buffer solution composed of phosphate, urea, magnesium
CTAB	Cetyltrimethylammonium Bromide
YMG	Culture medium composed of yeast, malt and glucose
TG	Triacylglycerols
C/N	Ratio between the quantity of carbon and nitrogen sources
STR	Stirred tank reactor
VVM	Volumetric air flow x volume of half x minute^{-1}
TLC	Thin layer chromatography
FTIR	Fourier Transform Infrared
NMR	Nuclear magnetic resonance
MS	Mass spectrometry
GC	Gas chromatography
GC-MS	Mass spectrometry coupled to gas chromatography
HPLC	High performance liquid chromatography
MALDI-TOF-MS	Matrix-assisted laser desorption ionization time of light mass spectrometry
PAH	Polycyclic aromatic hydrocarbons

AMF	Arbuscular mycorrhizal fungi
NO_3^-	Nitrate
O_2	Oxygen
SO_4^-	Sulfate
CO_2	Carbon dioxide
H_2O	Water
GC–MS	Mass spectrometry coupled to gas chromatography
CYP	Cytochrome P450 monooxygenases
Eh	Redox potential
HBT	1-Hydroxybenzotriazole
ABTS	2:2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
ROS	Reactive oxygen species
NER	Non-extractable residues
CMC	Critical micelle concentration
SL	Sophorolipids
MEL	Mannosylerythritol lipids
PL	Polyol lipids
RBBR	Remazol brilliant blue R
CTAB	Cetyltrimethylammonium bromide
DCPIP	2:6-Dichlorophenol indophenol
CG	Gas chromatography
MIC	Minimum inhibitory concentration
AAS	Atomic absorption spectrometry
ITS	Internal Transcript Spacer
DGGE	Denaturing gradient gel electrophoresis
FISH	Fluorescence in situ hybridization
T-RFLP	Terminal restriction fragment length polymorphism
STR	Stirred tank reactors
$CaCO_3$	Calcium carbonate
$Na_5P_3O_{10}$	Sodium tripolyphosphate
Al	Aluminum
Fe_3O_4	Iron Oxide
Pb^{2+}	Lead

Cu^{2+}	Copper
Zn^{2+}	Zinc
Mg^{+2}	Magnesium
K^{+}	Potassium
Na^{+}	Sodium
Fe^{+2}	Iron
As	Arsenic
PCR	Polymerase Chain Reaction
C23O	Catechol 2,3-dioxygenase
RNAi	Post-transcriptional gene silencing
Cd	Cadmium
Cr^{+6}	Chromium
BAP	Benzo[a]pyrene
PHE	Phenanthrene
ANT	Anthracene
PYR	Pyrene
FLU	Fluoranthene
CHRY	Chrysene
BS	Biosurfactant
LCC	Laccase
MnP	Manganese peroxidase
CAT	Catalase
Lip	Lignin peroxidase
Dh	Dehydrogenase
PPO	Polyphenoloxidase
1,2-CTD	Catechol 1,2-dioxygenase
SM	Soil microcosms
LC	Liquid culture
Ni^{2+}	Nickel
$CaCO_3$	Calcium carbonate
CYP52	Cytochrome P450 alkane hydroxylase
VRE	Vancomycin resistant
EM	Mass Spectrometry

HAP	Policíclicos aromáticos
ITS	Subunidade pequena do RNAr
TUB2	β-tubulina
CaM	Calmodulina
TEF-1α	Fator de alongamento de tradução
RPB1	RNA polimerase II- subunidade maior 1
RPB2	RNA polimerase II- subunidade maior 2
$NaNO_3$	Sodium nitrate
$(NH_4)_2SO_4$	Ammonium sulfate

SUMÁRIO

1 INTRODUÇÃO GERAL

1.1 FUNDAMENTAÇÃO TEÓRICA E CONTEXTUALIZAÇÃO

A contaminação ambiental por hidrocarbonetos de petróleo e metais tóxicos é um problema mundial que ameaça a saúde pública (ROSS, 1975; DAVIES; WESTLAKE, 1979; PANDEY et al., 2021). No ano de 2019, dois desastres ambientais ocorreram no Brasil, os quais causaram impactos ambientais, sociais e econômicos. O primeiro ocorreu por derramamento de petróleo devido transporte marítimo, ao longo da região costeira do Brasil; e o segundo ocorreu por metais tóxicos devido a liberação de rejeitos de uma mineradora no sudeste do país (ROTTA et al., 2020; MAGALHÃES et al., 2022). Estes contaminantes apresentam efeitos citotóxicos e mutagênicos, o que diminui o potencial biótico de ecossistemas terrestres e aquáticos. Portanto, há uma necessidade urgente em desenvolver tecnologias visando a remediação e monitoramento ambiental (QUINTELLA; MATA; LIMA, 2019; PANDEY et al., 2021).

A biorremediação microbiana é uma alternativa ecossustentável em que utiliza microrganismos e/ou produtos do metabolismo microbiano para remediação de hidrocarbonetos e metais tóxicos, em comparação aos outros tipos de tratamentos (por exemplo, físicos, químicos e/ou físico-químicos) (QUINTELLA; MATA; LIMA, 2019; DACCÒ et al., 2020).

Os microrganismos estão presentes nos três domínios taxonômicos Archea, Bacteria e Eukarya, e são importantes para todas as formas de vida. Alguns destes protagonistas nas pesquisas em microbiologia industrial e ambiental, uma vez que microrganismos podem atuar em processos industriais com objetivo de produzir bens (produtos) e serviços (processos) que promovam a manutenção da qualidade ambiental e/ou contribuam para sustentabilidade (NAJAFPOUR, 2015).

Os fungos estão incluídos no domínio Eukarya. Quando comparados aos outros eucariotos (algas, protozoários), se destacam no desenvolvimento de bioprocessos e bioprodutos aplicados a micorremediação de hidrocarbonetos e metais tóxicos, devido sua plasticidade metabólica e morfológica (QUINTELLA; MATA; LIMA, 2019; SILVA et al., 2021a; SILVA et al., 2022). No entanto, uma pesquisa bibliométrica sobre análise de artigos científicos e patentes publicadas entre 1997-2017, reportou que os fungos têm recebido menos atenção do que o domínio Bacteria em pesquisas focadas em biorremediação (QUINTELLA; MATA; LIMA, 2019).

Biosurfactantes e enzimas são os bioprodutos fúngicos comercialmente disponíveis para biorremediação, embora haja limitações nesta produção biotecnológica devido aos custos

e baixo rendimento (SILVA et al., 2022). A biotecnologia industrial é um campo da ciência para extrapolar recursos disponíveis na formulação destes bioprodutos, bem como para superar limitações inerentes a cada etapa de bioprocessos (BAJPAI, 2020). Avanços tecnológicos em desenvolvimento de biosurfactantes são alcançados por meio da biotecnologia industrial em diferentes etapas envolvidas no bioprocessamento, tais como: isolamento, triagem e identificação de linhagens; engenharia genética; otimização de condições nutricionais e operacionais; recuperação, purificação e caracterização dos produtos (SILVA et al., 2021a).

Embora estudos em biologia molecular (via técnica independente de cultura) tem permitido extrapolar a bioprospecção de microrganismos/metabólitos (WILLIAMS; TRINDADE, 2017; HAQUE et al., 2022); o uso de microrganismo, ou seja, o inóculo é essencial na grande maioria dos bioprocessos. O inóculo pode ser obtido por meio de isolamento de recursos naturais (contaminados ou não), coleções de culturas e/ou modificação genética (STANBURY; WHITAKER; HALL, 2017a).

Solos contaminados ou não, plantas, animais e/ou ambientes extremos foram reportados como fontes de isolamento de linhagens fúngicas que foram usadas como inóculo para desenvolvimento de bioinoculantes ou biosurfactantes (MOUSAVI; BEHESHTI-MAAL; MASSAH, 2015; YALÇIN; ERGIN-TEPEBAŞI; UYAR, 2018; BUENO et al., 2019; MARTINHO et al., 2019). Neste contexto, embora nenhum estudo tenha sido reportado sobre isolamento de fungos de unidades experimentais de biotratamento de solos e água subterrânea contaminados com hidrocarbonetos, que estão localizadas no Núcleo Ressacada de Pesquisas em Meio Ambiente (REMA) da Universidade Federal de Santa Catarina (UFSC); provavelmente promissores inóculos fúngicos prosperam ali.

A domesticação e caracterização (morfológica, molecular e bioquímica) de isolados fúngicos tem permitido explorar seu potencial em estudos de bioprospecção, e garantir o fornecimento de condições (nutricionais ou operacionais) apropriadas para produção de biosurfactantes (SENA et al., 2018; YALÇIN; ERGIN-TEPEBAŞI; UYAR, 2018). Em síntese, o inóculo e nutrientes requeridos para produção de biosurfactantes, precisam ser devidamente selecionados e padronizados para alcançar maior produtividade e rendimento de processo (ALMEIDA et al., 2017; FERREIRA et al., 2020).

Portanto, a formulação de meio de cultura com composição e concentração apropriadas de nutrientes (por exemplo, fontes de carbono e nitrogênio) é imprescindível para selecionar potenciais isolados microbianos, aumentar a produtividade de biosurfactantes e consequentemente garantir a viabilidade de processos (ALMEIDA et al., 2017; PELE et al., 2019). Por exemplo, sais de nitrato, sais de amônio e extrato de levedura tem sido reportados

como fontes de nitrogênio para produção de biosurfactantes fúngicos (REIS et al., 2018; BECK; ZIBEK, 2020). A alteração do tipo e concentração de fonte de nitrogênio (orgânico ou inorgânico) pode influenciar o rendimento, composição e propriedades físico-químicas de biosurfactantes (JOSHI-NAVARE; SINGH; PRABHUNE, 2014; ISHAQ et al., 2015; ALMEIDA et al., 2017).

1.2 APRESENTAÇÃO E METODOLOGIA DA PESQUISA TEÓRICA-EXPERIMENTAL

Pesquisadores de todo o mundo estão engajados e firmando parcerias com universidades e empresas públicas/privadas para desenvolver conhecimento científico e tecnologias que possam impulsionar o mercado de bioprodutos fúngicos para remediação de ambientes contaminados (FREITAS et al., 2016; SILVA et al., 2021a; RATHANKUMAR et al., 2021). A produção de conhecimento científico e desenvolvimento destas tecnologias dependem do uso de métodos científicos que são selecionados de acordo com a estrutura e características do objeto de pesquisa (MOXLEY, 1974; THOMAS; JAMES, 2006).

Em geral, pesquisas teóricas em desenvolvimento de bioprodutos/bioprocessos são fundamentadas no método empírico-indutivo (MEI), enquanto pesquisas experimentais no método hipotético-dedutivo (MHD) (MOXLEY, 1974; FARDET; LEBREDONCHEL; ROCK, 2021). É válido reportar que a compreensão geral da teoria envolvida no problema de pesquisa (alcançada por MEI) lança luzes para o delineamento de metodologias para testar as hipóteses de pesquisa experimental (via HDM) (MOXLEY, 1974; THOMAS; JAMES, 2006). Em ambos os tipos de pesquisa, os seus resultados são apresentados a comunidade acadêmica por meio da publicação de artigos científicos (SANDERS, 2020).

Embora a literatura científica seja expansiva em estudos sobre micorremediação e biosurfactantes fúngicos (AKHTAR; MANNAN, 2020; SINGH et al., 2020); há uma lacuna em relação a artigos de revisão delineados a partir de uma perspectiva integral em biotecnologia industrial. Portanto, é empírico-indutivo que artigos de revisão sobre esta lacuna, fomentarão o desenvolvimento de tecnologias e/ou aprimoramento de novas, e lançarão luzes a diversas hipóteses de pesquisa devido a abrangência do tema.

Pesquisas experimentais são etapas obrigatórias e preliminares no desenvolvimento de bioprocessos e bioprodutos para alcançar uma futura produção em larga escala (FREITAS et al., 2016; FARIAS et al., 2019). Portanto, é hipotético-dedutivo que o isolamento de fungos (de diversos ambientes), sua caracterização (morfológica e molecular) e bioprospecção para

produção de biosurfactantes permite a descoberta e seleção de potenciais inóculos a serem usados em bioprocessos.

Neste contexto, esta tese é um estudo teórico-experimental em desenvolvimento de bioprocessos a partir de linhagens fúngicas para aplicação em micorremediação ou produção de biosurfactantes. Este manuscrito foi estruturado em quatro capítulos para melhor sistematizar e dispor o objeto de pesquisa aqui investigado por meio de MEI e HDM.

Capítulo I - artigo de revisão que apresenta os avanços recentes e tecnologias emergentes em desenvolvimento de bioprocessos para uso e aplicação de produtos fúngicos como bioinoculantes, enzimas e biosurfactantes (e seus mecanismos de ação) para remediação de hidrocarbonetos e metais tóxicos.

Capítulo II - artigo de revisão que explora as classes estruturais de biosurfactantes fúngicos e suas potenciais aplicações; e apresenta metodologias e tecnologias relacionadas a todas as etapas no bioprocessamento destas moléculas, o que abrange desde o isolamento de linhagens fúngicas até formulação de biosurfactantes comerciais.

Capítulo III - artigo de pesquisa experimental sobre o isolamento e caracterização (morfológica e molecular) de linhagens fúngicas de solos contaminados com hidrocarbonetos (REMA/UFSC).

Capítulo IV - artigo de pesquisa experimental sobre a bioprospecção de isolados fúngicos de solos contaminados (REMA), solos antárticos e endofíticos de plantas de mangue (oriundos de coleção de cultura) para produção de biosurfactantes; bem como sobre o perfil cinético desta produção sob a influência do tipo e concentração de fonte de nitrogênio.

Para finalizar a tese, descreveu-se as considerações finais, realizando um retrospecto geral acerca dos assuntos abordados nos quatro capítulos. A bibliografia completa de cada capítulos foi disposta apenas na seção final referências devido ao elevado número de documentos e possíveis repetições entre os capítulos.

O presente trabalho foi realizado partir da parceria entre pesquisadores das instituições brasileiras UFSC, Universidade Federal do Tocantins (UFT), empresa Petrobras-BR e a instituição norte-irlandês School of Biomedical Sciences, Faculty of Life and Health Sciences. As seções experimentais foram desenvolvidas no Centro Ciências Biológicas (CCB), Núcleo Ressacada de Pesquisas em Meio Ambiente (REMA) e Departamento de Engenharia Química (EQA) da UFSC.

2 OBJETIVOS

2.1 OBJETIVO GERAL

O objetivo desta tese foi apresentar os avanços técnicos-científicos em bioprocessos e bioprodutos fúngicos para remediação ambiental e aplicações industriais, e produzir biosurfactantes por fungos filamentosos.

2.2 OBJETIVOS ESPECÍFICOS

i. Explorar a literatura sobre os avanços recentes e tecnologias emergentes em desenvolvimento de bioprocessos para uso e aplicação de produtos fúngicos como bioinoculantes, enzimas e biosurfactantes (e seus mecanismos de ação) para remediação de hidrocarbonetos e metais tóxicos.

ii. Explorar a literatura sobre as classes estruturais de biosurfactantes fúngicos e suas potenciais aplicações; e apresentar metodologias e tecnologias relacionadas a etapas bioprospecção e bioprocessamento destas moléculas até alcançar o mercado.

iii. Isolar linhagens fúngicas de solos contaminados do REMA/UFSC com diesel, gasolina e etanol, diesel e biodiesel de soja, e biodiesel de palma; e identificá-las para obter uma coleção de cultura.

iv. Prospectar isolados fúngicos de solos contaminados, solos antárticos e endofíticos de plantas de mangue para produção de biosurfactantes; e avaliar a cinética desta produção.

CAPÍTULO I

Bioprodutos fúngicos para remediação de hidrocarbonetos de petróleo e metais tóxicos: avanços recentes e tecnologias emergentes

Este capítulo é uma reprodução do artigo intitulado "**Fungal bioproducts for petroleum hydrocarbons and toxic metals remediation: recent advances and emerging technologies**", publicado em 09 de agosto de 2022, pela revista "*Bioprocess and Biosystems Engineering*" (ISSN: 1615-7591).

Bioprocess and Biosystems Engineering
https://doi.org/10.1007/s00449-022-02763-3

RESEARCH PAPER

Fungal bioproducts for petroleum hydrocarbons and toxic metals remediation: recent advances and emerging technologies

André Felipe da Silva[1,2] · Ibrahim M. Banat[3] · Diogo Robl[1] · Admir José Giachini[1]

Received: 12 May 2022 / Accepted: 22 July 2022

Qualis: A2

Fator de Impacto: 3.434 (2021)

Disponível em: https://link.springer.com/article/10.1007/s00449-022-02763-3

ABSTRACT

Petroleum hydrocarbons and toxic metals are sources of environmental contamination and are harmful to all ecosystems. Fungi have metabolic and morphological plasticity that turn them into potential prototypes for technological development in biological remediation of these contaminants due to their ability to interact with a specific contaminant and/or produced metabolites. Although fungal bioinoculants producing enzymes, biosurfactants, polymers, pigments and organic acids have potential to be protagonists in mycoremediation of hydrocarbons and toxic metals, they can still be only adjuvants together with bacteria, microalgae, plants or animals in such processes. However, the sudden accelerated development of emerging technologies related to the use of potential fungal bioproducts such as bioinoculants, enzymes and biosurfactants in the remediation of these contaminants, has boosted fungal bioprocesses to achieve higher performance and possible real application. In this review, we explore scientific and technological advances in bioprocesses related to the production and/or application of these potential fungal bioproducts when used in remediation of hydrocarbons and toxic metals from an integral perspective of biotechnological process development. In turn, it sheds light to overcome existing technological limitations or enable new experimental designs in the remediation of these and other emerging contaminants.

Keywords: Mycoremediation, Contamination, Bioinoculants, Enzymes, Biosurfactants.

RESUMO

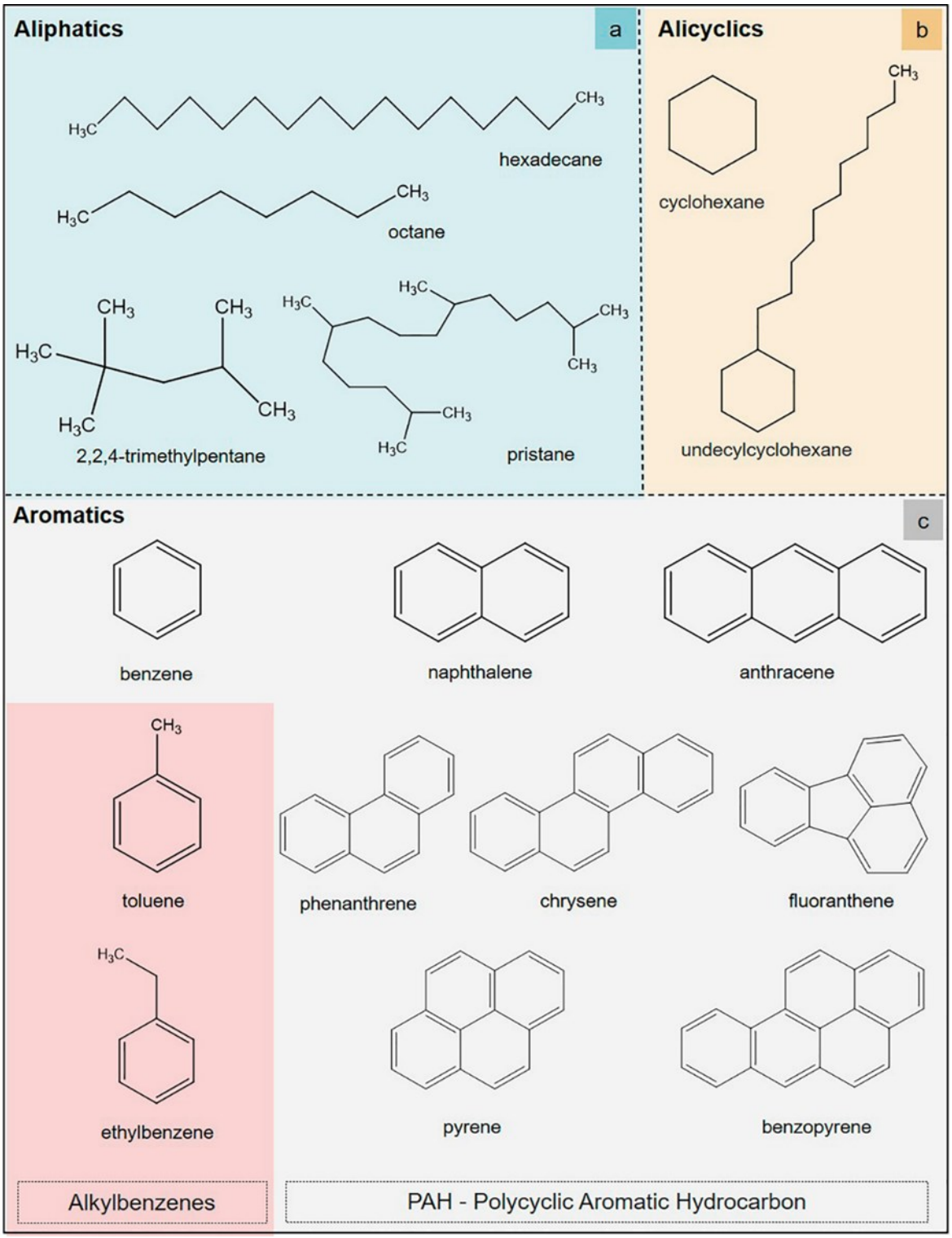
Os hidrocarbonetos de petróleo e os metais tóxicos são fontes de contaminação ambiental e são prejudiciais a todos os ecossistemas. Os fúngicos possuem plasticidade metabólica e morfológica que os tornam potenciais protótipos para o desenvolvimento tecnológico na remediação biológica desses contaminantes devido à sua capacidade de interagir com um contaminante específico e/ou de produzir metabólitos. Embora os bioinoculantes fúngicos que produzem enzimas, biosurfactantes, polímeros, pigmentos e ácidos orgânicos tenham potencial para serem protagonistas na micorremediação de hidrocarbonetos e metais tóxicos, eles ainda podem ser apenas adjuvantes junto com bactérias, microalgas, plantas ou animais em tais processos. Entretanto, o súbito desenvolvimento acelerado de tecnologias emergentes relacionadas ao uso de potenciais bioprodutos fúngicos, tais como bioinoculantes, enzimas e biosurfactantes na remediação desses contaminantes, impulsionou os bioprocessos fúngicos para alcançar maior desempenho e possível aplicação real. Nesta revisão, exploramos os avanços científicos e tecnológicos em bioprocessos relacionados à produção e/ou aplicação destes potenciais bioprodutos fúngicos quando usados na remediação de hidrocarbonetos e metais tóxicos a partir de uma perspectiva integral do desenvolvimento de processos biotecnológicos. Por sua vez, ela lança luz para superar as limitações tecnológicas existentes ou permitir novos projetos experimentais na remediação destes e de outros contaminantes emergentes.

Palavras-chave: Micorremediação, Contaminação, Bioinoculantes, Enzimas, Biosurfactantes.

1 INTRODUCTION

Environmental contamination by petroleum hydrocarbons and toxic metals is a worldwide problem that threatens public health. Petroleum industrial activities, anthropogenic actions and environmental disasters are frequent and represents major sources for such contamination (PANDEY et al., 2021; BEVITÓRIO et al., 2022; MAGALHÃES et al., 2022). Petroleum is a recalcitrant compound with a complex composition of saturated, unsaturated and ramified hydrocarbons, which includes aliphatic, alicyclic, monoaromatic, polycyclic aromatic hydrocarbons (PAHs), resins, and asphaltenes (VARJANI, 2017; PRENAFETA-BOLDÚ; HOOG; SUMMERBELL, 2019). Aliphatics have lower toxicity than aromatics (alkylbenzenes, PAH) and alicyclics. Low molecular weight PAHs have up to three fused benzene rings, while high molecular weight PAHs have four rings or more (Figure 1) (VARJANI, 2017). The volatility of these hydrocarbons decreases with increasing molecular weight and exhibit low solubility and availability, especially in long-term contaminated soils (FAYEULLE et al., 2019). In living cells some metals such as cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn) are essential for various biochemical functions in appropriate concentrations, but at high concentrations these metals are toxic. Other non-essential metals such as arsenic (As), lead (Pb), chromium (Cr), mercury (Hg), and cadmium (Cd) are toxic even at very low concentrations (SINGH et al., 2018; AKHTAR; MANNAN, 2020).

Figure 1 – Chemical structures of the principal petroleum hydrocarbons: aliphatics (a), alicyclics (b) and aromatics (c)



Source: Elaborated by the author (2022).

In this context, hydrocarbons and toxic metals exhibit cytotoxic, carcinogenic and mutagenic effects, which tend to decrease biotic potential in terrestrial and aquatic ecosystems (CHORMARE; KUMAR, 2022). These contaminants can be bioaccumulate in animal or plant

cells (TEKE et al., 2020; MAGALHÃES et al., 2022), and particularly metals have the potential to be transported through the food chain undergoing biomagnification (BEVITÓRIO et al., 2022). Thus, there is an urgent need for the development of technologies to remediate hydrocarbons and toxic metals.

Biological remediation is an eco-sustainable and more cost-effective technology compared to physical or chemical treatments of hydrocarbons and toxic metals (QUINTELLA; MATA; LIMA, 2019; DACCÒ et al., 2020). Patents and articles on biological remediation reported between 1997 and 2017 point out that the main technologies available are those intended for the remediation of hydrocarbons (38%) and toxic metals (21%) in soils (QUINTELLA; MATA; LIMA, 2019). Furthermore, the number of articles and patents on fungi as bioremediation agents is only surpassed by those based on bacteria (QUINTELLA; MATA; LIMA, 2019). This specific fact can be partially explained by the 30-year gap for such purposes between the initiation of fungal bioprocesses (1970s) compared to bacteria (1940s) (ROSS, 1975; DAVIES; WESTLAKE, 1979). Furthermore, technological development involving the production and/or use of fungal metabolites for such specific purposes has only begun in the past 30 years (in 1990s) (LEAHY; COLWELL, 1990). Thus, it is presumed that although fungi of various morphologies such as arbuscular mycorrhizal (AMF) (VERGARA-FERNÁNDEZ; YÁNEZ et al., 2018a), filamentous (BENGUENAB; CHIBANI, 2021; NJOKU; AKINYEDE; OBIDI, 2020), unicellular yeast (GHORBANNEZHAD; MOGHIMI; DASTGHEIB, 2018; CHEN; HU; WANG, 2020) or mushrooms (DAMODARAN; VIDYA SHETTY; RAJ MOHAN, 2013; WOLLENBERG et al., 2021) have been reported as promising prototypes for technological development in hydrocarbon and toxic metal remediation, they still remain underexplored for such purposes.

Prototypes, in technological development of services or products, are structures projected as a proof concepts to validate their feasibility, verify their performance, and collect feedback that may enhance their features before making them commercially available (SILVA et al., 2021b). In this context, fungi are prototypical service providers in remediation due to their ability to assimilate hydrocarbons and obtain energy or produce biomass through passive mechanism (without energy expenditure), as well as toxic metals interaction (e.g., biosorption) (XIAO et al., 2010; SAIKA et al., 2017; BARANGER et al., 2021), which in turn also makes them product providers as (bio)-inoculants for mycoremediation. Alternatively, through active metabolism, fungi are also prototype providers of potential valuable metabolites used in mycoremediation, which includes enzymes (BETTIN et al., 2019), biosurfactants (FERREIRA et al., 2020), organic acids (BODIN; ASP; HULTBERG, 2017), polymers (VERGARA-

FERNÁNDEZ et al., 2018a), pigments (OH et al., 2021) and siderophores (GANESH KUMAR et al., 2021). Fungal strains produce these metabolites to promote their survival, growth and protection in diverse environments. However, this ecological potential to colonize and/or remediate environments can be enhanced when the use of these prototypes is exploited through biotechnological processes (SÁNCHEZ-VÁZQUEZ et al., 2018; AKHTAR; MANNAN, 2020).

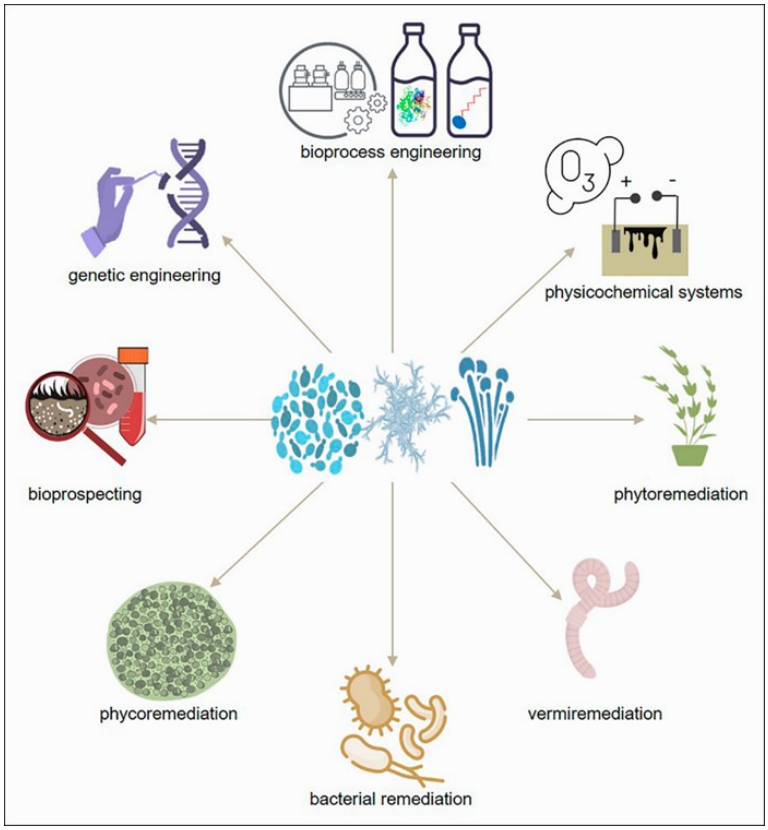
The potential of fungi belonging to the phyla Ascomycota (BARNES et al., 2018; KUMAR; DWIVEDI, 2021), Basidiomycota (DAMODARAN; VIDYA SHETTY; RAJ MOHAN, 2013; WULANDARI et al., 2021), Glomeromycota (LIU et al., 2015; WANG; WANG; MA, 2022), and Mucoromycotina (incertae sedis) (BIROLLI et al., 2018; NJOKU; AKINYEDE; OBIDI, 2020) to remediate hydrocarbons and toxic metals arises from their ability to thrive in such environments and the capability to engage in natural attenuation processes. However, higher performance compared to natural attenuation can be achieved when the biotic potential of these fungal strains is increased through changes in physicochemical parameters (pH, moisture and aeration) at the site and/or by the addition of nutrients to favor biostimulation of the indigenous microbiota (CHAPRÃO et al., 2015; LI et al., 2021a). Furthermore, these autochthonous fungi can be isolated, domesticated and also used as bioinoculants to carry out bioaugmentation of the microbiota involved in remediation through passive or active mechanism (MEDAURA et al., 2021; LI et al., 2021b). In other words, fungi bioprospecting allows the selection of those that have potential to be used to produce bioinoculants or metabolites applied to environmental remediation (SOUZA et al., 2016; LEE et al., 2020). Bioinoculants are selected strains that resist the toxicity and presence of other microorganisms in the contaminated site and still have the ability to interact with contaminants and/or produce metabolites for remediation (FERREIRA et al., 2020; MEDAURA et al., 2021). These metabolites can be different biochemical molecules that interact with contaminants to carry out or enhance the steps involved in environmental decontamination (BENGUENAB; CHIBANI, 2021; OH et al., 2021).

The degradation pathways and interaction of fungal metabolites with hydrocarbons and toxic metals are sources of speculation that enable more assertive bioproduct development (BARANGER et al., 2021; GANESH KUMAR et al., 2021). Biotechnological processes are under constant development to enable large-scale production of enzymes and biosurfactants, but the market for these fungal bioproducts to remediate hydrocarbons and toxic metals is still incipient (SILVA et al., 2021c; FASIM; MORE; MORE, 2021). Only fungal biosurfactants are already marketed for this purpose (FENG et al., 2021; SHAH; DAVEREY, 2021). The main

obstacle to the practical application of products containing enzymes and/or bioinoculants as metabolite producers is related, respectively, to the sensitivity to environmental and nutritional parameters of these products in contaminated environments (EIBES et al., 2015; PANDEY et al., 2021). The high costs, low productivity and technological limitations related to the production/application of fungal bioproducts also still restrict their use (SILVA et al., 2021c; PANDEY et al., 2021).

Biotechnology companies and universities worldwide have boosted fungal prototyping from the development of emerging technologies to propose, elucidate and/or enhance bioprocesses focused on the containment/removal/degradation/detoxification of hydrocarbons or toxic metals (SILVA et al., 2021c; MISHRA et al., 2021; RATHANKUMAR et al., 2021). These emerging technologies are the result of technical-scientific advances and innovations in various fields that promote "biotic and abiotic resource optimization" or "performance enhancement" in various steps of fungal bioprocesses focused on environmental remediation (SÁNCHEZ-VÁZQUEZ et al., 2018; ELSHAFIE et al., 2020). Figure 2 shows the main bioprocess fields in mycoremediation.

Figure 2 – Main current insights in scientific-technical development from the use of fungi in mycoremediation



Source: Elaborated by the author (2022).

In this context, technological development is premised on exploring the biodiversity of fungal strains that allows the expansion of resources as follows: (1) elucidate mechanisms of action and degradation pathways (BARANGER et al., 2021; PEIDRO-GUZMÁN et al., 2021); (2) enhance existing technologies involved in process steps (SÁNCHEZ-VÁZQUEZ et al., 2018), (3) minimize negative effects related to abiotic and biotic parameters (GANESH KUMAR et al., 2021; MEDAURA et al., 2021); and (4) propose alternative strategies through physicochemical-biological systems or biosystems to add resources or overcome technological limitations (ELSHAFIE et al., 2020; BAO et al., 2021). In summary, such biotechnological resources serve as supports in mycoremediation processes and generally have as "starting point" the bioprospecting in different environments, with a possible "end point" being the formulation of bioproducts and ecotoxicological tests to ensure environmental safety (BENGUENAB; CHIBANI, 2021; SILVA et al., 2021b; WULANDARI et al., 2021).

Although the mycoremediation of hydrocarbons and toxic metals is not a new subject in the scientific literature (CHAPRÃO et al., 2015; LI et al., 2021a), its approach is promising and current, which corroborates with the high number of research articles proposing technologies and review articles presenting the scientific state of the art in different fields of knowledge (MISHRA; MALIK, 2014; MARCHAND et al., 2017; WANG; WANG; MA, 2022). No reviews however, were found regarding scientific advances and emerging technologies from a mechanistic perspective focused on the integral mycoremediation processes from bioinoculants, enzymes and biosurfactants. This gap, when overcame, can extrapolate the current interface between available and innovative technologies for fungal bioprocesses in hydrocarbon and toxic metal remediation. This review therefore aimed to compile and explore information on the main mechanistic actions and use of fungal bioproducts such as bioinoculants, enzymes and biosurfactants for the remediation of hydrocarbons and toxic metals. We also comprehensively scrutinized the scientific and technological advances to promote, enhance and increase the performance of these bioproducts when used for the remediation of such contaminants.

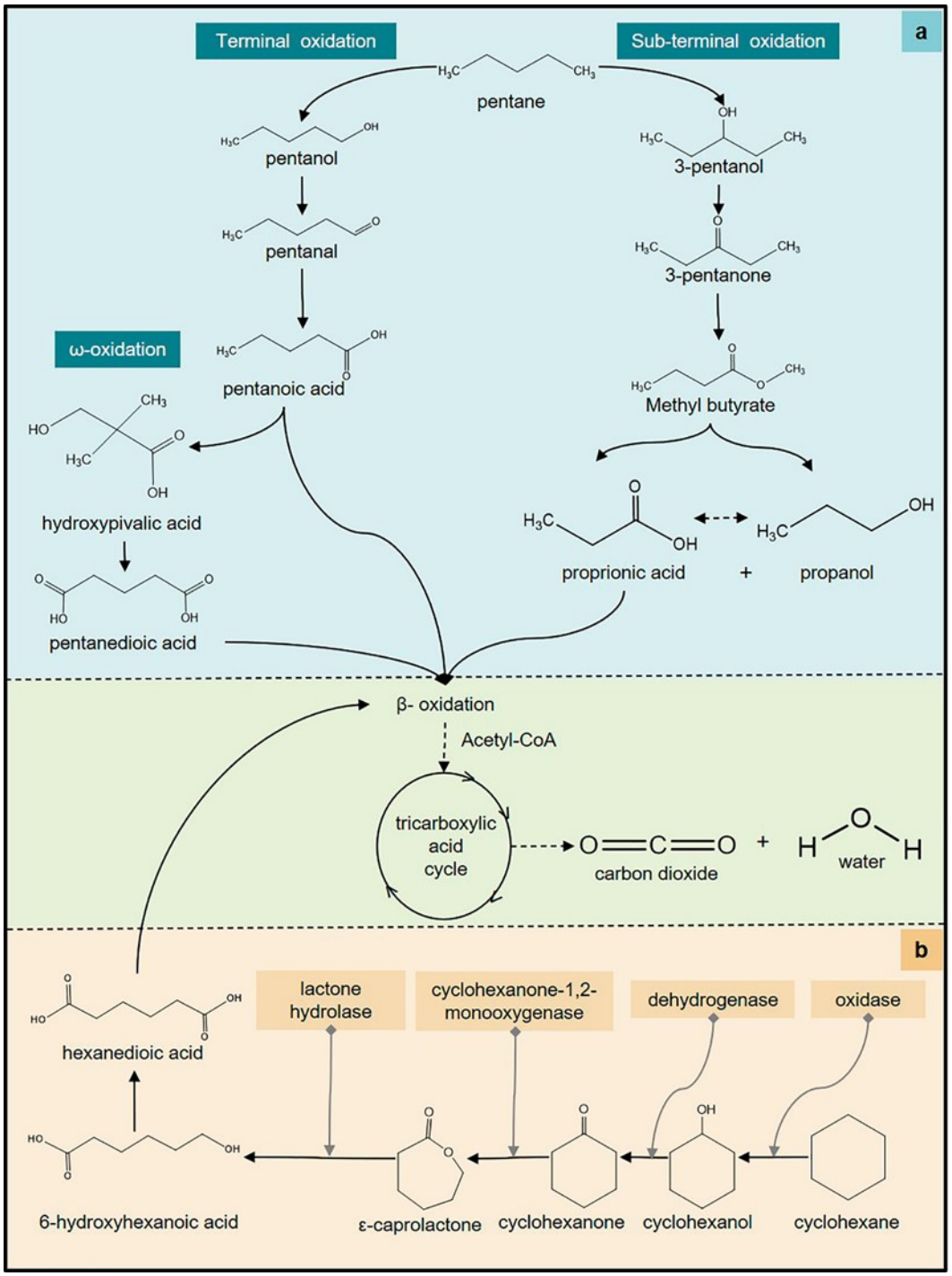
2 FUNGAL MECHANISMS AND ENZYMES FOR HYDROCARBONS REMEDIATION

Oxygen, the second most available element in air, is involved in natural hydrocarbon degradation processes, and acts as the final electron acceptor in aerobic microbial metabolism, which promotes higher energy production compared to anoxic conditions (WILSON; JONES,

1993; GHOSAL et al., 2016). In general, fungal bioinoculants under oxygen-limited or anaerobic conditions (e.g., aquifers, sludge or mangroves) utilize various inorganic radicals or compounds such as nitrate (NO_3^-), sulfate (SO_4^{-1}), Fe^{+2}, Mn^{+2} and carbon dioxide (CO_2) as final electron acceptors (WILSON; JONES, 1993; DACCÒ et al., 2020). Anoxic conditions can also be generated during hydrocarbon degradation due to an increase in microbial respiration during the consumption of readily assimilable substrates used to support microbial growth (GHOSAL et al., 2016). Anaerobic degradation however, can occur at negligible rates and produce metabolites that are more toxic than their original counterpart and/or inhibit other strains during degradation (FAYEULLE et al., 2019; KHATOON; RAI; JILLANI, 2021).

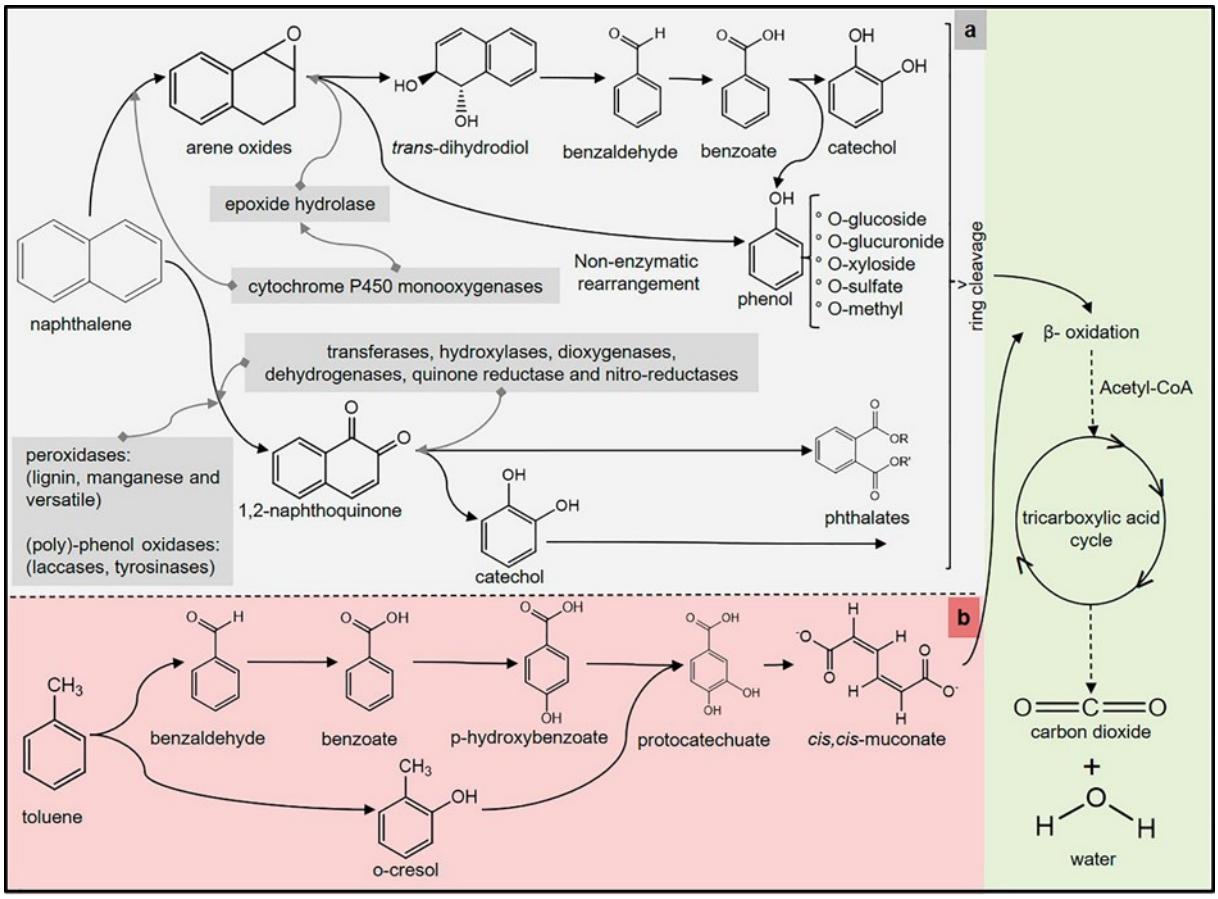
One of the main ecological contributions of fungi is their ability to produce enzymes that cleave chemical bonds and/or transfer functional groups in different hydrocarbon structures. The catalysis involved in hydrocarbon degradation, besides providing energy for strains, assists the transfer of electrons from a reduced organic substrate (hydrocarbons-donor) to another chemical compound (acceptor) (BENGUENAB; CHIBANI, 2021; KHATOON; RAI; JILLANI, 2021). Although the same set of fungal enzymes partially or completely degrade or detoxify hydrocarbons, the degradation pathways and mechanisms involved in these processes may be different for each hydrocarbon (KADRI et al., 2017; PRENAFETA-BOLDÚ; HOOG; SUMMERBELL, 2019). Susceptibility to hydrocarbon degradation by fungal enzymes can be classified by the following sequence: linear aliphatic>branched aliphatic>aromatic>alicyclic (CHAILLAN et al., 2004; VARJANI, 2017). Possible hydrocarbon degradation pathways are deduced through combined analyses of genome annotation/transcriptomic and gas chromatography coupled to a mass spectrometry (GC-MS) profile (GANESH KUMAR et al., 2021; PEIDRO-GUZMÁN et al., 2021). Thus, possible metabolic pathways involved in degradation of aliphatic and alicyclic hydrocarbons, PAHs and alkylbenzene are shown in Figures 3 and 4, respectively.

Figure 3 – Possible peripheral pathways for degradation of aliphatic and alicyclic hydrocarbons by fungi are shown in a and b, respectively



Source: Adapted from Dallinger et al. (2016), Varjani, (2017), and Prenafeta-Boldú; Hoog; Summerbell (2019).

Figure 4 – Possible peripheral pathways for degradation of PAHs and alkyl-benzene by fungi are shown in a and b, respectively



Source: Adapted from Kadri et al. (2017) and Prenafeta-Boldú; Hoog; Summerbell (2019).

When incorporated within the fungal cell, hydrocarbons are degraded by intracellular enzymes via cytosolic or mitochondrial pathways (SINGH et al., 2020; PEIDRO-GUZMÁN et al., 2021). Mass transfer of hydrocarbon into cells by a passive mechanism can be restricted to specific structural fractions of the molecule (FAYEULLE et al., 2014). Hydrocarbon degradation and intracellular lipid metabolism is speculated in fungal cells, since hydrocarbons can be stored in lipid bodies and be oxidized simultaneously in neighboring peroxisomes (DELSARTE et al., 2018; BARANGER et al., 2021). Extracellular enzymes also partially degrade hydrocarbons, producing different intermediate metabolites that may be assimilated by the fungus itself or by other strains for further detoxification (KADRI et al., 2017; PEIDRO-GUZMÁN et al., 2021).

Intracellular enzymes such as epoxide hydrolases, quinone oxidoreductases and cytochrome P450 monooxygenases (CYPs) catalyze several reactions involved in hydrocarbon degradation, such as hydroxylation, epoxidation, dealkylation, sulfoxidation, deamination, desulfurization, dehalogenation and N-oxide reduction in fungal cell (WILSON; JONES, 1993;

SINGH et al., 2020). The number of CYPs genes can vary according to morphological characteristics of the fungus. Saccharomycotina yeasts (*Candida maltosa C. tropicalis, C. apicola, Yarrowia lipolytica*) have relatively few CYPs genes, while filamentous Eurotiales fungi (*Aspergillus flavus, Monascus ruber* and *Penicillium chrysogenum*) tend to have high numbers of CYPs (CHEN et al., 2014a; DACCÒ et al., 2020). Other intracellular enzymes such as transferases (glutathione system), hydroxylases, dioxygenases, dehydrogenases, reductive dehalogenases, nitro-reductases and tyrosinases (sometimes extracellular) may also be involved in hydrocarbons degradation (SINGH et al., 2020; GANESH KUMAR et al., 2021; PEIDRO-GUZMÁN et al., 2021).

Extracellular enzymes such as peroxidases (lignin, manganese and versatile) and the (poly)-phenol oxidases (laccases, tyrosinases) have low substrate specificity, since they are involved in the degradation of lignin (composed of phenolic molecules), which also promotes their catalytic action in hydrocarbon degradation (HADIBARATA; TACHIBANA; ITOH, 2009; SINGH et al., 2020). The redox potential (Eh) of these enzymes is directly related to hydrocarbon oxidation. Peroxidases have high Eh (1.0-1.5 V) and are dependent and sensitive to H_2O_2 concentrations, while laccases have lower Eh (0.4-0.8V) and can be produced by basidiomycetes and ascomycetes (OSTREM LOSS; YU, 2018; PARK et al., 2019). Although laccases and tyrosinases have catalytic similarities such as containing Cu in their structures and requiring O_2 for catalysis, fungal tyrosinases are still less studied for remediation of hydrocarbons compared to laccases (BA; KUMAR, 2017; ROSHANDEL et al., 2021). Tyrosinases oxidize a smaller range of hydrocarbons than laccases and there is a risk of their irreversible inactivation during the degradation of these contaminants (CATHERINE; PENNINCKX; FRÉDÉRIC, 2016; MOHAMMADI et al., 2018). Additionally, hydrolytic enzymes such as lipases and esterases also promote hydrocarbon biotransformation, as genes encoding for these enzymes were expressed by *Dentipellis* sp. (PARK et al., 2019) and *Aspergillus sydowii* (PEIDRO-GUZMÁN et al., 2021), respectively, during degradation of PAHs.

In summary, the reactions involved in enzymatic catalysis of hydrocarbons are aimed to initially incorporate molecular oxygen into their structure and produce more polar pre-intermediate metabolites, which also stimulates their subsequent degradation (WILSON; JONES, 1993; VARJANI, 2017). This goal is achieved through the formation/transfer of hydroxyl groups and/or cleavage of C-C bonds between adjacent phenolic hydroxyl groups of the hydrocarbon, which through specific metabolic pathways form other more soluble and less toxic intermediate metabolites, such as carboxylic acids and aldehydes (GANESH KUMAR et

al., 2021; PEIDRO-GUZMÁN et al., 2021). These, in turn, can be excreted to be degraded by other microbial strains or enzymes; or are stored in lipid vesicles, or biotransformed by β-oxidation and the tricarboxylic acid cycle to produce simpler organic compounds such as pyruvate, acetate, and subsequently CO_2 and water (H_2O) (WILSON; JONES, 1993; VARJANI, 2017; DELSARTE et al., 2018).

2.1 ENZYMATIC MYCOREMEDIATION

When enzyme-producing fungal bioinoculants are applied to contaminated soils, the produced enzymes diffusion is probably low, since excreted enzymes tend to concentrate near the cells and/or where substrates are available (KÜES, 2015). The practical use of enzyme-producing fungal bioinoculants for bioaugmentation in non-sterile contaminated soils may have their enzyme activity influenced due to microbial co-metabolism (KADRI et al., 2017). On the other hand, enzymatic mycoremediation occurs through the use of concentrated enzymes (crude or purified extract), and may present advantages compared to the use of bioinoculants (EIBES et al., 2015; VIPOTNIK; MICHELIN; TAVARES, 2022a). This is corroborated by the fact that enzymes can exhibit: (1) higher catalytic activity; (2) smaller size, which improves their diffusion; and (3) higher stability and coverage over a wide range of physicochemical gradients and contaminant concentrations (EIBES et al., 2015; BETTIN et al., 2019). For instance, the use of crude laccases extract (60 $U \bullet mL^{-1}$) produced by *Pleurotus sajor-caju* at pH 3.2 and 30 °C promoted 55% removal of phenol (3.0 $mmol \bullet L^{-1}$) from an aqueous solution, which represents a higher percentage value when compared to the use of this strain as bioinoculant (BETTIN et al., 2019).

Enzymatic mycoremediation of hydrocarbons is mainly related to the use of laccases that are already commercially available (ZENG et al., 2018; 2021). Although enzymatic degradation of hydrocarbons is more easily carried out in liquid culture compared to the same strains growing in soil, studies of enzymatic mycoremediation in soil microcosms have also shown promising results for hydrocarbon remediation (WU et al., 2008; VIPOTNIK; MICHELIN; TAVARES, 2022b). In summary, higher efficiencies in enzymatic mycoremediation are achieved when parameters such as pH, temperature, enzyme loading and Eh are properly controlled (LI et al., 2010a; VIPOTNIK, MICHELIN, TAVARES, 2022a).

Although fungal laccases show higher Eh than those of bacteria and plants, this potential is still low for oxidation of high molecular weight hydrocarbons (MATE; ALCALDE, 2015). Alternatively, mediating compounds (natural or synthetic) such as 1-

hydroxybenzotriazole (HBT), 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), coumaric acid and ferulic acid can be added to the reaction medium, which are initially oxidized (by laccase) forming reactive oxygen species (ROS) that increase the Eh for oxidation of higher molecular weight hydrocarbons (DENG et al., 2022; VIPOTNIK; MICHELIN; TAVARES, 2022b). The oxidation of ABTS by laccases may be influenced by mediator concentration and water availability (LI et al., 2010a; VIPOTNIK; MICHELIN; TAVARES, 2022a); since greater degradation of benzo[a]pyrene was speculated to occur in aqueous solutions than in soil microcosms, both containing ABTS (LI et al., 2010a). The practical use of enzymatic mediators, however, may be unfeasible due to high costs and possible increase in toxicity (WU et al., 2008; VIPOTNIK; MICHELIN; TAVARES, 2022b).

In soils natural phenolic compounds may act as redox mediators for laccases and possibly enhance enzymatic degradation of hydrocarbons (CAÑAS; CAMARERO, 2010). However, laccases from *Trametes versicolor*, when adsorbed on natural soil minerals such as Fe and aluminum (Al), decreased their enzymatic activity. Catalytic activity however, was increased under acidic conditions (WU et al., 2014), which exemplifies the impact of parameters in enzymatic mycoremediation. Laccases can also have their catalytic activity inhibited or be irreversibly inactivated due to oligomerization of the products formed from oxidation of PAHs such as quinone derivatives and phenolic compounds via self-coupling reactions (CATHERINE; PENNINCKX; FRÉDÉRIC, 2016; MOHAMMADI et al., 2018). Furthermore, enzymatic mycoremediation of solid matrices requires a mixing process to promote a great enzyme dispersion (mass transfer), since the restricted spatial location of the enzyme may differ with the distribution of contaminants (KÜES, 2015; PANDEY et al., 2021).

Several technologies have been proposed to evaluate and/or promote enhancements in enzymatic mycoremediation. For example, the use of packed-bed bioreactors and intermittent feeding of laccases (every 5 days) achieved higher degradation rates of chrysene (78%) and benzo[a]pyrene (35%) in soils within 10 days, while a slowdown of this rate was maintained up to 35 days of mycoremediation (VIPOTNIK; MICHELIN; TAVARES, 2022a). In addition, organic solvents and chemical surfactants are used to increase the availability of hydrocarbons prior to enzymatic mycoremediation, although such chemicals at high concentrations may alter stability or lead enzyme inhibition (LI et al., 2010a; RATHANKUMAR et al., 2021). The conversion of anthracene to anthraquinone reached titers greater than 95% within 12h when the laccases and HBT (mediator) system was added surfactant Tween 80 (YANG et al., 2021). Laccase can also cleave C-O and C=C bonds present in the chemical structure of Tween 80,

promoting the formation of ROS such as RO- and ROO-; which contribute to hydrocarbon degradation (YANG et al., 2021).

Some factors such as fate of degraded hydrocarbons, changes in ecotoxicity and soil microbial community, are criteria that evaluate the feasibility for practical use of enzymatic mycoremediation (ZENG et al., 2018; WULANDARI et al., 2021). Laccases from *T. versicolor* promoted a lower mineralization rate of anthracene, benzo[a]anthracene and benzo[a]pyrene in soils compared to disposal of these PAHs (covalently bound to organic matter) as non-extractable residues (NER) (ZENG et al., 2018; 2021). NERs also enable the detoxification of PAHs due to their recalcitrance to breakdown and transport in organic matter (KÄSTNER et al., 2014). Furthermore, the action of these laccases altered bacterial diversity during mycoremediation of these soils; and increased the toxicity of those contaminated with anthracene, probably due to intermediate metabolites that form from reactions subsequent to the initial oxidation of anthracene (ZENG et al., 2021).

Biotechnological enzyme production with environmental interest presents limitations in terms of low productivity and concentration steps (FASIM; MORE; MORE, 2021; PANDEY et al., 2021). Enzymatic mycoremediation also presents limitations in a large-scale application due to costs (related to enzyme production, use of mediators, etc.), operational instability and non-recovery of enzymes after use (EIBES et al., 2015; KÜES, 2015). Thus, alternative substrates for production and extraction of laccases (LI et al., 2010b; VIPOTNIK; MICHELIN; TAVARES, 2022b); protein engineering and computational simulation (ALCALDE, 2015; CHIADÒ et al., 2021); use of natural mediators (CHEN et al., 2021) and enzyme immobilization are strategies developed to overcome such limitations (MOHAMMADI et al., 2018; DENG et al., 2022).

In this context, laccases extracted from spent mushrooms such as *Agaricus bisporus* and *Pleurotus eryngii* promoted degradation of anthracene, benzo[a]pyrene and benzo[a]anthracene between 66-100% in aqueous solution in 24h (LI et al., 2010b). Laccases from *T. versicolor* (expressed in *Pichia pastoris*) when rationally engineered (via computational simulation) by modification of amino acid residues to have a larger binding pocket, exhibited ability to degrade higher molecular weight hydrocarbons compared to non-mutant laccase, even in the absence of mediators (additional cost) (CHIADÒ et al., 2021). Furthermore, immobilization of laccases on Fe_3O_4 nanospheres coated on silica and chitosan (Fe_3O_4@SiO_2-chitosan) promoted the degradation of anthracene and benzo[a]pyrene by 81% and 69% in 48 h, respectively; and improved operational stability performance (DENG et al., 2022). The recovery of these Fe_3O_4 particles containing laccases in their cores, was facilitated through

magnetic field; which allowed their reuse, achieving over 50% performance for degradation of these PAHs in three catalytic runs (DENG et al., 2022). Additionally, the co-immobilization of laccase and natural redox mediator (phenolic compounds extracted from soybean meal) on Ca-modified chitosan-alginate support promoted a degradation rate of phenanthrene (94%) about 20-30% higher than that of free laccase and immobilized laccase beads without mediator (CHEN et al., 2021). Therefore, the aforementioned technologies tend to boost the enzymatic mycoremediation due to these improvements in operational conditions, and possible reductions in process costs (SAIKA et al., 2017; MOHAMMADI et al., 2018; VIPOTNIK; MICHELIN; TAVARES, 2022b).

3 FUNGAL MECHANISMS AND METABOLITES FOR TOXIC METALS REMEDIATION

Bioinoculant-metal interaction triggers enzymatic and non-enzymatic mechanisms related to metal detoxification in fungal cells (ZHANG et al., 2015; MOTA et al., 2020). Fungal exposure to metal promotes oxidative stress that induces ROS formation as well as the elimination of thiols (glutathione and cysteine), important non-enzymatic antioxidants (ZHANG et al., 2015). Antioxidant enzymes such as catalases, glutathione peroxidases and superoxide dismutase are also produced by some strains as a primary defense mechanism (CHAKRABORTY; MUKHERJEE; DAS, 2013). The remediation of heavy metals from fungal bioinoculants can occur by altering metal mobility via immobilization or solubilization mechanisms, or also via enzymatic by reducing metal toxicity due to its transformation from one oxidative or organic complex state to another (MOSA et al., 2016; SINGH et al., 2018).

Fungal bioinoculants can immobilize toxic metals in their biomass by biosorption or bioaccumulation mechanisms, as well as by excretion of metabolites such as chelating agents and pigments (OH et al., 2021; WOLLENBERG et al., 2021). Biosorption does not necessarily depend on an active mechanism, i.e., immobilization of the metal can also be achieved by dead fungal cells (MOSA et al., 2016; SINGH et al., 2018). The potential of fungal bioinoculants as biosorbents of metals emerges from the interaction between metals with chemical groups such as carboxylate, hydroxyl, amino and phosphate of macromolecules (polysaccharides, pigments) that compose their cell wall (XIAO et al., 2010; FERNÁNDEZ et al., 2018). For instance, chitosan polymer produced from deacetylation of chitin extracted from *Cunninghamella elegans* showed almost similar adsorption capacity for Pb^{2+} and Cu^{2+} (300 $mg \bullet kg^{-1}$) in aqueous solutions or contaminated soils (ALSHARARI; TAYEL; MOUSSA, 2018). Likewise, melanin

extracted from *Amorphotheca resinae* consisting of indole-based functional groups showed capacity for biosorption of Cu^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+} in aqueous solutions, as well as constant adsorption/desorption capacity for five cycles (OH et al., 2021). Biosorption mechanisms therefore, can involve ion exchange, adsorption, electrostatic interaction, complexation and precipitation processes (MA et al., 2015; LU et al., 2020). In turn, these are highly dependent on parameters such as pH, metal ion concentration and biomass, as biomasses with strong negative charge can adsorb metal ions (DUSENGEMUNGU et al., 2020). Additionally, metal immobilization can also occur through its biomineralization, when it forms metal-metabolite complexes with precipitating agents produced by fungal bioinoculants, e.g., oxalic acid produced by *Phanerochaete chrysosporium* under metal stress promoted metal detoxification by immobilizing soluble metal ions as metal oxalate crystals (XU et al., 2015).

Bioaccumulation process depends on the toxicokinetics and sensitivity of the bioinoculant to absorb the metal in its structure, which may require a longer time for remediation compared to biosorption (MOSA et al., 2016; FERNÁNDEZ et al., 2018). It is speculated that metal bioaccumulation may occur via a mechanism similar to the influx of metabolically important ions such as Mg^{+2}, K^{+} and Na^{+} into the fungal cells (SINGH et al., 2018). Some fungi immobilize toxic metals via intracellular peptides such as glutathione, phytochelatin and metallothionein, as these chelating peptides have conserved cysteine residues that allow the formation of metal-thiolate clusters (HARMS; SCHLOSSER; WICK, 2011). Bioinoculants composed by AMF, besides the synergistic contribution with plant roots, can carry out bioaccumulation and phytostabilization of metals present in the rhizosphere due to the internalization of metals in their structures (vacuole, cell wall) (HASSAN; HIJRI; ST-ARNAUD, 2013; LIU et al., 2015). The main advantage of fungal bioinoculants to accumulate metals is related to their active metabolism that allows their reproductive capacity and modification of the cell surface to absorb them. However, their subsequent recovery is limited due to their internal compartmentalization or precipitation (MUÑOZ et al., 2012; FERNÁNDEZ et al., 2018). Several mushrooms (edible, inedible and poisonous) are also bioaccumulators of toxic metals, since different metal concentrations can be accumulated along their fruiting bodies (stipe, cap), which depends on the physiology of each species (DAMODARAN; VIDYA SHETTY; RAJ MOHAN, 2013; BAREA-SEPÚLVEDA et al., 2022). In particular, wild edible mushrooms pose a risk to human health due to their high potential for toxic metal accumulation (BAREA-SEPÚLVEDA et al., 2022).

Bioinoculants composed of *Glomus versiforme* or *Rhizophagus irregularis* can alter the mobility of metal present in rhizospheres by phytoextraction as they assist metal-plant

translocation (HASSAN; HIJRI; ST-ARNAUD, 2013; LIU et al., 2015). Metal mobility can also be altered by the action of fungal metabolites such as organic acids, biosurfactants and siderophores (hydroxamates), which promote metal solubilization and sorption/desorption processes (AHMED; HOLMSTRÖM, 2014; MOTA et al., 2020; MISHRA et al., 2021). Although the acid bioleaching by *Aspergillus niger* or *Cladosporium halotolerans* showed promising results for removal of metals such as Pb^{+2}, Zn^{+2} or Mn^{+2} in aqueous solutions, the leaching of sediments can be a source of natural contamination due to the release (solubilization) of metals present in its composition (YANG et al., 2009; MOTA et al., 2020). Siderophores increase bioavailability of metals in soils as a mechanism to facilitate Fe^{+2} uptake, which also promotes solubilization of toxic metals (AHMED; HOLMSTRÖM, 2014).

Fungal bioinoculants can carry out oxidation/reduction reactions that biotransform toxic metals into their more stable, less toxic and volatile speciation. These reactions involve the linkage of methyl or alkyl groups through enzymatic mechanisms, which promotes biovolatilization of the metal in its surrounding environment (BORIOVÁ et al., 2014; FERNÁNDEZ et al., 2018; KHATOON; RAI; JILLANI, 2021). However, toxic metals can affect biochemical mechanisms involved in their detoxification, as they can replace appropriate metal cofactors for enzyme catalysis (LI; LIU; GADD, 2020). In general, less focus has been placed on enzymatic mechanisms in toxic metal remediation, since metals are not decomposed into intermediate metabolites such as petroleum hydrocarbons (ZHANG et al., 2015; MOTA et al., 2020). Laccases from *T. versicolor*, using ABTS, promoted the removal of Fe and Al contained in contaminated soils respectively by 90% and 99% within 35 days (VIPOTNIK; MICHELIN; TAVARES, 2022a).

4 FUNGAL BIOSURFACTANTS: THE MULTIFUNCTIONAL MOLECULES

Chemical surfactants are used to increase the availability and mobility of hydrocarbons and toxic metals strongly associated with non-polar domains of soil (i.e., organic matter and micropores), and this promotes better conditions for subsequent remediation of these contaminants (CHAPRÃO et al., 2015; MISHRA et al., 2021). Although the combination of chemical surfactants with fungal bioinoculants has promoted better performance in mycoremediation, high concentrations of surfactants can decrease the biotic potential of native soil microbiota (SOUZA; VESSONI-PENNA; SOUZA; VESSONI-PENNA; OLIVEIRA, 2014; RATHANKUMAR et al., 2021). These chemicals may also concomitantly cause another environmental problem due to their toxicity and retention in the soil matrix (SILVA et al., 2021c;

MISHRA et al., 2021). Thus, fungal biosurfactants or biosurfactant-producing fungal bioinoculants are ecological alternatives to reduce environmental toxicity, since fungal metabolism is induced to produce such amphiphilic molecules when the strain is exposed to hydrocarbons (SARUBBO et al., 2015; KAMYABI; NOURI; MOGHIMI, 2017; AL-HAWASH; ZHANG; MA, 2019). The main physicochemical properties of biosurfactants for environmental remediation are related to the reduction of surface/interfacial tension and increase of surface area in different fluid phases, which results in better mobility, solubility, emulsification and bioavailability of contaminants (SOUZA; VESSONI-PENNA; OLIVEIRA, 2014; BAMI et al., 2022).

Fungal biosurfactants are directly related to the mechanisms for contaminant absorption by bioinoculants. These molecules can favor hydrocarbons uptake even if the fungal hyphae do not directly touch the contaminant. This fact was demonstrated in a microsystem with *Talaromyces helicus* (biosurfactant producer) and benzo[a]pyrene which were introduced in separate compartments, and even so there was PAH uptake by the fungal strain (BARANGER et al., 2021). Biosurfactants interact with the fungal cell surface through its hydrophilic moiety, which exposes its hydrophobic moiety to the outside and increases the hydrophobicity of the cell surface for contaminant permeation (SILVA et al., 2021c). Similarly, fungal exopolysaccharides interconnect individual cells into a complex mass tangle when these are produced by the strain and/or adhered to its cell wall, which facilitates hydrocarbon uptake (CHANDRAN; DAS, 2012).

The action of biosurfactants as agents for mobilization of hydrocarbons depends on its Critical Micelle Concentration (CMC), since that at concentrations below CMC there is a reduction of the interfacial force between soil and oil, facilitating diffusion (BAMI et al., 2022). On the other hand, at concentrations above CMC, aggregated structures are formed as micelles due to its self-assembly property (BAO et al., 2021; SINGH et al., 2021). In this context, micelles promote solubilization of hydrocarbons (incorporation), while hydrophilic groups are directed to the surface, i.e., the hydrophobic contaminant is coated by a hydrophilic surface (BAO et al., 2021; SINGH et al., 2021). However, higher efficiency process is achieved at higher concentrations compared to CMC, since at lower concentrations surfactants may be degraded or lost on adsorption to the soil (RATHANKUMAR et al., 2021).

The interaction performance between fungal biosurfactants and toxic metals depends on the chemical structure and ionic charge of the molecule. Biosurfactants can have heteroatoms and/or functional groups (such as carboxyl, hydroxyl and amino) in their structure that by Van der Waals electrostatic interaction form metal-biosurfactant complexes with toxic metal ions

(SANTOS et al., 2017a; MISHRA et al., 2021). Biosurfactants self-assembled as micelles can also interact with oppositely charged metals, which increases the solubility and mobility of the metal and hence its availability (MULLIGAN; YONG; GIBBS, 2001; BAMI et al., 2022). Anionic biosurfactants have a strong chelating action for cationic metal ions, while cationic biosurfactants for anionic metal ions (MISHRA et al., 2021). Some cationic biosurfactants are toxic to microorganisms and inhibitory to the biodegradation process even at low concentrations (PINO-HERRERA et al., 2017). Non-ionic surfactants exhibit lower CMC values and higher stability in the presence of electrolytes and divalent cations than ionic surfactants, which may require lower surfactant concentrations in the process. However, high adsorption of non-ionic surfactants on soil particles may result in their lower availability (RATHANKUMAR et al., 2021).

Fungal biosurfactants are molecules with versatile chemical structures, which influence their molecular weight and physicochemical properties. These molecules are composed of polysaccharides, lipids and/or proteins conjugates (SILVA et al., 2021c). Sophorolipids, mannosylerythritol lipids, polyol lipids, hydrophobins and glomalin are the classes of fungal biosurfactant reported with potential for hydrocarbons or toxic metals remediation (PUGLISI et al., 2012; GAŁĄZKA et al., 2020; SILVA et al., 2021c). However, SL have attracted more attention for such purpose probably due to the fact that they are a commercially available (FENG et al., 2021; SHAH; DAVEREY, 2021). In sum, fungal biosurfactants present functional stability even in adverse conditions (temperature, pH, salinity), i.e., they maximize the possible places to be applied and maintain surfactant action even at suboptimal conditions (FERREIRA et al., 2020; MISHRA et al., 2021).

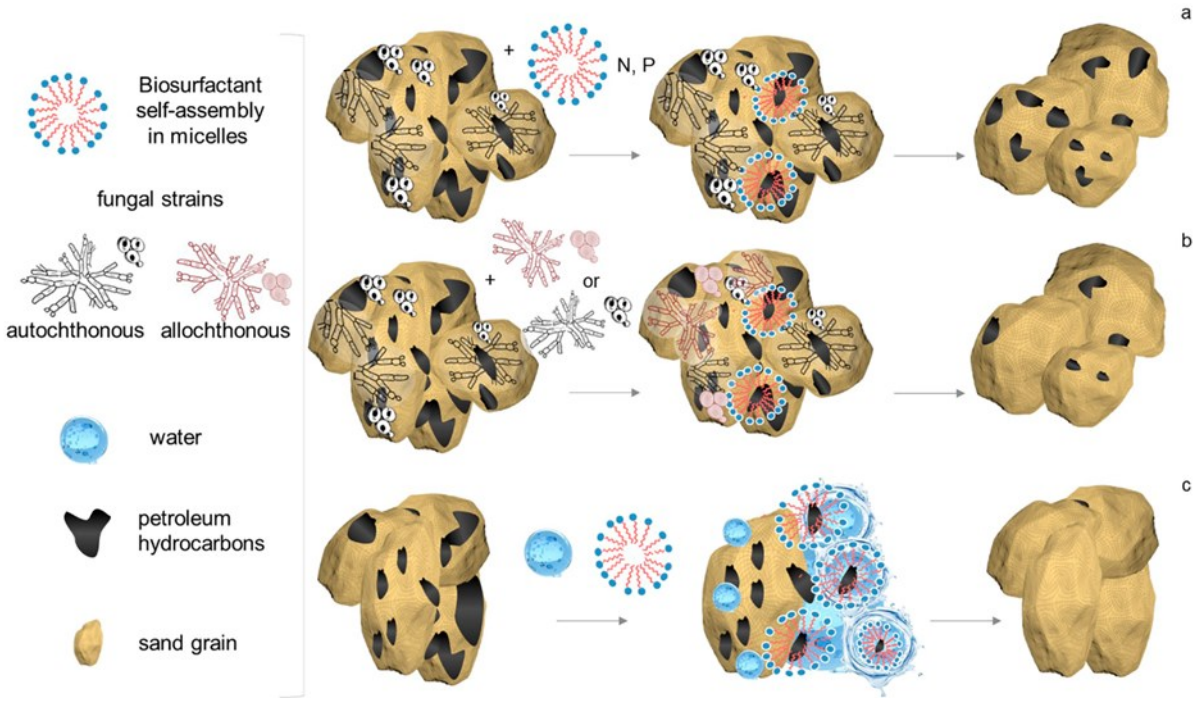
According to the literature, biosurfactants should be able to reduce water surface tension from 72 $mN \bullet m^{-1}$ to values lower than 35 $mN \bullet m^{-1}$ (SILVA et al., 2021c); surfactin (bacterial surfactant) presents values close to 28 $mN \bullet m^{-1}$ (ARIMA; KAKINUMA; TAMURA, 1968). SL, MEL and PL produced respectively by *Meyerozyma* sp., *Ceriporia lacerate* and *Rhodotorula paludigena*, showed surface tension reduction between 31-33 $mN \bullet m^{-1}$, and emulsification index for hydrocarbons between 77-84%; which corroborates the potential of these biosurfactants for environmental remediation (NIU et al., 2017; WANG et al., 2019a; REHMAN et al., 2021). Hydrophobins and glomalin are hydrophobic proteins with surfactant and chelating action (PUGLISI et al., 2012; CHEN et al., 2020). Hydrophobin production has been speculated to alter the cellular hydrophobicity of *Aspergillus brasiliensis* during hexadecane uptake (SÁNCHEZ-VÁZQUEZ et al., 2018). Overexpression of genes encoding hydrophobins during cultivation of *Trichoderma harzianum* in mercury-containing medium has

also been reported to facilitate bioaccumulation of the metal (PUGLISI et al., 2012). Glomalin is produced by AMF, and acts in the maintenance of soil properties (particle aggregation and stability) (GAŁĄZKA et al., 2020). This protein showed ability to immobilize Zn^{+2} and Pb^{+2} in aqueous solutions (YUAN et al., 2022), and to reduce phenanthrene adsorption in soil (increasing bioavailability for remediation) (CHEN et al., 2020).

4.1 ADJUVANTS FOR BIOSTIMULATION AND BIOAUGMENTATION PROCESSES

Fungal biosurfactants are adjuvants that can enhance biostimulation and/or bioaugmentation processes. A schematic chart on strategies for the use of fungal biosurfactants in remediation of soils contaminated with petroleum hydrocarbons is presented in Figure 5. The addition of biosurfactants increases the availability of the contaminant, which promotes its subsequent assimilation/degradation by the autochthonous microbial community (biostimulation) (Figure 5-a) (SOUZA; VESSONI-PENNA; OLIVEIRA, 2014; ZADEH; MOGHIMI; HAMEDI, 2018). Fungal biosurfactants can also be assimilated as substrates for autochthonous microbiota (FENG et al., 2021). On the other hand, the addition of biosurfactant-producing fungal bioinoculants compatible with native soil microbial communities can maximize remediation efficiency (bioaugmentation) (Figure 5-b). Combining the use of biosurfactants and bioinoculants can minimize the microbial load (inoculum size) required in the mycoremediation process and achieve similar results (SOUZA; VESSONI-PENNA; OLIVEIRA, 2014; PINO-HERRERA et al., 2017). Furthermore, it is assumed that fungal biosurfactants in biostimulation/bioaugmentation processes increase bioavailability of substrates due to solubilization of organic matter, which can stimulate fungal growth. However, surfactant concentration may also influence the activity of enzymes involved in the process (RATHANKUMAR et al., 2021).

Figure 5 – Schematic chart on strategies for the use of fungal biosurfactants in remediation of soils contaminated with petroleum hydrocarbons



Caption: Fungal biosurfactants can be added with micro- and macro nutrients for biostimulation (a) of autochthonous fungi involved in remediation, while the addition of fungal bioinoculants (autochthonous or allochthonous) that produce biosurfactant can increase efficiency due to microbial bioaugmentation (b). Fungal biosurfactants can also be added to washing solutions to facilitate contaminant desorption/solubilization, and achieve higher removal rates (c).
Source: Elaborated by the author (2022).

Mannoproteins produced by *Saccharomyces cerevisiae* promoted a twofold higher degradation of soy biodiesel when added to unsterilized contaminated soils compared to sterilized soils, i.e., the biosurfactant potentiated the biostimulation process (KRELING et al., 2020a). The use of biosurfactants produced by *Candida sphaerica* (CHAPRÃO et al., 2015) and *Starmerella bombicola* (SILVA et al., 2021b) combined to processes of biostimulation with sugarcane molasses and bioaugmentation with their respective producing yeast, besides reducing the time required to reach the same level of degradation in these processes without biosurfactant, also promoted the degradation of 50% and 88% of motor oil in sand, respectively (CHAPRÃO et al., 2015; SILVA et al., 2021b). Moreover, bioaugmentation processes applied to soils contaminated with biodiesel (20%) by inoculation (10%) of *A. niger* in solid fermented medium (containing biosurfactants and lipases) promoted a 10% higher degradation compared with natural attenuation process within 60 days duration (KRELING et al., 2020b).

4.2 ADJUVANTS FOR BIOSTIMULATION AND BIOAUGMENTATION PROCESSES

Soil washing processes containing surfactants even at concentrations below their CMC increases the solubility of hydrocarbons and toxic metals (Figure 5-c) (LUNA; RUFINO; SARUBBO, 2016; GOSWAMI et al., 2020). Application of surfactant-containing wash solutions can be carried out by techniques including flooding, basin infiltration system, infiltration well, and leach field (RATHANKUMAR et al., 2021). Biosurfactants increase the transfer of the contaminant to the aqueous phase. They reduce the adhesion between the contaminant and the soil matrix, as well as contaminant viscosity (SOUZA; VESSONI-PENNA; OLIVEIRA, 2014; FERREIRA et al., 2020). SL, when added to washing solutions, increase the capacity for Fe, Cu and As removal compared to water alone (ARAB; MULLIGAN, 2018), as well as show superior potential to synthetic surfactants (sodium dodecyl sulfate and Tween 80) for Cd and Pb removal during soil washing (QI et al., 2018).

Biosurfactants from *C. sphaerica*, *C. tropicalis* and *Rhizopus arrhizus*, when applied to artificially contaminated sand, were effective in removing adsorbed engine oil (65%), petroleum (78%) and diesel oil (79.45%), respectively (SOBRINHO et al., 2008; BATISTA et al., 2010; PELE et al., 2019). Likewise, SL produced by *S. bombicola* showed superior results (68%) when compared to the chemical surfactant Triton-X (38%) for the removal of kerosene (C10 - C40) from a contaminated soil (GOSWAMI et al., 2020). The performance of washing soils with SL in packed columns depends on the granulometry, porosity and permeability of soils, since the removal of motor oil in sandy soil and beach sand was approximately three- and fourfold higher than in silt and clay soil, respectively, due to a better mass transfer during percolation of the washing solution (SILVA et al., 2021b).

Toxic metal removal from soils by washing can be enhanced with prior speciation of components, shapes or phases, in which the metallic elements occur (OLANIRAN; BALGOBIND; PILLAY, 2013). Metals in exchangeable hydroxides state, carbonates and reducible oxides are more easily removed, unlike residual fractions, during the washing process (MULLIGAN; YONG; GIBBS, 2001). Soil washing processes do not necessarily require highly purified biosurfactants, those produced by a *Y. lipolytica* removed 30-40% Cu and Pb in artificially contaminated sand, while the same purified ones also removed approximately 30% (SANTOS et al., 2017a).

The washing of soils contaminated with toxic metals can have its performance influenced by the chemical composition and concentration of biosurfactant, temperature (ARAB; MULLIGAN, 2018; QI et al., 2018) and the use of other adjuncts such as acids and

bases (LUNA; RUFINO; SARUBBO, 2016). Acidic SL produced by *S. bombicola* at 8% concentration when added to washing solutions promoted removal of Cd (83%) and Pb (45%) in artificially contaminated soils. However, due to their lower water solubility, lactonic SL at 1% concentration promoted lower removal of Cd (10%) and Pb (4%) in these soils (QI et al., 2018). Biosurfactants from *C. sphaerica* were superior to chemicals used in acid and basic leaching for the removal of Pb (70%), Fe (89%) and Zn (87%) from contaminated soils, even at concentrations lower than the CMC (LUNA; RUFINO; SARUBBO, 2016). The temperature of the wash solution containing acidic and lactonic SL when increased from 15 to 23 °C also increased the removal of arsenic by 11%, but this potential was decreased by 22% when the temperature was increased from 23 to 35 °C, probably due to changes in properties involving metal-biosurfactant interaction (ARAB; MULLIGAN, 2018).

5 SELECTION OF FUNGAL BIOINOCULANTS AND/OR METABOLITES FOR REMEDIATION

The convergence between the premises that nature still has under-exploited resources and fungi have a ubiquitous habitat is the driving force for bioprocesses development aimed at exploiting biodiversity through bioprospecting technologies, which involves the isolation and screening of potential bioinoculants and/or metabolites for environmental remediation. The isolation of fungal strains can promote the discovery of new species or species not yet described for mycoremediation, as well as promote the domestication of these isolates for use in bioprocesses related to the production/application of bioinoculants and metabolites for environmental remediation (AL-HAWASH et al., 2018a; BECARELLI et al., 2021). Although extreme environments such as cold (FERNÁNDEZ et al., 2017), saline and marine (high pressure and depth) (SHETAIA et al., 2016; GANESH KUMAR et al., 2021) have been resource to isolate potential fungal bioinoculants and metabolites for remediation of hydrocarbons and toxic metals, these environments are under explored compared to contaminated soils and mine tailings (BENGUENAB; CHIBANI, 2021; MOTA et al., 2020).

It is not a fallacy to report that technologies related to bioprospecting and screening of bioinoculants, and metabolites are exhaustive and tiresome similar to the route of a treasure map, but these technologies give accessibility to find possible fungal treasures to be used in bioprocesses focused on environmental remediation. Thus, the development of methodologies to reach such treasures is based on consolidated scientific methods and on the current scientific and technological progress to provide technologies related to the bioprospecting of fungal

strains in order to effectively isolate and screen a larger number of strains and present them in environmental remediation strategies.

5.1 ISOLATION, THE FIRST STEP TOWARDS UNVEILING HIDDEN POTENTIAL

Although contaminated environments can promote a toxic effect on the local microbiota, they can also promote an enriching effect to isolate more resistant autochthonous fungi than allochthonous ones to be used in remediation of hydrocarbons and toxic metals (BIK et al., 2012; CHANG et al., 2019; CHAUDHARY et al., 2022). Fungi thrive in contaminated environments as their metabolic pathways are modulated to respond to both the nutrient and the stress state (e.g., presence of contaminants) in order to efficiently allocate energy from constitutive to conditional expression (OSTREM LOSS; YU, 2018). Thus, enrichment of soils (contaminated or not) with hydrocarbons or toxic metals; and/or with micro and macronutrients for a period of time before isolation may promote greater chances of selecting potential fungi able to assimilate/interact with contaminants or produce metabolites (BENGUENAB; CHIBANI, 2021; CHAUDHARY et al., 2022). This statement is true considering that fungal metabolism is induced to produce enzymes and/or biosurfactants under such conditions for the strain to thrive in the nutrient medium (with or without a specific contaminant) (KAMYABI; NOURI; MOGHIMI, 2017; AL-HAWASH et al., 2019).

The fungal biodiversity, when explored through cultivation-dependent techniques, results in a practically "negligible" number of recovered individuals in comparison to the real "hidden community" that thrives there, since some microbial strains may be neglected because they are not cultivable under laboratory conditions and/or require selective technologies to promote appropriate conditions for their isolation (PRENAFETA-BOLDÚ et al., 2001; WILLIAMS; TRINDADE, 2017). These selective technologies can be based on aspects related to cellular composition (cellular hydrophobicity) or microbial metabolism (growth rate) to promote a targeted isolation of fungal strains that potentially interact with contaminants or that would be presumably hidden (PRENAFETA-BOLDÚ et al., 2001; SATOW et al., 2008). For example, the mineral oil flotation technique may preferentially select the isolation of fungal strains with a hydrophobic surface (assimilate hydrocarbons by passive mechanism), as these strains tend to concentrate at the oil/salt solution interface due to the hydrophobicity of their cell wall (SATOW et al., 2008). Moreover, microbial exposure to the atmosphere of aromatic compounds selects fungi that are resistant to the toxic gases, as well as prevents possible slow-

growing, low-competitive bioinoculants from being overlooked due to the rapid growth of other fungal strains during isolation (PRENAFETA-BOLDÚ et al., 2001).

5.2 SCREENING/BIOPROSPECTING

After isolation, the selection of possible bioinoculants and/or metabolites are screened from technologies that may involve colorimetric, gravimetric, respirometric, chromatographic, spectroscopic, enzymatic and/or tensiometric analysis (MARCHAND et al., 2017; AL-HAWASH et al., 2018a; LEE et al., 2020). However, screening a high number of fungal isolates requires simple, fast, inexpensive, and most importantly accurate and reproducible techniques for all of them (LEE et al., 2014; POYNTNER et al., 2018). The main qualitative and quantitative methods for screening potential fungi to be applied in hydrocarbon remediation are based on the growth of strains in specific media, both solid and liquid, to analyze their ability to assimilate the contaminant and/or ability to produce enzymes or biosurfactants (SOUZA et al., 2016; KAMYABI; NOURI; MOGHIMI, 2017; MEDAURA et al., 2021). The combination of screening techniques provides a more accurate answer on the selection of fungi for bioprocesses focused on environmental remediation, since each individual technique has different sensitivity and coverage, which may overlook some potential strains if not selected by a specific method (SATOW et al., 2008; LEE et al., 2020; CHAUDHARY et al., 2022). For example, solid media are more susceptible to false-positive results for metabolite screening and may limit mass transfer of metabolite in the media (SILVA et al., 2021c).

In this context, solid media containing hydrocarbons are commonly used for an initial screening of strains able to resist or tolerate contaminants (ORTEGA-GONZÁLEZ et al., 2015; BENGUENAB; CHIBANI, 2021). Colorimetric tests such as phenolic oxidation (gallic acid, tannic acid, guaiacol) and Remazol Brilliant Blue R (RBBR) decolorization are used to qualitatively screen fungi that degrade hydrocarbons (LEE et al., 2014). The phenolic oxidation test analyses the excretion of phenol oxidases, which results in the formation of a brown halo around the mycelium (LEE et al., 2014; 2020). The ability of the fungus to decolorize RBBR is due to its metabolism in excreting enzymes (principally laccases) involved in the degradation of aromatic contaminants, which promotes decolorization from blue to yellowish white (LEE et al., 2020). Regarding the bioprospecting of biosurfactant-producing fungi, the screening can be carried out by colorimetric methods such as blood hemolysis and methylene blue - cetyltrimethylammonium bromide (CTAB) that involves the excretion of this metabolite in solid culture media (SILVA et al., 2021c).

In liquid culture media, screening of strains for degradation/assimilation of hydrocarbons commonly occurs by simple tests such as gravimetric analysis of the degraded contaminant (BENGUENAB; CHIBANI, 2021), profile for enzyme and biosurfactant production, and biomass dry weight (MARCHAND et al., 2017; BENGUENAB; CHIBANI, 2021). The 2,6-dichlorophenol indophenol (DCPIP) is an indicator (redox dye) that analyses excretion of oxide-reductases by fungal strains in the presence of hydrocarbons (SOUZA et al., 2016; BOVIO et al., 2017). DCPIP in its oxidized form shows a blue color, which becomes colorless when this indicator is reduced by oxido-reductases during hydrocarbon biodegradation (SOUZA et al., 2016; MARCHAND et al., 2017; BENGUENAB; CHIBANI, 2021). Tensiometric analysis and/or emulsification index of liquid culture supernatants of fungal strains provides more accurate results than colorimetric analysis for screening fungi producing biosurfactants, as these technologies allow the measurement of surface tension and/or ability to emulsify hydrocarbons (KAMYABI; NOURI; MOGHIMI, 2017; AL-HAWASH et al., 2018a; SILVA et al., 2021c).

The measurement of the fungal dry biomass weight after its growth in liquid medium containing hydrocarbons allows estimating which strains can better assimilate or degrade contaminants, as well as which chemical structure profiles the strain has more capacity to degrade (BENGUENAB; CHIBANI, 2021; BECARELLI et al., 2021). It is roughly believed that fungal dry biomass weight reveals the ability of the fungus to convert hydrocarbon into energy to produce biomass, even if not all carbon source was targeted for such purpose (AMEEN et al., 2016; BOVIO et al., 2017). This dry biomass method is analogous to the gravimetric method for screening fungal bioinoculants that assimilate hydrocarbons in liquid media. However, the difference between these technologies is related to the biomass or contaminant that will be measured in the final screening step (SHETAIA et al., 2016; BARNES et al., 2018). Additionally, fungal biomass can have the hydrophobicity of its cell surface measured to correlate with its potential to produce biosurfactants and absorb hydrocarbons (AL-HAWASH et al., 2019; ATAKPA et al., 2022).

Other quantitative methods, such as gas chromatography (GC) and respirometry, are less used to screen fungal isolates as possible bioinoculants for hydrocarbon remediation (CHAILLAN et al., 2004; MARCHAND et al., 2017). Although GC provides accurate information on the conformational changes in hydrocarbon structure after mycoremediation, this technology is still expensive for screening processes (CHAILLAN et al., 2004). The respirometry technique can screen fungi by quantifying CO_2 produced by the strain during mycoremediation, its main product generated when hydrocarbons are mineralized by aerobic

degradation (MARCHAND et al., 2017; GHORBANNEZHAD; MOGHIMI; DASTGHEIB, 2021).

The screening of possible fungal bioinoculants for the remediation of toxic metals also occurs from the growth of strains in liquid or solid culture media containing toxic metals to initially measure the tolerance of strains to metals through analysis of minimum inhibitory concentration (MIC) (MISHRA; MALIK, 2014; KHAN et al., 2019). However, only more robust and sophisticated techniques such as atomic absorption spectrometry (AAS) can quantify metal removal, as well as elucidate the type of strain-metal interaction (XIAO et al., 2010; CHAUDHARY et al., 2022).

6 MOLECULAR APPROACHES: THE KEY TO UNRAVELLING ANSWERS AND EXTRAPOLATING RESULTS

It is conceivable that fungal strains have evolved in the direction of ecological fitness rather than biotechnological efficacy, i.e., bioengineering of strains is a prerequisite for increasing the metabolic efficiency in mycoremediation (GHOSAL et al., 2016). DNA decoding of fungal strains and other molecular approaches will certainly be needed in every mycoremediation process that involves bioprospecting or attempts to propose degradation steps, elucidate regulatory mechanisms, monitor microbial profile, enhance strains for productivity increases, etc. There was a gap of 20 years between the use of fungal bioinoculants to the proper use of their metabolites for mycoremediation, as more attention was paid to this purpose after the sudden accelerated expansion of molecular techniques. Currently, -omics technologies are technological resources for expanding molecular approaches in the development of bioinoculants and metabolites for remediation of hydrocarbons and toxic metals, as well as for understanding microbial interaction on contaminated sites or during remediation.

6.1 MOLECULAR CHARACTERIZATION AND MICROBIAL MONITORING TECHNIQUES

Fungal isolates with potential to be used in mycoremediation can also be potentially opportunistic pathogens or be closely related at molecular level to plant, animal and/or human pathogens; e.g., *Fusarium oxysporum* and *Paecilomyces variotii*. This fact promotes biological risk due to virulence factors and spore respiration in bioaerosols (SHETAIA et al., 2016;

PRENAFETA-BOLDÚ; HOOG; SUMMERBELL, 2019). Any fungal bioprocess must meet biosafety criteria. Thus, morphological and molecular identification of the isolate is essential to previously verify its pathogenic potential before proceeding with its use in mycoremediation (PRENAFETA-BOLDÚ; HOOG; SUMMERBELL, 2019; LIU et al., 2020). However, a single fungus isolated from a contaminated environment can show large morphological differences, since these variations can be due to mutations caused by the high concentration of contaminants and broad resistance/tolerance mechanisms that apply to each isolate (REYES-CÉSAR et al., 2014; FAZLI et al., 2015). Therefore, the fungal strain should preferably be identified from its gene sequence through the Internal Transcript Spacer (ITS) region and, if necessary, other genes could be used, such as beta tubulin, actin, elongation factor (BARNES et al., 2018; FAYEULLE et al., 2019). The results are compared with similar type strains of different species within the genus through multiple sequence alignment, ensuring correct taxonomic identification (GHOSAL et al., 2016; PEIDRO-GUZMÁN et al., 2021).

Several PCR-based fingerprinting genotyping techniques are available to profile fungal isolates, as well as to monitor diversity and abundance of microbial communities and bioinoculant survival during mycoremediation (LIU et al., 2011; ZAFRA et al., 2017). In synthesis, these technologies are related to denaturing gradient gel electrophoresis (DGGE) (ZAFRA et al., 2017; MEDAURA et al., 2021), fluorescence in situ hybridization (FISH) (HESHAM et al., 2012) and terminal restriction fragment length polymorphism (T-RFLP) analysis (LIU et al., 2011). Quantitative PCR (qPCR) has been used to quantify bacterial (16S) and fungal (ITS) genes as indicative of microbial abundance, monitor catabolic activity and gene expression during mycoremediation (FAYEULLE et al., 2019; LEE et al., 2020; LI et al., 2021a). Moreover, DNA microarray technique has identified genes regulated in response to exposure to high concentrations of toxic metals (GORFER et al., 2009).

6.2 GENETIC ENGINEERING AND HETEROLOGOUS PRODUCTION

Advances in technologies related to fungal genome sequencing have been a shortcut to achieve improvements in bioprospecting for potential bioinoculants, enzymes and biosurfactants through data mining as well as to infer functions of genes involved in hydrocarbon degradation (HESHAM et al., 2012; TRIPPE et al., 2014; SILVA et al., 2021c). For example, the screening of bioinoculants that degrade aromatic hydrocarbons can be carried out from the search of sequences encoding the *C23O* (catechol 2,3-dioxygenase) gene (HESHAM et al., 2012). Furthermore, silencing of the *CYP52L1* gene (CYPs family) by post-

transcriptional gene silencing (RNAi) in *Graphium* sp. showed that the reduced monooxygenase activity, as well as the ability of this fungus to grow on alkanes and ethers was extinguished (TRIPPE et al., 2014).

The elucidation of catabolic genes related to hydrocarbon or heavy metal remediation allows designing fungal strains of higher performance due to the possibility of recombination/expression of these genes in another/single host (SAIKA et al., 2017; ZAFRA et al., 2017; OLIVEIRA et al., 2020). Although post-translational modifications during the processing of phenol oxidases are more effectively carried out by native lignolytic fungi than in host microorganisms, several host fungi have shown promising results in heterologous production of these enzymes for mycoremediation (ALCALDE, 2015; MÄKELÄ et al., 2020). Genes encoding for laccases from the basidiomycete *Trametes* sp. were expressed in ascomycete hosts such as *A. niger* (TÉLLEZ-JURADO et al., 2006), *Trichoderma atroviride* (BALCÁZAR-LÓPEZ et al., 2016), *Y. lipolytica*, *S. cerevisiae* and *P. pastoris* (MÄKELÄ et al., 2020). The bioinoculant *A. niger* genetically engineered for expression of genes encoding lignin peroxidase and manganese peroxidase from *P. chrysosporium* enhanced the degradation of phenanthrene, pyrene and benzo[a]pyrene in soil microcosms (ZAFRA et al., 2017). The yield of recombinant thermophilic tyrosinase from *Thermothelomyces thermophila* was increased twofold when this enzyme was cloned and expressed in *P. pastoris* rather than in other microbial cells (NIKOLAIVITS et al., 2018).

Insertion and/or deletion of some genes of specific interest in host strains enhance mycormediation processes. For example, increasing the active site of the non-lignolytic enzyme CYP63A2, among other CYP family enzymes, was achieved by expressing CYPs together with cytochrome P450 reductases from *P. chrysosporium* into *P. pastoris*, which promoted the oxidation of different contaminants such as PAHs, alkylphenols and long-chain alkanes (SYED et al., 2013). Similarly, the insertion of the lipase gene *PaLIPAp* from *Pseudozyma antarctica* T-34 into *Pseudozyma tsukubaensi* increased the uptake of oily contaminants, besides promoting a 1.7-fold yield of MEL production (SAIKA et al., 2017), a biosurfactant that has already been regarded as promising for mycoremediation (SILVA et al., 2021c). Furthermore, the insertion of the metallothionein gene *PtMT2b* from *Populus trichocarpa* into *S. cerevisiae* increased approximately tenfold the tolerance and bioaccumulation of Cd when compared to its wild counterparts (OLIVEIRA et al., 2020). Additionally, the deletion of the *crpA* gene (P-type ATPase) in *Aspegillus nidulans* increased Cd biosorption capacity by 2.7-fold (BOCZONÁDI et al., 2020).

6.3 THE "-OMICS" TECHNOLOGY

Several complete or near complete genomic sequences of culturable microorganisms with potential in mycoremediation are available in online databases, as well as sequences of catabolic genes also involved in such processes (GHOSAL et al., 2016; PANDEY et al., 2021). However, it is estimated that a 99% gene pool of microbial diversity remains unknown due to limitations of culture-dependent techniques (OLIVEIRA et al., 2015; WILLIAMS; TRINDADE, 2017).

In this context, genomic approaches of fungal isolates allow access to the possible metabolic pathways related to enzyme and biosurfactant production (SILVA et al., 2021c; GANESH KUMAR et al., 2021). For instance, the whole genomic analysis of *A. sydowii* allowed deducing possible enzymatic pathways of hydrocarbon degradation when combined with GC-MS, and revealed a variety of genes involved in the biodegradation of aromatics, such as monooxygenases, dioxygenases, glutathione transport system, peroxidases and semialdehyde dehydrogenase (GANESH KUMAR et al., 2021).

On the other hand, metagenomic approaches allow elucidating the microbial interaction at the contaminated site or during mycoremediation without the need for microbial cultivation (GHOSAL et al., 2016; WILLIAMS; TRINDADE, 2017). Metagenomic analysis of total DNA from contaminated environmental samples allows the screening of metabolite-producing fungi applied to remediation through genomic data mining or functional screening of specific genes (DHANJAL; SHARMA, 2018; SUN et al., 2020). However, metagenomic studies on fungi remain sparsely explored, and the results of annotated sequences for eukaryotes is much lower than for bacteria (SUN et al., 2020). For instance, functional metagenomic analysis of petroleum contaminated soils when bioaugmented with *Ciboria* sp. revealed synergy between fungal bioinoculant and different taxa of hydrocarbonoclastic bacteria (*Streptomyces*, *Nocardoides*, *Pseudonocardia*, *Solirubrobacter*, *Parvibaculum*, *Rhodanobacter*, *Luteiomonas*, *Planomicrobium* and *Bacillus* spp.) during contaminant degradation (BECARELLI et al., 2021). A metagenomics study also revealed that the fungal community possibly involved in PAHs remediation, differed by approximately 30% between soils collected at 0.5-m (m) distance from oil wells compared to those collected at 3 m. Furthermore, the genera *Tetracladium*, *Exophiala*, *Schizothecium* and *Ilyonectria* identified in samples collected at 0.5 m, showed strong correlation with PAH content, which classifies them as potential bioinoculants for mycoremediation (GAŁĄZKA et al., 2020).

Transcriptomic (mRNA profile), proteomic (proteins) and metabolomic (metabolites) analyses elucidate changes in the composition, regulation (up-regulated or down-regulated) and abundance of differentially expressed genes, proteins and metabolites under a given environmental/specific condition (LI et al., 2021c; KOLHE et al., 2021; PEIDRO-GUZMÁN et al., 2021). These omics technologies provide important information to maximize performance in using fungal bioproducts to remediate hydrocarbons and toxic metals (LI et al., 2021c; KOLHE et al., 2021; PEIDRO-GUZMÁN et al., 2021).

The transcriptional response of *Y. lipolytica* after exposure to uranium (50 μM) revealed the expression of 33 upregulated genes, which are involved in metal transport, DNA repair and oxidative stress response, as well as another 23 downregulated genes, which are involved in cell wall and cell cycle (KOLHE et al., 2021). Through transcriptomic analysis, the differential expression of the chloroperoxidase enzyme-related *cpo* gene, not previously described for PAH degradation, was identified during benzo[a]pyrene degradation under hypersaline conditions by *A. sydowii* (PEIDRO-GUZMÁN et al., 2021). Furthermore, enzymatic mechanisms involving mitochondria membrane-bound enzymes (CYPs, dehydrogenases, quinone reductases) and cytosol-soluble enzymes (dioxygenase, glutathione transferase) for PAHs degradation by *A. sydowii* were proposed through a combination of transcriptomics and metabolomics (PEIDRO-GUZMÁN et al., 2021).

Recently, proteomic analysis allowed the elucidation of n-hexadecane transmembrane transport mechanisms in *C. tropicalis*, since the 231 proteins differentially expressed by this strain are clearly enriched in endocytosis and phagosome pathways, as well as probable involvement in the energetic metabolism of the strain (LI et al., 2021c). Metabolomics analysis detected intermediate metabolites such as hydroxy naphthoic acid and catechol synthesized from oxidation of phenanthrene and benzo[a]pyrene by *A. sydowii*, as well as allowed the prediction that degradation of these contaminants occurred in approximately 10 days, since no aromatic metabolite was detected in samples taken after this period (PEIDRO-GUZMÁN et al., 2021).

7 BIOPROCESS ENGINEERING TO BOOST MYCOREMEDIATION: AN OVERVIEW OF BIOPROCESSING

The use of technologies integrated with engineering principles and appropriate maintenance of requirements related to bioprocessing involved in production or application of bioproducts will probably promote more and more possibilities for consolidation of

mycoremediation. Bioprocess engineering and its technologies investigate and/or enhance the performance of steps related to the development of bioinoculants and fungal metabolites in order to ensure efficiency in production, application and ecotoxicological safety (MEDAURA et al., 2021; TOLEDO et al., 2021).

Fungal bioprocesses for the production or application of bioinoculants and metabolites for remediation of hydrocarbons and toxic metals present an attribute of alterity. Thus, although there are intrinsic particularities related to the production process, as type of cultivation (submerged, semi-solid or solid), or issues related to the application, as type of site (soil, sludge, or water), these bioprocesses also present similarities related to subjects like the influence of nutrition and environmental parameters, complexity of each step along the process, physiological state, and metabolism of the inoculum (SILVA et al., 2021c; FASIM; MORE; MORE, 2021). Alternatively, experimental designs combined with statistical tools such as response surface methodology has allowed to select/evaluate optimal operating conditions (e.g., inoculum size, pH, etc.) and interaction between nutritional and environmental parameters to enhance the performance of fungal bioinoculants and/or metabolite production for mycoremediation (HAN et al., 2020; CHAUDHARY et al., 2022). However, although technologies related to batch, fed-batch and semi-continuous cultivation systems promote yield or efficiency enhancements in bioinoculant or metabolite production, the use of these technologies for application in mycoremediation is still under-explored probably due to practical limitations (VIPOTNIK; MICHELIN; TAVARES, 2022a).

In this topic, technologies developed for production and application of bioinoculants, enzymes and biosurfactants for remediation of hydrocarbons, and for toxic metals will be approached. Those related to bioprocessing for production of enzymes and biosurfactants will be presented only when these metabolites were produced exclusively for remediation purposes. If the reader is interested in the large-scale bioprocessing of fungal surfactants and enzymes, these have been described in detail by Silva et al. (2021c) and Fasim, More and More (2021), respectively.

7.1 INOCULUM AS BIOINOCULANT PRODUCT

The inoculum is the active portion of the microorganism used in remediation processes. When the inoculum is the bioproduct to be used, this is termed bioinoculant. Thus, the inoculum is the main promoting agent or product for mycoremediation, therefore its efficiency is essential to achieve better performances (ROBICHAUD et al., 2019; BECARELLI

et al., 2021). On a laboratory scale, mycoremediation requires the use of a standardized inoculum, especially when the aim is to evaluate efficiency after remediation. The initial steps involved in fungal inoculum preparation include strain growth in solid medium and its sequential growth in liquid medium (ARISTE et al., 2020; FERREIRA et al., 2020). After recovery of fungal biomass (inoculum), washing steps with water (COVINO et al., 2015), saline solution (BECARELLI et al., 2021) and buffer solution (FAN et al., 2014) can be carried out to standardize the inoculum.

As a rule of thumb, filamentous bioinoculants are generally used at a cell concentration ranging from 104 to 108 spores • [mL or g]$^{-1}$ (POTIN; RAFIN; VEIGNIE, 2004; ARISTE et al., 2020). Yeast bioinoculants are quantified indirectly by optical density of the culture medium, and an absorbance between 0.6 and 1.0 (at 600 nm) is indicated for better process performance (GHORBANNEZHAD; MOGHIMI; DASTGHEIB, 2018; KRELING et al., 2020a). Modulation of inoculum size for bioinoculant application or metabolite production ranges from 5 to 10% of the total process volume (BETTIN et al., 2019; FERREIRA et al., 2020). A seven- and fivefold increase in the inoculum size of *Ciboria* sp. and *Mucor hiemalis* increased oil degradation in soils by 27% (BECARELLI et al., 2021) and biosurfactant production by 560% (FERREIRA et al., 2020), respectively. In general, a practical application of fungal bioaugmentation will certainly require a greater inoculum amount than under controlled conditions, which would aim to minimize effects of microbial competition and ensure their development under adverse conditions (KADRI et al., 2017; PINO-HERRERA et al., 2017).

In this context, alternative technologies for preparation/use of bioinoculants have been developed in uncontrolled conditions that resemble the real environment of an application, and/or in conditions that can induce the fungal metabolism to increase its performance before the beginning of mycoremediation (SÁNCHEZ-VÁZQUEZ et al., 2018; ROBICHAUD et al., 2019). For example, the bioinoculant *Pleurotus ostreatus*, when previously submitted to an acclimation treatment in lignocellulosic biomass, promoted greater efficiency in diesel degradation after its inoculation in soil (ROBICHAUD et al., 2019). Regarding metabolites production, the application of an electric field to the developing inoculum (spores) of *A. brasiliensis* increased the bioemulsifier production (19.5%) applied to PAH and aliphatic remediation (SÁNCHEZ-VÁZQUEZ et al., 2018).

Morphological structures of fungal bioinoculants directly influence mycoremediation. Various fungal structures such as spores, mycelia, pellets and/or fruiting bodies of mushrooms were analyzed for remediation of PAHs and metals in soils (VALENTÍN et al., 2007;

DAMODARAN; VIDYA SHETTY; RAJ MOHAN, 2013). Mycelial inoculation promoted higher degradation rates of PAH compared to the use of spores of *Coniothyrium* sp. (26.5%) and *Fusarium* sp. (27.5%) (POTIN; RAFIN; VEIGNIE, 2004). The use of mycelium from *Bjerkandera adusta* increased PAH degradation by up to 29% when compared to the use of pellets, probably due to higher oxygen transfer (VALENTÍN et al., 2007). Similarly, the inoculum of *Aspergillus ochraceus* with concentration at 1% ($m \bullet v^{-1}$) in its fresh mycelium state ($v \bullet v^{-1}$) showed higher performance (13%) compared to its spores at 5% ($v \bullet v^{-1}$) for oil degradation in liquid culture (OZYUREK; AVCIOGLU; BILKAY, 2021). Fungal bioinoculants of *Galerina vittiformis* showed approximately fourfold higher bioaccumulation capacity of Cd^{+2}, Cu^{+2}, Cr^{+6}, Pb^{+2} and Zn^{+2} in its fruiting body state than in its mycelium, i.e., fungi that form fruiting bodies may also have an advantage as they are easily separated from soil (DAMODARAN; VIDYA SHETTY; RAJ MOHAN, 2013).

There are implications related to fungal morphology in bioprocesses for environmental remediation, which directly influences the process and can presume its best morphological structure for specific application conditions (DAMODARAN; VIDYA SHETTY; RAJ MOHAN, 2013; OZYUREK; AVCIOGLU; BILKAY, 2021). In this sense, dimorphic fungi (e.g., black fungi) in their filamentous structure may be more viable to be applied as bioinoculants, because hyphae can penetrate into the contaminated soil matrix pores, which allows greater accessibility and permeation to contaminants between different regions of the mycelium and through the air (POTIN; RAFIN; VEIGNIE, 2004; HARMS; SCHLOSSER; WICK, 2011). On the other hand, dimorphic fungi in their yeast-like form may be more viable to be applied in bioprocesses as producers of metabolites to be applied in mycoremediation, because the yeast morphology facilitates biomass recovery (RUSINOVA-VIDEVA et al., 2020).

Although bioinoculants of different phyla and order have shown high efficiency for mycoremediation of hydrocarbons and toxic metals due to their biochemical mechanisms or metabolites produced, the main process conditions (e.g., contaminant type, bioprocess type) and parameters (e.g., time, pH, temperature, agitation) under which each experiment was carried out should be individually considered in detail to conclude on this efficiency more accurately. In this context, Table 1 presents data on a diversity of possible fungal bioinoculants, process condition and parameters involved in hydrocarbon remediation. Similarly, Table 2 presents data on toxic metal remediation.

Table 1 – An overview of hydrocarbon remediation processes by bioinoculants and metabolites

Phylum/Order	Strain	Contaminant	Metabolite	Removal efficiency (%)	pH/ (%) mois-ture	Tempe-rature (°C)	Mixing (rpm)	Time (days)	Data source	Reference
Mucoromycotina										
Mucorales	*Mucor racemosus*	ANT	ND	12-14	ND	32	130	14	LC	Birolli et al. (2018)
Ascomycota										
Hypocreales	*Fusarium solani*	BAP	-	16	ND/25	-	-	9	SM	Fayeulle et al. (2019)
	Fusarium neocosmos-poriellum	crude oil	LCC/BS	43	ND/60	-	-	150	SM	Azin; Moghimi; Heidarytabar (2018)
	Purpureocillium lilacinum	crude oil	-	44	7/100	30	-	40	LC	Benguenab; Chibani (2021)
	Acremonium sclerotigenum	n-alkanes	-	52	-	28	80	23	LC	Barnes et al. (2018)
	Trichoderma tomentosum	PHE, ANT, PYR	-	60-90		25	100	49	LC	Marchand et al. (2017)
Pleosporales	*Alternaria alternata*	diesel oil	LCC, MnP	28	-	26	-	28	LC	Ameen et al. (2016)
	Coniothyrium sp.	PHE, ANT, PYR	-	26	-	-	-	30	LC	Potin; Rafin; Veignie (2004)
	Ulocladium sp.	PHE, BAP, PYR	LCC, MnP	40-98	ND/20	-	-	120	SM	Medaura et al. (2021)
Eurotiales	*Penicillium citrinum*	n-alkanes	-	80	-	28	80	23	LC	Barnes et al. (2018)
	Aspergillus ustus	crude oil	-	30	7/100	30	-	40	LC	Benguenab; Chibani (2021)
	Aspergillus niger	biodiesel	lipase/BS	64	ND/60	-	-	60	SM	Kreling et al. (2020b)
	P. variotii	diesel oil	MnP, CAT	30	-	26	-	28	LC	Ameen et al. (2016)
	Talaromyces helicus	BAP	-	33	-	-	-	9	SM	Fayeulle et al. (2019)

Phylum/Order	Strain	Contaminant	Metabolite	Removal efficiency (%)	pH/ (%) mois-ture	Tempe-rature (°C)	Mixing (rpm)	Time (days)	Data source	Reference
Saccharomycetales	*Candida maltosa*	alicyclic hydrocarbons	-	30-38	-	30	300	5	LC	Dallinger et al. (2016)
	Lipomyces tetrasporus	crude oil	-	68		28	150	30	LC	Shetaia et al. (2016)
	C. tropicalis	saturated and aromatic hydrocarbons	DH, PPO, BS	42-96	02/60	-	-	180	SM	Fan et al. (2014)
	Yamadazyma mexicana	octane, PYR	-	24	-	30	150	360	LC	Hashem et al. (2018)
Capnodiales	*Cladosporium* sp.	PYR, PHE, ANT, FLU	ND	47-71	-	32	130	21	LC	Birolli et al. (2018)
	Cladosporium sphaeros-permum	diesel oil	LCC, LiP, CAT	40	-	26	-	28	LC	Ameen et al. (2016)
Microascales	*Pseudoallescheria* sp.	aliphatic hydrocarbons	LCC/hydrol ases	79	ND/50	28	-	60	SM	Covino et al. (2015)
Helotiales	*Cadophora* sp.	PHE, BAP	LCC, MnP	74-99	-	25	150	10	LC	Batista-García et al. (2017)
incertae sedis	*Trematophoma* sp.	PHE, ANT, PYR	LCC	56-90	-	28	180	15	LC	Moghimi et al. (2017)
	Pseudogymnoascus sp.	PHE, BAP	LCC, MnP	53-93	-	25	150	10	LC	Batista-García et al. (2017)
Basidiomycota										
Agaricales	*Pleurotus dryinus*	PHE, FLU, PYR	LCC	25-60	7	27	150	30	LC	Ariste et al. (2020)
	P. ostreatus	PHE, BAP	LCC, MnP	64-98	-	30	150	10	LC	Batista-García et al. (2017)
	Pleurotus florida	saturated and aromatic hydrocarbons	LCC, tyrosinase, BS	55	-	24	120	30	LC	Roshandel et al. (2021)
	Crucibulum laeve	PHE, PYR, BAP	DH, LCC, PPO	42-64	ND/60	20-28	-		SM	Ma et al. (2021)

Phylum/Order	Strain	Contaminant	Metabolite	Removal efficiency (%)	pH/ (%) mois-ture	Tempe-rature (°C)	Mixing (rpm)	Time (days)	Data source	Reference
Polyporales	*Trametes polyzona*	PHE	LCC	98	4,5/100	-	150	1	LC	Wulandari et al. (2021)
	Pleurotus sajor-caju	phenol	LCC	36-82	6,5/100	28	-	4	LC	Bettin et al. (2019)
	Megasporoporia sp.	BAP	LCC, MnP	54	-	30	150	14	LC	Souza et al. (2016)
	Microporus vernicipes	PHE, ANT, FLU, PYR	LCC, MnP, LiP	15-38	-	27	150	14	LC	Lee et al. (2020)
	Phlebia acerina	PHE, ANT, FLU, PYR	LCC, MnP, LiP	42-52	-	27	150	14	LC	Lee et al. (2020)
	Trametes hirsuta	phenol, PHE, ANT, FLU, PYR	LCC	30-85	7	27	150	30	LC	Ariste et al. (2020)
	Polyporus sp.	CHRY	1,2-CTD	65	-	25	120	30	LC	Hadibarata; Tachibana; Itoh (2009)
Sporidiobolales	*Rhodotorula ingeniosa*	octane, PYR	-	35	-	30	150	360	LC	Hashem et al. (2018)
Russulales	*Peniophora incarnata*	PHE, ANT, FLU, PYR	LCC, MnP, LiP	68-95	-	27	150	14	SM	Lee et al. (2020)

Caption - ND: not described, BAP: benzo[a]pyrene; PHE: phenanthrene; ANT: anthracene; PYR: pyrene; FLU: fluoranthene; CHRY: chrysene BS: biosurfactant; LCC: laccase; MnP: manganese peroxidase; CAT: catalase; LiP: lignin peroxidase; DH: dehydrogenase, PPO: polyphenoloxidase; 1,2-CTD: Catechol 1,2-dioxygenase; SM: soil microcosms; LC: liquid culture.

Table 2 – An overview of toxic metals remediation processes by bioinoculants and metabolites

Phylum/Order	Strain	Metal	Mechanisms	Removal efficiency (%)	pH/(%) moisture	Tempera-ture (°C)	Mixing (rpm)	Time (h)	Assay source	Reference
Glomeromycota										
Glomerales	*G. versiforme*	Cd	Phytoextraction	25-74	ND/60	22-28	ND	1680	SM	Liu et al. (2015)
	R. irregularis	Cd	Phytoextraction	ND	ND	22-28	ND	1680	SM	Hassan et al. (2013)
Mucoromycotina										
Mucorales	*Circinella* sp.	Ni	Biosorption	6	6/ND	40	ND	1	LC	Alpat et al. (2010)
	Cunninghamella sp.	Pb	Biosorption	95	ND	28	180	168	LC	El-Morsy (2004)
	Mucor circinelloides	Pb, Cd, As	Biosorption/Bio-accumulation	2-87	6/ND	25	170	35	LC	Li et al. (2021b)
	Rhizopus stolonifer	Pb, Cd, Ni	Bioaccumulation	17-59	ND	30	150	96	LC	Njoku; Akinyede; Obidi (2020)
Ascomycota										
Coniochaetales	*Lecythophora* sp.	Hg	Biovolatilization/ Bioaccumulation	86	ND	30	150	96	LC	Chang et al. (2019)
	Lecythophora sp.	Hg	Bioaccumulation	13-26	ND/50			1344	SM	Chang et al. (2019)
Eurotiales	*Aspergillus niger, A. fumigatus, A. flavus*	Hg, Pb	Bioaccumulation	96	ND	30	120	120	LC	Khan et al. (2019)
	Aspergillus lentulus, A. terreus	Cu, Cr	Biosorption	65-95	ND	30	150	120	LC	Mishra; Malik (2014)
	Paecilomyces sp.	Cd	Bioaccumulation	35	6/ND	28	120	240	LC	Fazli et al. (2015)
Hypocreales	*Talaromyces islandicus*	Pb	ND	80	ND	30	ND	120	LC	Sharma et al. (2020)
	Trichoderma lixii	Cu	Biosorption	25-85	7/ND	28	80	120	LC	Kumar; Dwivedi (2021)
	Beauveria bassiana	Pb	Biomineralization	8	7/ND	30	ND	504	LC	Purchase et al. (2009)

Phylum/Order	Strain	Metal	Mechanisms	Removal efficiency (%)	pH/(%) moisture	Tempera-ture (°C)	Mixing (rpm)	Time (h)	Assay source	Reference
Microascales	*Scopulariopsis brevi-caulis*	Hg, Se	Bioaccumulation /Biovolatilization	46-50	5,6/ND	25	ND	720	LC	Boriová et al. (2014)
Saccharomycetales	*Saccharomyces cerevisi-ae*	U (VI)	Biosorption	75	3/ND	30	150	24	LC	Chen et al. (2020)
Pleosporales	*Microsphaeropsis* sp.	Cd	Biosorption	90	6.5/ND	30	ND	72	LC	Xiao et al. (2010)
Basidiomycota										
Polyporales	*Phanerochaete chrysos-porium*	Cd	Biosorption	47	ND	30	120	432	LC	Xu et al. (2015)
	Trametes hirsuta	As, Cu, Fe, Cd, Zn, Pb	Biosorption	23-98	7/ND	27	150	720	LC	Ariste et al. (2020)
	P. ostreatus	Cd	Biosorption	54	6/ND	30	100	168	LC	Xu et al. (2021)
Agaricales	*Schizophyllum com-mune, Leucoagaricus naucinus*	U (VI)	Biosorption/Bio-accumulation	10-60	ND	ND	120	168	LC	Wollenberg et al. (2021)
	G. vittiformis	Cd, Cu, Cr, Pb, Zn	Bioaccumulation	10-90	ND/85	22	ND	600	SM	Damodaran; Vidya Shetty; Raj Mohan, (2013)
Tremellales	*Cryptococcus laurentii*	Al, Cr, In, Ga, Fe, Bi	Biosorption	60-80	4-7/ND	ND	500	1,5	LC	Rusinova-Videva et al. (2020)
	Trichosporon sp.	Pb, Zn, Ag	Biosorption	ND	ND	28	ND	48	LC	Muñoz et al. (2012)
Sporidiobolales	*Rhodotorula mucilagi-nosa*	Zn, Pb	Biomineralization	2-16	7/ND	30	ND	504	LC	Purchase et al. (2009)

Caption: - ND: not described, SM: soil microcosms; LC: liquid culture.

According to Tables 1 and 2; bioinoculants belonging to the phyla Ascomycota and Basidiomycota are the most explored for mycoremediation of hydrocarbons and toxic metals, while the phyla Glomeromycota and Mucoromycotina have been more applied to remediate metals. Trials with bioinoculants for mycoremediation of metals are generally carried out in shorter time when compared to hydrocarbon degradation. This is probably due to the mechanisms of interaction with toxic metals (e.g., biosorption and bioaccumulation) are faster than mechanisms involving the action of enzymes and biosurfactants for hydrocarbon remediation (MUÑOZ et al., 2012; KRELING et al., 2020b; VIPOTNIK; MICHELIN; TAVARES, 2022a).

The measurement of the efficiency rate in mycoremediation is the main criteria to analyze the performance of bioinoculants, although this rate is influenced by several operational parameters; which are difficult to maintain under conditions appropriate to the bioinoculant during a real application. In summary, high efficiency rates in mycoremediation are achieved through the use of bioinoculants under controlled conditions, mainly in liquid culture simulating remediation of aquatic environments. However, experiments in soil microcosms better mimics contaminated environmental conditions, since they simulate contaminant-fungus-soil interactions, which promotes results closer to real conditions (FAYEULLE et al., 2019; MEDAURA et al., 2021). Furthermore, technologies related to the use of bioinoculants in bioreactors to carry out mycoremediation or to produce metabolites for this purpose are constantly developing (SÁNCHEZ-VÁZQUEZ et al., 2018; CHAUDHARY et al., 2022). The next sections discuss in detail this information that concerns the bioprocessing and formulation of bioinoculants and strategies to improve their use in mycoremediation of hydrocarbons or toxic metals.

7.2 BIOREACTORS: CONTROLLED SYSTEM CONFIGURATIONS

Bioreactors are engineered equipment projected with control systems to ensure the maintenance of appropriate operating conditions for the process. Engineering principles such as mass transfer, mixing, aeration, etc., promote the versatility of different bioreactor configurations that can provide specific conditions related to the type of process in order to promote greater efficiency (MORALES-BARRERA; CRISTIANI-URBINA, 2006; PANDEY et al., 2021). Fungal bioprocesses related to hydrocarbon and toxic metal remediation, when conducted in bioreactors, are to produce metabolites or analyze influence and behavior of these metabolites under different physicochemical and/or biological conditions during remediation

(SÁNCHEZ-VÁZQUEZ et al., 2018; VERGARA-FERNÁNDEZ et al., 2018a). Thus, mycoremediation processes for hydrocarbons or toxic metals have been carried out in submerged (water), semi-solid (soil sludge) or solid (air biofilters) state bioreactors (MORALES-BARRERA; CRISTIANI-URBINA, 2006; VALENTÍN et al., 2007; ZHANG et al., 2019). The use of submerged culture bioreactors allows scale-up and process control, as well as being manageable, predictable, and easier to handle in a confined environment than in situ or solid phase developed systems (FERNÁNDEZ et al., 2018; PANDEY et al., 2021).

Stirred tank reactors (STR) have mechanical stirrers that promote high shear stresses, which can cause fragmentation of the mycelium and/or pellet formation (FERNÁNDEZ et al., 2018). Higher phenol degradation was achieved in STR via laccases and other phenol-oxidases produced by *P. sajor-caju* compared to the same enzyme when produced in shaking flasks (BETTIN et al., 2019). Differentially, air lift reactors are pneumatically agitated by air injection that impose low shear stress, which maintains the integrity of dispersing hyphae and mycelia and increases the surface contact area, requiring less energy compared to STR (FERNÁNDEZ et al., 2018; PANDEY et al., 2021). Air lift reactors have been used to scale up bioemulsifier production from *A. brasiliensis* applied to PAHs remediation (SÁNCHEZ-VÁZQUEZ et al., 2018). A maximum removal at 92% of phenol (4.0 mmol•L^{-1}) in liquid medium was achieved from laccases excreted by *P. sajor-caju* in STR, while in air lift reactor the maximum removal was 82% (BETTIN et al., 2019). Moreover, STR and air lift were used for the development of a *Trichoderma viride* bioinoculant involved in Cr^{+6} biotransformation. A higher biotransformation capacity was achieved in air-lift reactor, since mechanical agitation in STR caused negative effects on the mycelium (MORALES-BARRERA; CRISTIANI-URBINA, 2006).

Slurry phase stirred tank reactor represent a highly engineered treatment system for soil remediation, since this system allows increased surface phenomena such as gas/liquid and solid/liquid mass transfer due to agitation (EIBES et al., 2015; PINO-HERRERA et al., 2017). Biotreatment in sludge-phase reactor can occur with the input of contaminated soil, water, air, fungal bioinoculant, nutrients and biosurfactants, while the output streams will be decontaminated soil, sewage, biomass, CO_2 and by-products (PINO-HERRERA et al., 2017). The use of the bioinoculant *B. adusta* in sludge phase reactor promoted the degradation of several PAHs, probably due to the higher availability of oxygen and nutrients. This technology also maintains sludge homogeneity, which avoids solids separation during mycoremediation (VALENTÍN et al., 2007).

Bioreactor configurations such as packed bed, trickle bed and bioscrubbers are the main forms used as fungal biofilters for remediation of air containing volatile hydrocarbons (VERGARA-FERNÁNDEZ et al., 2018b; SRIVASTAVA; SINGH; SINGH, 2021). Packed bed bioreactors have an absorption column configuration and can be multiphase; they also have inlet for contaminated air stream in upward or downward flow, which permeates the pores of the packing material where the fungus is immobilized, promoting the exit of a decontaminated air stream due to microbial degradation (PRENAFETA-BOLDÚ; HOOG; SUMMERBELL, 2019; BRUNEEL et al., 2020). Trickle bed bioreactors have a configuration almost like the packed bed type; however, they are smaller and have spray nozzles on their top for intermittent addition and recirculation of nutrient solution and water. Furthermore, these reactors operate in countercurrent flow and can generate liquid waste (VERGARA-FERNÁNDEZ et al., 2018b; ZHANG et al., 2019). The bioscrubber-type differ from the other biofilters previously mentioned only because their operation depends on a prior mass transfer step from the contaminant from the gas phase to a liquid phase that must be subsequently decontaminated (SRIVASTAVA; SINGH; SINGH, 2021). In general, the efficiency of a biofilter-type bioreactor depends on the air inlet flow rate, nutrient starvation state and physicochemical properties of the packing material (BRUNEEL et al., 2020; LIU et al., 2020).

7.3 NUTRITIONAL PARAMETERS

Fungal metabolism have its metabolic pathways altered according to the availability (presence or limitation) of substrates, so nutritional parameters such as carbon sources, nitrogen, trace elements, etc. must be supplied appropriately to ensure bioinoculant growth, which in turn influences the rate of hydrocarbon degradation (DALLINGER et al., 2016; DELSARTE et al., 2018; GANESH KUMAR et al., 2021). For instance, supplementation with carbon sources is required during degradation of alicyclics by fungal bioinoculants, as these hydrocarbons do not support microbial growth due to their high recalcitrance (DALLINGER et al., 2016). The degradation of benzo[a]pyrene by the bioinoculant *Fusarium solani* increased approximately ninefold when the carbohydrate carbon source (glucose) was replaced by a lipid substrate (olive oil) in liquid medium (DELSARTE et al., 2018). In addition, supplementation with nitrogen sources such as urea and ammonium dihydrogen phosphate increased engine oil degradation by 72% and 54% by the bioinoculant *A. sydowii*, respectively (GANESH KUMAR et al., 2021). However, high concentrations of NPK nutrients can reduce microbial hydrocarbon degradation activities (GANESH KUMAR et al., 2021; PANDEY et al., 2021). Supplementation of fungal

bioinoculants with substrates such as citric acid, ferulic acid, glycerol, veratrole alcohol and copper sulphate can induce increased production of peroxidases and oxidoreductases in liquid cultures or soil microcosms (AKHTAR; MANNAN, 2020). Additionally, the bioinoculant *P. chrysosporium* modified its enzymatic mechanism involved in hydrocarbon degradation according to nutrient availability: the strain preferentially expressed peroxidases in nutrient-limited media and CYPs in nutrient-sufficient media (SYED et al., 2010).

The addition of lignocellulosic biomass to contaminated soil can enhance the performance of fungal bioinoculants as the biomass supports fungal growth, induces the excretion of oxidative enzymes and acts as a texturizing agent to enhance porosity and oxygen transfer (REYES-CÉSAR et al., 2014; COVINO et al., 2015; ZAFRA et al., 2017). When wood chips were added to the treatment of water contaminated with PAHs and phenols, laccases produced by *P. dryinus* showed a higher catalytic activity compared to those produced with glucose. However, the phenol concentrations were increased due to the depolymerization of lignin (ARISTE et al., 2020). Furthermore, cometabolic substrates such as lignin and vanilla, when added to benzo[a]anthracene contaminated soils, promoted the stimulation of autochthonous fungi, which may cause hydrocarbon degradation (SUN et al., 2020; GU et al., 2022). Additionally, fungal bioinoculants that produce biosurfactants can have their productivity increased when there is a supplementation with a carbohydrate source, because the combination with the available hydrocarbon induces the excretion of biosurfactants (SILVA et al., 2021c).

7.4 OPERATIONAL CONDITIONS AND ABIOTIC PARAMETERS

In general, abiotic parameters such as temperature, pressure, Eh, pH, moisture, salinity, aeration and mixing directly influence the performance of fungal bioinoculants, as these parameters affect microbial physiology regarding enzymatic pathways, balance of catalytic reactions, contaminant transport (desorption, diffusion) and excretion of metabolites (AL-HAWASH et al., 2018b; DACCÒ et al., 2020).

When the bioinoculant *A. sydowii* was subjected to high pressure reactors (10 MPa) to check its ability to degrade spent motor oil under such conditions, this resulted in an increase of 11.3% compared to the degradation under mild pressure conditions (0.1 MPa) (GANESH KUMAR et al., 2021). This was explained by the higher aggregation and reduction in the size of mean diameter of hyphal filament under high pressure condition, which may have increased the surface area of active sites for hydrocarbon assimilation and degradation (GANESH

KUMAR et al., 2021). Moreover, high saline concentrations can negatively affect mycoremediation, as this condition tends to decrease solubility of enzymes and oxygen in aqueous solution, as well as decrease bioavailability of hydrocarbons (PEIDRO-GUZMÁN et al., 2021). Bioinoculants composed of halotolerants such as *L. tetrasporus* and *P. variotii* would be suitable for use in saline soils as they showed 38% and 31% efficiency for petroleum hydrocarbon degradation under high salinity (45 $g \bullet L^{-1}$) conditions, respectively (SHETAIA et al., 2016).

The pH of soils contaminated with PAHs ranges from pH 3 to 9 (MEDAURA et al., 2021; ZHANG et al., 2021). Higher efficiencies of PAH degradation were reported at neutral pH, although generally fungal bioinoculants and enzymes can interact with PAHs under slightly acidic conditions (Tables 1 and 2) (LI et al., 2010a; OZYUREK, AVCIOGLU; BILKAY, 2021). However, *Talaromyces* sp. isolated from contaminated soils showed good performance in the degradation of aliphatic and aromatic hydrocarbons under alkaline conditions (pH=9), which are extreme conditions for other microorganisms (ZHANG et al., 2021). Additionally, alkaline pH induce the formation of negatively charged groups in the fungal biomass, which favors the biosorption of cationic metals (DUSENGEMUNGU et al., 2020).

The Eh measures the oxidizing (Eh positive) or reducing (Eh negative) capacity of the microenvironment in which the biotransformation of contaminants occurs, and influences biochemical mechanisms involved in the action of bioinoculants and/or enzymes applied to mycoremediation (OLANIRAN; BALGOBIND; PILLAY, 2013). In addition, the moisture/water activity of the environment influences fungal growth, excretion of metabolites and biochemical reactions involved in mycoremediation, as these depend on a specific water level (KRELING et al., 2020b; KHATOON; RAI; JILLANI, 2021). Addittionally, low temperatures can increase the viscosity of hydrocarbons and inhibit fungal enzymes activities involved in their degradation, although oxygen solubility is favored at low temperatures for aerobic degradation (WILSON; JONES, 1993; ROBICHAUD et al., 2019). Higher temperatures increase the bioavailability and solubility of hydrocarbons (ZHANG et al., 2019), and increase the capacity of fungal bioinoculants for biosorption due to increased activation of adsorption surfaces and diffusivity of toxic metals (DUSENGEMUNGU et al., 2020). Trials on mycoremediation of hydrocarbons and toxic metals are commonly conducted at temperatures between 20 and 32 °C (Tables 1 and 2).

The agitation is a parameter that considerably influences the production/application of fungal bioinoculant and metabolites for environmental remediation (HADIBARATA; TACHIBANA; ITOH, 2009; ROCHA JUNIOR et al., 2019). Bioinoculants composed of

filamentous fungi generally require high oxygen demand and the minimization of mechanical disturbance for real application in soil remediation (HARMS; SCHLOSSER; WICK, 2011; ORTEGA-GONZÁLEZ et al., 2015). Under static conditions, air biofilters inoculated with filamentous fungi may exhibit hydraulic/gas retention or loss in interstitial fluid volume due to overgrowth of hyphae, which also promotes higher pressure drop (SRIVASTAVA; SINGH; SINGH, 2021). Regarding to enzymatic mycoremediation, *Polyporus* sp. produced ligninolytic enzymes and degraded chrysene about two times more efficiently when orbitally shaken than in a stationary culture (HADIBARATA; TACHIBANA; ITOH, 2009). This was probably due to fungus morphology that in the form of pellets increases the mass transfer between cells and medium, a condition not met in the stationary culture characterized by the formation of a mycelial layer on the substrate surface (HADIBARATA; TACHIBANA; ITOH, 2009). Nevertheless, in relation to biosurfactant application, those produced by *Y. lipolytica* removed 98% of engine oil when applied under agitation, as opposed to 30% under static conditions (RUFINO et al., 2013). Likewise, biosurfactants from *C. tropicalis* were able to remove Zn (80%), Cu (70%) and Pb (15%) when applied under orbital mixing conditions and at double CMC. However, under static and at normal CMC, its capacity was reduced considerably: Zn (32%), Cu (20%), and Pb (0.5%) (ROCHA JUNIOR et al., 2019).

7.5 DOWNSTREAM PROCESSING: EFFICIENCY, ECOTOXICOLOGICAL SAFETY, RECOVERY AND FORMULATION

The development of fungal bioprocesses in environmental remediation involving the production or application of bioinoculants may include downstream processing to analyze their efficiency and ecotoxicological safety, as well as to propose recovery and formulation of bioinoculants on a market scale.

Under laboratory conditions, the efficiency of bioinoculants in remediation of hydrocarbons and toxic metals are measured using high resolution chemical techniques such as GC-MS and AAS, respectively (KUMAR; DWIVEDI, 2021; MEDAURA et al., 2021). Lower resolution techniques such as gravimetry and respirometry also present less accurate quantitative data on hydrocarbon remediation efficiency (KRELING et al., 2020a; ATAKPA et al., 2022). However, the efficiency of bioinoculants is certainly impossible to measure in a real application due to the complexity and dynamics of soil-fungus-contaminant interactions along small interfaces of the contaminated environment, as well as different distributions of contaminant concentration gradients may imply inconsistent results (LI; LIU; GADD, 2020).

Exotic fungal bioinoculants may pose risks, such as decreasing microbial and plant biodiversity in a natural habitat, due to their behavior as an invasive species (THOMSEN; HART, 2018). Fungal bioproducts must not promote an additional ecotoxicological risk for contaminated environments after their application, i.e., they must not affect the metabolism of terrestrial or aquatic plants and animals (SANTOS et al., 2017a; ROSHANDEL et al., 2021). Some fungi can excrete intermediate metabolites such as oxygenated, alkylated and nitro-PAHs that are formed during enzymatic degradation of PAHs and are more toxic than their original counterpart (PRENAFETA-BOLDÚ; HOOG; SUMMERBELL, 2019; ZENG et al., 2021). Thus, technologies to verify the ecotoxicological safety via mutagenicity, phytotoxicity and genotoxicity tests on the use of bioinoculants or biosurfactants in mycoremediation should be based on fast growing organisms that are very sensitive to toxic chemicals (SANTOS et al., 2017a; ROCHA JUNIOR et al., 2019; MEDAURA et al., 2021; ROSHANDEL et al., 2021; WULANDARI et al., 2021). For example, vegetables like watercress (*Lepidium sativum* L.), cabbage (*Brassica oleracea*), beans (*Vigna radiata*); animals like fish (*Poecilia vivipara*) and shrimp (*Artemia salina*); and bacteria like *Vibrio fischeri* (bioluminescence test) and *Salmonella typhimurium* (AMES test) are organisms that allow an easy response in environmental toxicology assays (SANTOS et al., 2017a; ROCHA JUNIOR et al., 2019; MEDAURA et al., 2021; ROSHANDEL et al., 2021; WULANDARI et al., 2021).

Downstream processing technologies such as recovery, purification, and characterization in developing bioinoculants do not require high resolution techniques presumably due to their environmental purpose, thus purification steps are not necessarily required. Thus, filamentous and yeast bioinoculants can be adequately recovered by unit operations involving solid/liquid separation such as filtration and centrifugation (RUSINOVA-VIDEVA et al., 2020; MEDAURA et al., 2021). However, the application of drying techniques can increase the storage feasibility and shelf life of metabolically inactive bioinoculants compared to those with active metabolism.

Although the formulation and use of fungal bioinoculants in environmental remediation is not yet consolidated for a practical application, the technologies related to this formulation are predictably similar to those required for formulation of bioinoculants used in agriculture, preferably with minimal unit operations of preparation (CHAUDHARY et al., 2020). Besides containing nutrients (C, N, P and K), the formulation of fungal bioinoculants may require the addition of preservatives (e.g., potassium sorbate), stabilizers (e.g., hydroxyethyl cellulose) and chelating agents (e.g., ethylenediaminetetraacetic acid, except metal ions) similarly to the formulation of bioproducts containing fungal biosurfactants

(SANTOS et al., 2017a; SILVA et al., 2021b). Bioinoculants can be formulated in liquid state, which promotes greater ease for application, as well as in solid state through the use of carriers (e.g., polymers) to provide stabilization and protection of the strain during transport, storage and application (CHAUDHARY et al., 2020). The solid carriers involved in formulation of fungal bioproducts are related to immobilization technology, which is discussed below.

7.6 IMMOBILIZATION: CELLS, METABOLITES AND NANOTECHNOLOGY

Immobilization systems for fungal bioinoculants and/or enzymes increase the mycoremediation performance in comparison to free cells as immobilized cells have their cell density, mechanical strength and structural rigidity increased (WU et al., 2014; FAYEULLE et al., 2019; TOLEDO et al., 2021). In other words, the immobilization process alters mass transfer between contaminant-bioproduct, which can generate a microenvironment possibly more favorable for remediation (MOHAMMADI et al., 2018; CHEN; HU; WANG, 2020). Furthermore, immobilization supports can also positively favor other or sequential mycoremediation steps such as solid-liquid separation and/or support regeneration for reuse (KADRI et al., 2017; TOLEDO et al., 2021).

Organic supports composed of fungal polysaccharides and pigments present potential to immobilize bioinoculants or hydrocarbons/metals (CHANDRAN; DAS, 2011; OH et al., 2021). For example, the immobilization of the biosurfactant-producing bioinoculant *C. tropicalis* in chitosan and its adsorption on a biofilm formed on gravel increased the degradation of diesel oil by 22% since the polymer favored the fungal metabolism (CHANDRAN; DAS, 2011). Similarly, the polymer (1→3)-α-d-glucans extracted from *Lentinus edodes* showed sorption capacity for Ni^{2+} (11%), Cd^{2+} (24%), Zn^{2+} (4%) and Pb^{2+} (72%) in aqueous solution due to the interaction of these metals with oxygen-containing functional groups of the biopolymer (NOWAK et al., 2019). The use of semipermeable cellulose membrane to immobilize *A. nidulans* by encapsulation also increased approximately 3.6-fold the capacity for biosorption/desorption of copper in aqueous solution by multiple adsorption-desorption cycles compared to free cells of this strain (TOLEDO et al., 2021).

Porous inorganic supports contribute to a better physical retention of bioinoculants and/or metabolites in their structures, which allows better contact between contaminant and immobilized cell without changing the physicochemical and thermodynamic properties of the process (COBAS et al., 2013; FAYEULLE et al., 2019). Several packing materials such as ceramic pellets (ZHANG et al., 2019), vermiculite (VERGARA-FERNÁNDEZ et al., 2018a),

perlite (BRUNEEL et al., 2020) and polyueratan (LIU et al., 2020) have been used to immobilize fungal cells for aromatic and/or aliphatic hydrocarbon degradation. *Fusarium solani* reduced by 18% the total PAH in soil microcosms when immobilized on expanded clay particles (FAYEULLE et al., 2019). *Trichoderma longibrachiatum* promoted a microbial biofilm formation that removed about 70% of phenanthrene in soils when immobilized on nylon sponge (COBAS et al., 2013). Immobilization of *S. cerevisiae* on cross-linked gel in boric acid-saturated calcium alginate also allowed maximum biosorption of radioactive uranium (113.4 $\mu mol \bullet g^{-1}$), besides intensifying the adsorption on both the surface and internal parts of the yeast (CHEN; HU; WANG, 2020). Additionally, mineral particles as support for intracellular immobilization of metal ions in *S. cerevisiae* (MA et al., 2015) and *P. chrysosporium* (LU et al., 2020) enhanced the removal of toxic metals in aqueous solution, since these strains, when functionalized with calcium carbonate ($CaCO_3$) in their internal structure, achieved higher rates (3 to 4x) for immobilization of Pb^{2+} and Cd^{2+} compared to untreated strains (MA et al., 2015; LU et al., 2020).

Enzyme immobilization can enhance the biodegradation of hydrocarbons because it promotes for greater enzyme stability, resistance to proteolysis, increased catalytic activities and enzyme recyclability and shelf life (MOHAMMADI et al., 2018; PANDEY et al., 2021). Laccases from *T. versicolor* immobilized on Fe and Al soil minerals showed increased catalytic activities compared to free laccase at low pH, which favors their application for soil remediation (WU et al., 2014). Covalent immobilization of laccase produced by *Myceliophthora thermophila* on support (silica) functionalized with epoxy promoted degradation of phenolic compounds derived from oxidation of PAHs, such as catechol (95% in 2 h) and phenol (13% in 24 h), as well as improved enzyme stability at different temperature profiles and organic solvents in comparison to free enzymes (MOHAMMADI et al., 2018). Membranes fabricated by emulsion electrospinning, composed of structural nanofibres in the shell (for contaminant adsorption) and with immobilized laccase in its core (for contaminant degradation) and pores in its shell (for mass transfer), promoted enhanced adsorption and removal of hydrocarbons in soils (DAI; YIN; NIU, 2011).

Nanotechnology has allowed achieving higher performance in metal immobilization using nanoparticles synthesized from fungal cells and/or fungal metabolites as reducing agents for biogenic synthesis of such miconanoparticles (MUKHERJEE et al., 2017; ALSHARARI; TAYEL; MOUSSA, 2018). Nanoparticles as metal immobilization support have high surface/volume ratio, which confers a higher expected number of ligands for metal sorption (MUKHERJEE et al., 2017; ALSHARARI; TAYEL; MOUSSA, 2018). Nanoparticles

synthesized from chitosan extracted from *C. elegans* and sodium tripolyphosphate ($Na_5P_3O_{10}$) showed better potential to immobilize Pb^{2+} than Cu^{2+} in contaminated soil or water (ALSHARARI; TAYEL; MOUSSA, 2018). Silver nanoparticles biofabricated/adsorbed on the carbonized mesoporous surface of *Aspergillus foetidus* increased approximately threefold the arsenic biosorption compared to the same fungal biosorbent without the nanoparticles (MUKHERJEE et al., 2017).

8 EXPERIMENTAL PROCESS DESIGN: A PROPOSAL FOR INTEGRATED TECHNOLOGIES

Although integrated technologies are even more challenging in a practical use, they are under constant development to overcome technological limitations and increase the efficiency of mycoremediation (TANG et al., 2018; ELSHAFIE et al., 2020). A combination of technologies involving production and application of bioinoculants or metabolites can extend the allocation of resources appropriately to fill small gaps that may still limit remediation, as well as increase the amount of resources available to better carry out the process (YANG et al., 2009; CAMARGO et al., 2018).

8.1 PHYSICOCHEMICAL-BIOLOGICAL SYSTEMS

Bioinoculants or metabolites when integrated into physicochemical technologies have promoted superior results compared to technology without such fungal bioproducts (TANG et al., 2018; ELSHAFIE et al., 2020). The electrokinetic remediation is a technology indicated for soils with low hydraulic permeability, in which it would be unfeasible to use fungal bioinoculants due to restrictions related to low oxygen availability and permeation of its hyphae (PRENAFETA-BOLDÚ et al., 2001; TANG et al., 2018). However, the combination of SL with electrokinetic remediation enhanced the removal of Cu (53.2%), Zn (62.0%), Cr (53.0%), Pb (52.4%), Ni (56.2%) and Fe (37.0%) due to the chelating agent action of the biosurfactant in acidic conditions, which allowed greater mobility of toxic metals through electro-migration and electro-osmosis during electrokinetic remediation (TANG et al., 2018). Similarly, the use of SL (20 $g \bullet L^{-1}$) increased Cd removal by 71.2% when combined simultaneously with an ultrasound technique (35 kHz) and temperature control (50 °C) during soil washing, as cavitation and high temperature promote greater dispersion and molecular movement of the metal, respectively (YE et al., 2016).

The oxidative process of ozonization promotes degradation of hydrocarbons by addition of ozone. The combination of this technology with the use of fungal bioinoculants can increase efficiency for the degradation of these contaminants (ELSHAFIE et al., 2020). After the mycoremediation of soils contaminated with diesel by *T. harzianum*, the application of ozone in these soils increased diesel degradation by two-fold compared to the only ozonation process. It is important however to mention that lower degradation rates were achieved after ozone application due to the antimicrobial activity of ozone (ELSHAFIE et al., 2020).

8.2 MULTIPLE BIOSYSTEMS

We define multiple biosystem applied to mycoremediation of hydrocarbons and toxic metals as any bioprocess involving the (1) combination of metabolite and bioinoculant, (2) a system composed of a single fungus producing multiple metabolites, the (3) combination of different metabolites and the (4) combination of bioinoculants or metabolites with other biological systems, such as plants, animals or other microorganisms.

The sequential combination of bioleaching (by organic acids) and biosorption can be an alternative to enhance the remediation of toxic metals since the bioinoculant *A. niger* promoted the solubilization of Pb and Zn during its cultivation in liquid medium (bioleaching) and its biomass was considered a good biosorbent for such metals when applied later (YANG et al., 2009).

The production of multiple metabolites such as enzymes and biosurfactants by a single fungus can occur because the substrate can be directed to different metabolic pathways of the strain that are activated in its presence. This can favor the economy of production process and efficiency of the process for simultaneous application of these two metabolites (MOGHIMI; TABAR; HAMEDI, 2017; AZIN; MOGHIMI; HEIDARYTABAR, 2018; KRELING et al., 2020b). *Pleurotus florida* degraded crude oil (55%) in liquid medium by simultaneous production of biosurfactants and intracellular and extracellular enzymes such as tyrosinase and laccases, respectively (ROSHANDEL et al., 2021). It is speculated that positive enzyme-biosurfactant synergy in hydrocarbon degradation relates to increased enzyme activity due to the higher bioavailability of the contaminant promoted by biosurfactants (ROSHANDEL et al., 2021). However, high concentrations of surfactants can also reduce the enzymatic activity (RATHANKUMAR et al., 2021). Additionally, due to nutritional and environmental parameters that can divert and/or induce towards other metabolic pathways, the production of multiple metabolites for mycoremediation is still underdeveloped (KRELING et al., 2020b).

The combination of different classes of biosurfactants can increase washing efficiency of petroleum sludge, as binary system composed of non-ionic SL and anionic (bacterial) lipopeptide promoted the formation of mixed micelles with superior heterogeneous and structural properties. These include lower CMC and better efficiency in reducing surface and interfacial tension compared to single surfactant system (BAO et al., 2021).

Commercial SL, when combined with phytoremediation processes (remediation by plants) of Cd by pot-grown *Bidens pilosa*, promoted higher bioaccumulation of this metal in roots (5.36 $\mu g \bullet pot^{-1}$) and shoots (15.34 $\mu g \bullet pot^{-1}$) of the plant, as their addition promoted higher metal availability (SHAH; DAVEREY, 2021). Commercial sophorolipid, can also when applied to bacterial remediation, increased the degradation of petroleum hydrocarbons in soil microcosms by 13% compared to bacterial remediation alone. This maybe the results of the fungal surfactants that may have biostimulated the bacteria activities (FENG et al., 2021). The combination of fungal bioinoculants with phytoremediation and/or vermiremediation (use of earthworms) are promising technologies for hydrocarbon/or toxic metal remediation (DENG; ZENG, 2017; MA et al., 2021; SHAH; DAVEREY, 2021).

Synergistic plant-fungus interaction can promote microbial growth due to secretion of root exudates (phenolic compounds, organic acid), while plants can obtain mineral nutrients and increase their defense potential due to fungal action (LI et al., 2018; MA et al., 2021; LI et al., 2021b). Bioinoculants composed of the AMF *G. versiforme* increased Cd bioaccumulation in shoots and roots of *Solanum nigrum*, as well as increased plant growth and resistance to metal toxicity (LIU et al., 2015). Another good example is the bioinoculant P. *chrysosporium*, which increased by almost twofold the phytoremediation efficiency by *Amaranthus hypochondriacus* in soils co-contaminated with hydrocarbons and toxic metals, an even further two-fold greater efficiency was achieved when biosurfactants were added to this process (LI et al., 2018). The interaction between *S. nigrum* and *M. circinelloides* reduced oxidative toxicity of Pb, Cd and As and allowed to alter the mobility of these metals through biosorption and bioaccumulation mechanisms, besides increasing plant growth (LI et al., 2021b). In addition, microbial-plant remediation carried out by the bioinoculant *C. laeve* and *Salix viminalis* increased pyrene degradation compared to the independent processes of mycoremediation (14%) (MA et al., 2021).

The bioavailability of organic contaminants and toxic metals to fungal strains and plant roots can be increased through the use of earthworms in soils. These animals increase porosity and modify soil physicochemical parameters, which in turn favors the remediation of the soil due to the greater contact surface reached (SHI et al., 2020; WANG; WANG; MA, 2022).

Although not necessarily reported for petroleum hydrocarbons and toxic metals, mechanisms for remediation of organic contaminants by vermiremediation include vermiacumulation, vermiextraction, vermitransformation and drilodegradation (SHI et al., 2020). Multiple biosystems consisting only of the combination of vermiremediation and mycoremediation are still unexplored, so are those combined with other technologies such as phytoremediation (DENG; ZENG, 2017; WANG; WANG; MA, 2022).

The combination of vermirremediation using *Eisenia fetida* with a multiple biosystem composed of *P. chrysosporium* and *Medicago sativa* degraded higher levels of phenanthrene (11%) in soils compared to the multiple biosystem only (DENG; ZENG, 2017). Likewise, although the individual potential of each remediation system was not evaluated, the use of the AMF *Rhizophagus intraradices* with *S. nigrum* with *E. fetida* reduced the initial Cd concentration in soils by 8% compared to the untreated soils (120 $mg \bullet kg^{-1}$ of Cd) (WANG; WANG; MA, 2022). The application of microbial remediation technologies combined with vermiremediation is still limited to low concentrations of hydrocarbons due to toxicity issues (CHACHINA; VORONKOVA; BAKLANOVA, 2015; SHI et al., 2020). Baykal-EM© is a mixed bioinoculant composed of fungi (*Saccharomyces, Aspergillus* and *Penicillium*), photosynthetic bacteria (Thiorhodaceae, Athiorhodaceae and Chlorobacteriaceae), nitrogen-fixing bacteria (*Azotobacter* and *Clostridium*) and Actinomycetales (CHACHINA; VORONKOVA; BAKLANOVA, 2015). This biotechnological product Baykal-EM©, when combined with annelids *E. fetida*, *Eisenia andrei* or *Dendrobena veneta*, achieved superior results for hydrocarbon degradation than when without the animals (CHACHINA; VORONKOVA; BAKLANOVA, 2015). However, the biotic potential of all these annelids at different hydrocarbon concentrations was decreased, which presumes that the microbial consortia may facilitate oil uptake in the digestive pathway of the earthworm, which in turn increases the toxic effects of the contaminants (CHACHINA; VORONKOVA; BAKLANOVA, 2015). Furthermore, ethical aspects and both environmental and ecological safety related to the use of animals (even invertebrates) discourage real application of the combination of myco- and vermiremediation.

8.3 MIXED FUNGAL BIOINOCULANTS: CONSORTIUM WITH OTHER FUNGI, BACTERIA OR MICROALGAE

Although axenic fungal bioinoculants have demonstrated high efficiency in remediating hydrocarbon and toxic metals, they may have their enzymatic mechanisms limited

to the degradation of specific/different fractions of petroleum hydrocarbons (FAN; XIE; QIN, 2014; DALLINGER et al., 2016; HASSAN et al., 2020). Furthermore, it is very likely that in a practical application their use will predictably not be under axenic conditions.

Alternatively, mixed cultures of fungi and/or bacteria can enhance efficiency/productivity and reduce the period required to achieve maximum hydrocarbon and toxic metal removal compared to axenic cultures (HESHAM et al., 2012; ORTEGA-GONZÁLEZ et al., 2015), particularly due to the combination of different genotypes and diversity of metabolic processes of the strains (AMEEN et al., 2016; ATAKPA et al., 2022). For example, co-culture of *Acinetobacter* sp. and *Scedosporium* sp. increased the abundance of genes involved in aliphatic degradation such as alkane monooxygenase (alkB) and cytochrome P450 alkane hydroxylase (CYP52) by 43% and 65% compared to axenic cultures, respectively. This fact was attributed to biosurfactant excretion by *Acinetobacter* sp., which increases hydrocarbon bioavailability (ATAKPA et al., 2022). Furthermore, the use of co-cultures of biosurfactant-producing yeasts such as *Sarocladium* sp. and *Cryptococcus* sp. promoted greater degradation of pyrene in a shorter time than with individual strains (KAMYABI; NOURI; MOGHIMI, 2017).

The symbiotic and co-metabolism relationships between fungi and bacteria that compose mixed bioinoculants can enhance hydrocarbon mycoremediation (GHORBANNEZHAD; MOGHIMI; DASTGHEIB, 2018; ZHANG et al., 2021). Mixed bioinoculant composed of *Talaromyces* sp. and *Acinetobacter baumannii* degraded different fractions of petroleum hydrocarbons, as mostly n-alkanes were degraded by the fungus, while aromatics and branched alkanes were better degraded by the bacteria (ZHANG et al., 2021). In addition, some bacterial bioinoculants produce exopolysaccharides as a secondary carbon source, which supports the growth of fungal strains (CHANDRA et al., 2013). Mycelia can also mobilize contaminants through cytoplasmic transport linked to ‘hyphae pipelines’ vesicles, acting as vectors for dispersal and transport of contaminant-degrading bacteria (HARMS; SCHLOSSER; WICK, 2011; ATAKPA et al., 2022).

Syntrophic action between different microbial strains complements metabolic reactions involved in hydrocarbon degradation when a single strain is unable to perform degradation without the cooperation of others, as the previously formed metabolite is a substrate for next catalysis (FAN; XIE; QIN, 2014; DALLINGER et al., 2016). For example, alicyclics are only degraded by mixed bioinoculants, since the enzyme cascade involved in this degradation is almost never found in the same strain (DALLINGER et al., 2016; VARJANI, 2017). The microbial consortia composed of eight filamentous fungi, three yeasts and four

bacteria, when inoculated sequentially (bacteria added after one week), promoted greater degradation of crude oil (GHORBANNEZHAD; MOGHIMI; DASTGHEIB, 2018), pyrene and tetracosane (GHORBANNEZHAD; MOGHIMI; DASTGHEIB, 2021) compared to consortia composed separately of only fungal or bacterial strains, as well as when compared to the concomitant inoculation of all microorganisms at the start of the process. Thus, enzymes produced by fungal bioinoculants produce more soluble and less toxic intermediate metabolites from hydrocarbon degradation, which in turn can be further degraded by bacterial bioinoculants (GHORBANNEZHAD; MOGHIMI; DASTGHEIB, 2018; GHORBANNEZHAD; MOGHIMI; DASTGHEIB, 2021).

Overall, microbial consortia of fungi and/or bacteria can enhance: enzyme and biosurfactant production (AMEEN et al., 2016; ZHANG et al., 2021), bioaugmentation processes (MEDAURA et al., 2021), air biofiltration (VERGARA-FERNÁNDEZ et al., 2018a) and metal mobility (MISHRA; MALIK, 2014; HASSAN et al., 2020) during mycoremediation. Fungal consortia consisting of *Alternaria alternata*, *Aspergillus terreus*, *C. sphaerospermum*, *Eupenicillium hirayamae* and *P. variotii* degraded diesel oil and increased enzymatic activity of laccase, manganese peroxidase, lignin peroxidase and catalase compared to using individual strains alone (AMEEN et al., 2016). Bioaugmentation with autochthonous fungi belonging to the genus *Penicillium*, *Ulocladium*, *Aspergillus* and *Fusarium* increased removal of petroleum hydrocarbons by 65% compared to biostimulation in soil microscosms (MEDAURA et al., 2021). Additionally, the mixed bioinoculant composed of *F. solani* and *Rhodococcus erythropolis* removed approximately 60% of toluene and benzo[a]pyrene in an upflow air biofilters (VERGARA-FERNÁNDEZ et al., 2018a). *Fusarium solani* predominantly colonized the first stage of the biofilter and *R. erythropoli* preferentially colonized the second and third stages, probably due to the feeding with mineral medium that occurred through the top of the reactor. This demonstrated that these different strains grew differently in the presence of high flow of contaminated air according to nutrient availability and carried out toluene and benzo[a]pyrene removal (VERGARA-FERNÁNDEZ et al., 2018a).

Regarding to toxic metals, fungal consortia composed of *Aspergillus niveus*, *A. flavus* and *A. niger* increased (on average) biosorption efficiency of Zn (20%), Pb (10%), Cd (24%) and Ni (16%) in aqueous solution compared to individual strains (CHAUDHARY et al., 2022). The fungal consortia of ascomycetes (*Aspergillus*, *Fusarium*, *Penicillium*, *Purpureocillium*, etc.) and basidiomycetes (*Phanerochaete*, *Polyporales*, *Perenniporia*, etc.) also enhanced bioaccumulation of Ni and Pb in soil microcosm since ascomycetes are known to have a potential as biosorbents, while basidiomycetes are known for the production of oxidative

enzymes (HASSAN et al., 2020). Moreover, mixed bioinoculant composed by the biosurfactant-producing yeast *Meyerozyma guilliermondii* and organic acid-producing bacteria *Acidithiobacillus* promoted the solubilization of Zn (76.5%), Ni (59.8%), Cu (22.0%), Cd (9.8%), Cr (99. 8%) and Pb (7.1%) in sewage sludge, since this microbial consortium promoted better bioleaching due to excretion of organic acids and biosurfactants that alter metal mobility (CAMARGO et al., 2018).

The formulation of a mixed bioinoculant for hydrocarbon and toxic metal remediation requires that there is no interspecies inhibition for the survival of each strain, as well as that there is temporal stability of the microbiota, which can be achieved through efficient substrate availability and transport (ZAFRA et al., 2017; ATAKPA et al., 2022). In this context, the use of *C. laeve* bioinoculant in PAH-contaminated soil increased the relative abundance of bacteria (*Rhizobium* and *Bacillus*) and fungi (*Hypocreales*, *Mortierellales*, *Mucorales*, and *Pezizales*) that may be involved in the degradation of this contaminant. However, this bioinoculant also antagonized other putative PAH-degrading bacteria (MA et al., 2021). Furthermore, although *T. longibrachiatum* is a promising bioinoculant for the remediation of phenanthrene-contaminated soil, bacteria native to this soil, such as *Sphingomonas*, *Sphingobacterium*, *Acidovorax*, *Massilia*, *Flavobacterium*, *Cupriavidus* and *Aeromicrobium*, enhanced the degradation of this contaminant. Prior sterilization of the contaminated soil decreased degradation by 82% (LI et al., 2021a). Hence, interactions between bioinoculant strains (MA et al., 2021), nutrient availability (ATAKPA et al., 2022) and the native microbial communities (LI et al., 2021a) influenced the efficiency of hydrocarbon degradation via mixed bioinoculants with respect to enhancing or retarding strain development.

Although bacterial-microalgae consortia have shown promising results for the remediation of petroleum hydrocarbons (AHMAD, 2022); the application of fungal-microalgae consortia to degrade such hydrocarbons is little explored compared to the use of fungi to enhance bioflocculation processes for microalgae harvesting (AL-FAWWAZ; JACOB; AL-WAHISHE, 2016; LENG et al., 2021). Regarding hydrocarbon degradation, the consortium between the microalgae *Desmodesmus* sp. and the fungus *Rhizopus* sp. showed a superior removal of phenol (25 $mg \bullet L^{-1}$) in aqueous solutions of 25% and 29% compared to axenic cultures of these microorganisms, respectively (AL-FAWWAZ; JACOB; AL-WAHISHE, 2016). Applied to the remediation of toxic metals, the microbial consortia of the microalgae *Chlorella vulgaris* and *A. niger* had a similar result to the use of only this fungus for the removal of Cd in aqueous solutions (BODIN; ASP; HULTBERG, 2017). However, the aqueous solutions from this consortium had its pH decreased during metal removal due to the excretion of organic acids

by the fungus, promoting metal bioleaching (BODIN; ASP; HULTBERG, 2017). Possible synergistic relationships between fungi and microalgae during hydrocarbon remediation may include the additional supply of organic carbon source (exopolymers) and oxygen produced by microalgae for fungal metabolism (AHMAD, 2022). In return, microalgae assimilate inorganic carbon as CO_2 released by the fungal aerobic respiration, or organic carbon produced from the degradation of complex substrates via fungal enzymes (AHMAD, 2022). Furthermore, the adherence and interaction between the cell surfaces of microalgae and fungi can promote a favorable microenvironment for hydrocarbon and toxic metal remediation, as there is an increase in the contact surface area, which also increases hydrocarbon uptake and metal sorption (AHMAD, 2022).

9 FINAL CONSIDERATIONS

Fungi are potential prototypes to be used in the biotechnological and environmental development of remediation processes for hydrocarbons and toxic metals. This is due to the metabolism, physiology and morphology of several fungi that make them able to interact with contaminants and/or to produce metabolites possibly scalable to an industrial production focused on the commercialization of fungal bioproducts for environmental remediation. Microbial enzymes (added chemical surfactants, nutrients) (http://www.osei.us/) and fungal biosurfactants (https://www.allied-c-s.co.jp/acs-sophor) are already produced and marketed on a large scale ($\approx$ 1000L) for environmental remediation. However, technological issues regarding to the direct application, storage and shelf life of these products require further research to enable the use of these technologies, mainly related to enzymatic mycoremediation. Fungal biosurfactants show promising results for washing contaminated soils even when they are used directly from the fermented liquid culture, without any purification step. Furthermore, although the potential of various fungal bioinoculants in the remediation of hydrocarbons and toxic metals is widely demonstrated under controlled conditions, their real application in contaminated environments remains, analogously to sporulated fungi, awaiting appropriate conditions for development, i.e., technical and economic feasibility for proper consolidation.

Among the fungal metabolites studied by the scientific community with focus on environmental remediation, enzymes and biosurfactants have received more attention for hydrocarbon remediation, while other metabolites such as pigments, organic acids, polymers or bioinoculant itself (due to cell wall composition) for toxic metals remediation. It is assumed that bioprocesses involving bioprospecting of fungal isolates as possible bioinoculants and/or

producers of enzyme or biosurfactants for environmental remediation can take years to obtain an effective and accurate answer about the potential of the isolates; since several isolates must undergo a combination of screening techniques and be molecularly characterized to ensure biosafety for handling in bioprocesses. In this regard, enzymes have received a little more attention than biosurfactants in studies on bioprospecting and integration of molecular biology techniques and bioprocess engineering for mycoremediation of hydrocarbons and toxic metals. Nutritional and operational parameters, when adequately supplied, can minimize negative effects on fungal metabolism to achieve maximum performance through the full utilization of available resources provided by the natural archetype of fungal bioinoculants for environmental remediation. However, only from bioengineering of fungal strains is it possible to extrapolate the maximum performance achieved via maintenance of process parameters. Thus, genetic engineering has allowed the heterologous production of metabolites with higher yield or superior physicochemical-biological properties compared to non-engineered fungi. Moreover, -omics technologies and bioinformatics tools have enabled the scientific elucidation of gaps to promote more efficient mycoremediation processes, mainly on: a) regulatory mechanisms (induction/repression) involved in enzyme and biosurfactant production; b) contaminant degradation pathways; and c) metabolic responses of fungal strains under multiple pollutant conditions.

Other emerging technologies to increase performance in mycoremediation are related to immobilization techniques and biogenic synthesis of nanoparticles that promote enhancements in structural and mechanical characteristics in bioinoculants and/or metabolites, which in turn favor their interaction with contaminants and mass transfer during remediation. Integrated technologies based on the combination of bioinoculants and/or fungal metabolites with physico-chemical remediation, such as ozonation or electrokinetic techniques, and the combination with other bioremediation methods such as phytoremediation, vermiremediation or mixed microbial bioinoculants have achieved superior results compared to the use of bioinoculants or metabolites alone in most cases. Furthermore, the simultaneous use of multiple metabolites such as enzymes and biosurfactants, or the combination of different classes of biosurfactants, can promote synergistic actions during remediation to achieve better performance since biosurfactants increase the bioavailability of contaminants for oxidative enzymatic action, as well as micelles with superior properties can be formed by mixed biosurfactants.

This review has not been restricted to remediation of specific types of hydrocarbons and toxic metals and/or potential fungal bioproducts, which has broadened the coverage of

different technologies to be applied in general. Although such technologies related to bioprocess and biomolecular engineering techniques may be trivial to any type of bioinoculant, metabolite or contaminant within its class, some of these technologies/methodologies discussed herein may not be applied to other bioprocesses due to their particularities, as well as divergence in operational parameters may decrease the coverage range or influence the response on the use of such technology. However, the high number of articles screened from the past 20 years of our survey brings robustness to the data discussed here, as well as relevance to the promotion of fungal bioprocesses for remediation of different hydrocarbons, toxic metals and/or possibly other emerging contaminants.

CAPÍTULO II

Biosurfactantes fúngicos, da natureza ao produto biotecnológico: bioprospecção, produção e potenciais aplicações

Este capítulo é uma reprodução do artigo intitulado "**Fungal biosurfactants, from nature to biotechnological product: bioprospection, production and potential applications**", publicado em 16 de junho de 2021, pela revista "*Bioprocess and Biosystems Engineering*" (ISSN: 1615-7591).

Bioprocess and Biosystems Engineering (2021) 44:2003–2034
https://doi.org/10.1007/s00449-021-02597-5

CRITICAL REVIEW

Fungal biosurfactants, from nature to biotechnological product: bioprospection, production and potential applications

André Felipe da Silva[1,2] · Ibrahim M. Banat[3] · Admir José Giachini[1] · Diogo Robl[1]

Received: 15 March 2021 / Accepted: 1 June 2021 / Published online: 16 June 2021

Qualis: A2

Fator de Impacto: 3.434 (2021)

Disponível em: https://link.springer.com/article/10.1007/s00449-021-02597-5

ABSTRACT

Biosurfactants are in demand by the global market as natural commodities that can be added to commercial products or use in environmental applications. These biomolecules reduce the surface/interfacial tension between fluid phases and exhibit superior stability to chemical surfactants under different physico-chemical conditions. Biotechnological production of biosurfactants is still emerging. Fungi are promising producers of these molecules with unique chemical structures, such as sophorolipids, mannosylerythritol lipids, cellobiose lipids, xylolipids, polyol lipids and hydrophobins. In this review we aimed to contextualize concepts related to fungal biosurfactant production and its application in industry and the environment. Concepts related to the thermodynamic and physico-chemical properties of biosurfactants are presented, which allows detailed analysis of their structural and application. Promising niches for isolating biosurfactant-producing fungi are presented, as well as screening methodologies are discussed. Finally, strategies related to process parameters and variables, simultaneous production, process optimization through statistical and genetic tools, downstream processing and some aspects of commercial products formulations are presented.

Keywords: Filamentous fungi; Yeasts; Biosurfactants; Bioprocess, Screening; Downstream.

RESUMO

Os biosurfactantes são demandados pelo mercado global como *commodities* naturais que podem ser adicionados a produtos comerciais ou utilizados em aplicações ambientais. Estas biomoléculas reduzem a tensão superficial/interfacial entre fases fluidas e exibem estabilidade superior aos surfactantes químicos sob diferentes condições físico-químicas. A produção biotecnológica de biosurfactantes ainda está emergindo. Os fungos são produtores promissores destas moléculas com estruturas químicas únicas, tais como soporolípidos, lipídios de manosileritritol, lipídios de celobiose, xilolípidos, lipídios de poliol e hidrofobinas. Nesta revisão, objetivamos contextualizar conceitos relacionados à produção de biosurfactantes fúngicos e sua aplicação na indústria e no meio ambiente. São apresentados conceitos relacionados às propriedades termodinâmicas e físico-químicas dos biosurfactantes, o que permite uma análise detalhada de sua estrutura e aplicação. São apresentados nichos promissores para o isolamento de fungos produtores de biosurfactantes, bem como são discutidas metodologias de triagem. Finalmente, são apresentadas estratégias relacionadas a parâmetros e variáveis de processo, produção simultânea, otimização de processos através de ferramentas estatísticas e genéticas, processamento downstream e alguns aspectos de formulações de produtos comerciais.

Palavras-chave: Fungos filamentosos; Leveduras; Biosurfactantes; Bioprocessos, Triagem; Downstream.

1 INTRODUCTION

Since the 1930s, chemical surfactants have been part of several commercial products. These molecules are structurally composed of a hydrophilic and a hydrophobic moiety, with variations in structure, which allows for applications in numerous environmental and industrial areas (SCOTT; JONES, 2000; COWAN-ELLSBERRY et al., 2014). Main physicochemical action of surfactants is to reduce the surface and interfacial tension at the interfaces between immiscible liquids, solids and gases, allowing distinct phases to mix and interact (COWAN-ELLSBERRY et al., 2014; OTZEN, 2017). They play a diverse and significant role in various industrial market segments, including products currently in demand due to the Covid-19 pandemic (ÇELIK et al., 2020; JOHNSON et al., 2021). Several products contain an significant amount of surfactants in their composition, such as toothpastes, soaps, detergents, fabric softeners, etc. (OTZEN, 2017). Most of the chemical surfactants are synthesized from petrochemicals products, which although economically viable, are ecologically undesirable (COWAN-ELLSBERRY et al., 2014; OTZEN, 2017). However, biotechnology and chemical companies are continuously engaged in research for safer and more environmentally friendly industrial bioprocesses preferably using ecological biomolecules with superior structural and functional properties (GEETHA; BANAT; JOSHI, 2018; EVONIK INDUSTRIES, 2020). In this context, biosurfactants represent a sustainable alternative to these requirements, since they are naturally synthesized by biological systems such as plants and microorganisms, offering lower toxicity and higher degradability (MARCHANT; BANAT, 2012a; 2012b; GEETHA; BANAT; JOSHI, 2018).

Bacterial biosurfactants (glycolipids, glycoproteins, lipopeptides) are produced by different strains mainly *Pseudomonas* and *Bacillus strains*, leading the largest volume of data and scientific output (RAAIJMAKERS et al., 2010). Fungal biosurfactants, in comparison, represent only 19% out of the total (12% from ascomycetes and 7% from basidiomycetes), while having the widest chemical structural variant of biosurfactants, some of these are produced exclusively by fungi, like sophorolipids, mannosylerythritol lipids, cellobiose lipids, xylolipids, lipid polyols and hydrophobins (SUNDE et al., 2017; ABDEL-MAWGOUD; STEPHANOPOULOS, 2018; GARAY et al., 2018). In general, fungal biosurfactants have chemical structures versatility and their properties allow a wide range of applications such as in personal care sector (BAE et al., 2019), food (MANIGLIA et al., 2019), agriculture (SHAH; DAVEREY, 2021), pharmaceutical (CHUO et al., 2019), biomedicine (GUERFALI et al., 2019),

materials engineering (RANJANA et al., 2019), bioenergies (MENON et al., 2010) and environmental remediation (LUNA et al., 2015; YE et al., 2016).

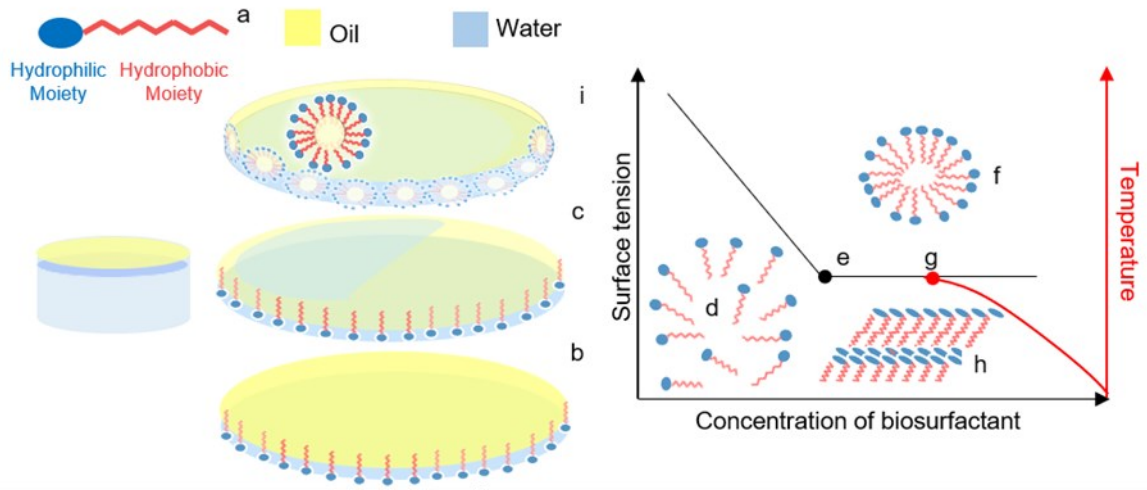
Both bacterial and fungal biosurfactant production is still quite restricted, due to the production and recovery costs that often are higher than chemical surfactants and the fact that most bioprocesses show low yield and productivity (JIMOH; LIN, 2019). In summary, industrial biotechnology can minimize limitations inherent to each stage, including isolation, screening, growth and product yield optimization, downstream processing, recovery, purification and characterization of the products (BAJPAI, 2020). In this context, after the isolation of the fungal strain from different environments, several techniques based on direct and indirect measurement of surface tension and/or emulsifying activity can be applied to select producing strains (WALTER; SYLDATK; HAUSMANN, 2010). In addition, the use of agro-industrial waste, non-conventional substrates and the optimization of bioprocess parameters through statistical designs have also been shown as a tool to enhance the viability of the production of these biomolecules (GEYS; SOETAERT; VAN BOGAERT, 2014; ASGHER et al., 2020). Downstream processing steps are also constantly being improved to ensure greater yields and better performance and to elucidate structural variants of these molecule (ANDRADE et al., 2017; DOLMAN et al., 2019).

The purpose of this review is to collate information related to fungal biosurfactants and bioemulsifiers and determine the main types and properties of these molecules. The review evaluates the bioprocesses involved in biosurfactants production considering the entire innovation chain, from isolation sources and selection tests for producing microorganisms, cultivation parameters, process optimization through statistical and genetic tools, to downstream processing; in addition to their potential in formulating commercial products.

2 STRUCTURAL CLASSES, PROPERTIES AND APPLICATIONS

The versatility of the chemical structures of fungal biosurfactants is directly correlated to their biochemical composition and molecular weight, these macromolecules carryout physicochemical and biological properties of biotechnological interest. Low molecular weight biosurfactants have their molar mass ranging between 0.5-1.5 kDa (MULLIGAN, 2005), while bioemulsifiers (high molecular weight) also known as Exopolysaccharides (EPS) can reach up to 500 kDa (PERFUMO et al., 2010; MUKHOPADHYAY et al., 2014; UZOIGWE et al., 2015). Figure 6 presents a schematic chart for understanding the action of biosurfactants and bioemulsifiers.

Figure 6 – Schematic diagram on the action of biosurfactants

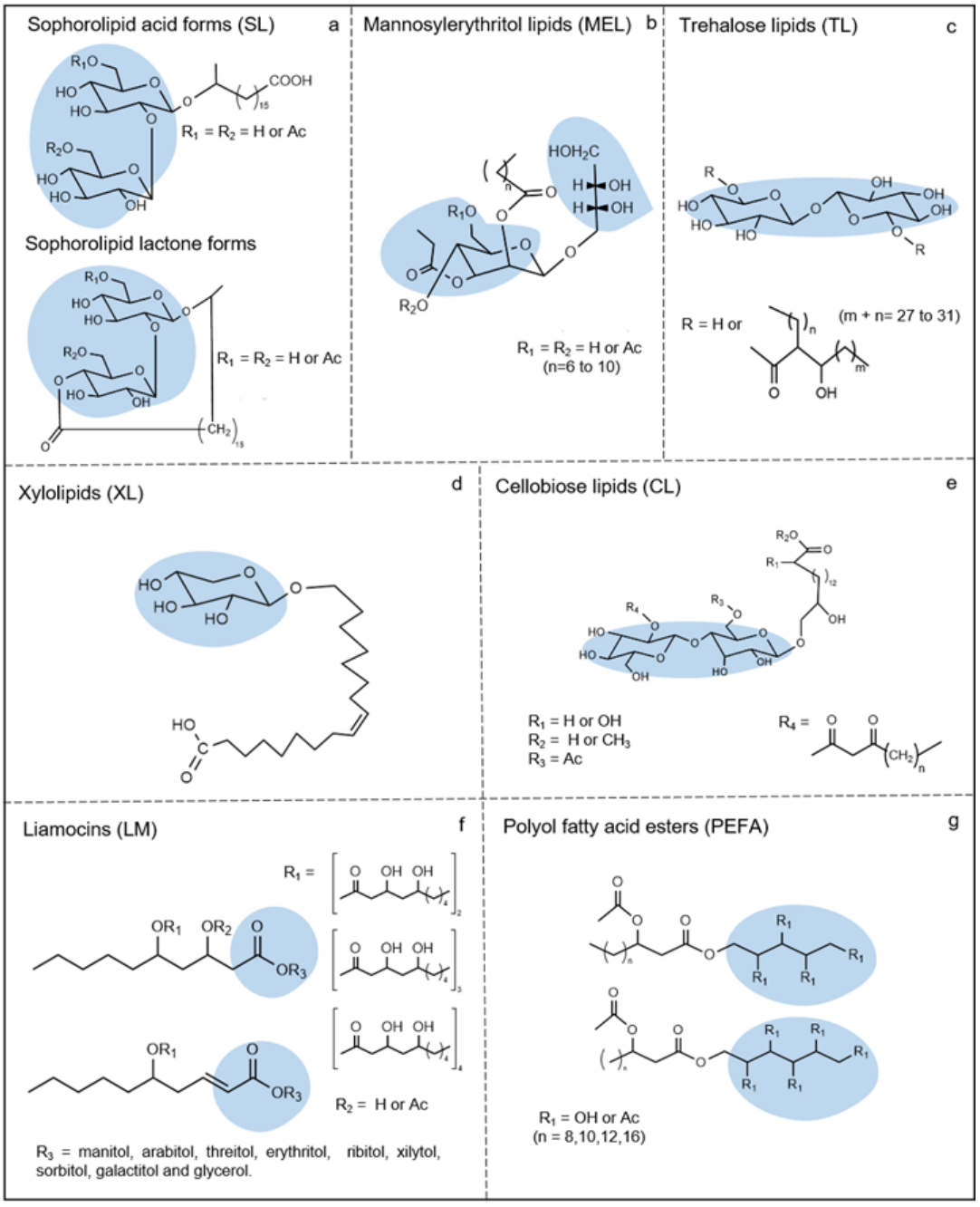


Caption: Biosurfactants are amphiphilic molecules, their composition consists of hydrophilic (carboxylic acids, alcohols, amino acids, phosphates, peptides, mono-, di- or polysaccharide cations or anions) and hydrophobic (hydrocarbon chains or saturated/unsaturated fatty acids) moieties (a). These molecules accumulate at phase interfaces of different polarities and stabilize stabilize heterogeneous phases (oil/air bubble droplets). (b). Thus, adsorption reduces the free energy per unit area needed to create a new surface, a reduction that is closely related to surface tension (liquid-air) and interfacial tension (liquid-liquid) [note the displacement of surface oil over water in c] (JAHAN et al., 2020). Biosurfactant monomers (d) due to their self-assembly properties on reaching the Critical Micellar Concentration (CMC) (e), can form aggregated micellar structures (f) (BANAT et al., 2010). Micelle formation and solubility occur just above the Krafft temperature (g), i.e., the critical temperature at which micellar self-assembly occurs due to dissolution of the hydrated surfactant crystals (h) (LU et al., 2018). Bioemulsifiers are less effective in reducing surface tension and are involved in the formation and stabilization of emulsions between two immiscible phases (i) (PERFUMO et al., 2010; UZOIGWE et al., 2015).
Source: Elaborated by the author (2021).

In summary, the main function of surfactants is to decrease the tension at the fluid interfaces (liquid/liquid [emulsion] and liquid/air [foam]) Likewise, emulsifiers promote the formation of emulsions and steric stabilization at the interfaces of small drops of emulsions and foams, providing long-term stability (SAŁEK; EUSTON, 2019). It is known that some biomolecules have both properties, surfactants and emulsifiers (PESSÔA et al., 2019). Biosurfactant types include glycolipids, polyol lipids, proteins, carbohydrate-lipid-protein complexes, lipoproteins (or lipopeptides) and glycoproteins (KITAMOTO et al., 2009; GARAY et al., 2018).

Figure 7 shows the classes of fungal biosurfactant glycolipids (low molecular weight), and polyol lipids based on their composition and chemical structure. Table 3 presents the classes of high molecular fungal biosurfactants based on their biochemical composition, structural characteristics and main producing microorganisms reported.

Figure 7 – Chemical structure of fungal glycolipids and polyol lipids



The hydrophilic portions are highlighted in blue.
Source: Adapted from Garay et al. (2018) and Kitamoto et al. (2009).

Table 3 – An overview of hydrocarbon remediation processes by bioinoculants and metabolites

Group	Biosurfactant	Composition	Producer Microorganism	References
Carbohydrate-lipid-protein complexes	Not classified	Molecules composed by the ligation of congeners of carbohydrates, lipids and proteins	*Galactomyces pseudocandidum, Candida tropicalis* and *Geotrichum candidum*	Eldin; Kamel; Hossam (2019)
			Aspergillus flavus	Ishaq et al. (2015)
			Penicillium citrinum	Camargo-De-Morais et al. (2003)
			Aspergillus ustus	Kiran et al. (2009)
Lipopeptides (or lipoproteins)	Not classified	Molecules composed of proteins and lipids	*Yarrowia lipolytica* MTCC9520	Radha et al. (2020)
			Penicillium chrysogenum SNP5	Gautam et al. (2014)
			Aspergillus mulundensis	Bills et al. (2016)
			Fusarium sp.	Qazi et al. (2014)
Polymeric-Glycoproteins	Liposan	Molecule composed of polysaccharide and protein	*Y. lipolytica*	Cirigliano; Carman (1984)
	Manoproteins	Mannose polymers covalently linked to the backbone main protein	*Saccharomyces cerevisiae*	Cameron et al. (1988)
			Kluyveromyces marxianus	Lukondeh; Ashbolt; Rogers (2003)
	Yasan	Low-lipid polysaccharide-protein complex	*Y. lipolytica*	Amaral et al. (2006)

2.1 SOPHOROLIPIDS

Among fungal glycolipids, Sophorolipids (SL) has taken the lead industrially due to its high production yields, which can exceed 300 gL^{-1} and ample applicability in the industrial, environmental and health sectors (KIM; YUN; KIM, 2009). SL has a hydrophilic portion of sophorose, a two β-1,2 glucose residues linked to a hydrophobic long fatty acid chain (C_{16} or C_{18}), terminally or subterminally hydroxylated (Figure 7-a). SL are synthesized metabolically in two main forms, lactonic or acidic, that lead to a mixture of different congeners and quantities in the final production lines (KULAKOVSKAYA; BASKUNOV; ZVONAREV, 2014; PAULINO et al., 2016). Acidic SL are alicyclic and have a carboxylic acid group (COOH) at the end of the hydrophobic portion, while lactonic SL are cyclic with ester functionality (LYDON et al., 2017; BANAT et al., 2021).

Although 20 or more congeners can occur of SL, generally a few forms are dominant (CLAUS; VAN BOGAERT, 2017). The structures of SL can be modified through chemicals or enzymes (DELBEKE et al., 2018), i.e., the combination of genetic manipulation techniques allows the modification in the hydrophilic and/or hydrophobic portions of the molecule (MARCHANT; BANAT, 2012b). The structural variation is due to the length and saturation of the fatty acid portion and acetylation of the sophorose, as well as the position of the hydroxyl group in both the lipid portion and the carbohydrate that is esterified with fatty alcohol (JADHAV; PRATAP; KALE, 2019; BANAT et al., 2021).

SL production occurs in several non-pathogenic yeasts, principally from *Starmerella* genus, which was previously classified as *Candida* and/or *Torulopsis* (WANG et al., 2019b). The diversity of species able to produce SL in this genus explains the SL production with different physico-chemical characteristics, since they have enzymes necessary to synthesize them when grown on hydrophobic substrates, forming fatty acids to be metabolized by β-oxidation (CORTÉS-SÁNCHEZ; HERNÁNDEZ-SÁNCHEZ; JARAMILLO-FLORES, 2013).

The SL congeners profile produced by a microorganism can have different types and extensions of bioactivity, therefore, the use of purified SL does not attribute to it an unambiguous activity (NAUGHTON et al., 2019). Recently, it was reported that the interfacial properties of acidic SL, such as leaching and chelating action, are pH-dependent and can promote its application in hydrometallurgical extraction of metals and separation of ions and minerals through foam flotation, respectively (DHAR et al., 2021). Lactonic SL in comparison have lower CMC than acidic forms, being more hydrophobic and less soluble in water molecules (KULAKOVSKAYA; BASKUNOV; ZVONAREV, 2014), in the same way that

acetylation decreases SL solubility (SHAH et al., 2005). In addition, lactonic SL have a better surface tension reduction compared to acid SL. However, both have recognized antimicrobial activity (VAN BOGAERT; ZHANG; SOETAERT, 2011) and have already been proven to be prominent compounds with antimicrobial action in topical creams and for oral hygiene (ELSHIKH et al., 2017; LYDON et al., 2017).

In general, SL have broad antimicrobial activity against several bacterial (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) (RIENZO et al., 2014; GARG; PRIYANKA; CHATTERJEE, 2018; CERESA et al., 2020) and fungal pathogens (*Colletotrichum gloeosporioides*, *Fusarium verticilliodes*, *Fusarium oxysporum*, *Corynespora cassiicola* and *Trichophyton rubrum*) (SEN et al., 2017; FERNANDES et al., 2020). In addition, SL have antimicrobial action against *Clostridium perfringens* and *Campylobacter jejuni* and their association with lactic acid is a promising natural disinfectant in the poultry industry (SILVEIRA et al., 2019).

In addition to antimicrobial activity, when sophorolipids was added to the bioactive films composed of polylactic acid for food, it enhanced their thermomechanical properties (SILVEIRA et al., 2020). In this context, SL are promising ingredients in the food industry as they also have emulsifying properties over edible oils and are stable at a wide range of pH, temperature and salinity (DAVEREY; PAKSHIRAJAN, 2010). *Candida albicans* SC5314 and *Candida glabrata* CBS138 sophorolipids have been reported to have emulsifying activities in olive oil, soy oil, almond oils and mustard by 40 to 50% (GAUR et al., 2019). In addition, SLs have sensory properties on the palate and responses induced in human taste buds cell cultures, enabling their application to modulate the taste of food and drugs (OZDENER et al., 2019).

Applied as adjuvants in antibiotic recovery processes (erythromycin and amoxicillin), SLs are alternatives for increasing the solubility and transferring to the organic phase through the formation of reverse micelles, in a liquid-liquid extraction processes (CHUO et al., 2019). SL were also used to remove tetracycline (88.2%), sulfadiazine (96.6%) and roxithromycin (100%) from soils contaminated by these antibiotics (YE et al., 2016).

In agriculture, the addition of SL as adjuvants in pesticide formulations resulted in the US patent 2017/0094968 A1, which describes strategies on the use of SL to increase yield in the production of plants of agricultural interest (SIEVERDING, 2017). SLs are also employed at preferably 100 to 500 g/ha, allowing a better formation of total biomass or improving harvestable parts in plants (SIEVERDING, 2017). The *Medicago sativa* and *Bidens pilosa* plants when grown in soils augmented with SL had an increase in the growth of their shoots

and roots, which may have been due to greater microbial activity in the soil after this addition (SHAH; DAVEREY, 2021).

In materials engineering, SL can be used as stabilizers and reducing/capping agents in the synthesis of metal-based nanomaterials, since they are generally used to improve colloidal stability in water or in aqueous solutions containing salt, many nanoparticles syntheses are carried out in organic media (KASTURE et al., 2007; BACCILE et al., 2013). SL use in the synthesis of copper (Cu) nanowires through hydrothermal synthesis, together with these nanomaterials, showed the potential for the substitution of components in transparent conductive electrodes (RANJANA et al., 2019).

The increase in the yield of enzymatic saccharification applied to vegetable biomass, as in wheat bran (20%) and steam-exploded wood (67%) was increased with the addition of SL in enzymatic cocktails, opening applications also aimed at bioethanol production (HELLE; DUFF; COOPER, 1993; MENON et al., 2010).

2.2 MANNOSYLERYTHRITOL LIPIDS

The mannosylerythritol lipids (MEL) are composed of 4-O-D-mannopyranosyl-meso-erythritol with various patterns of acylation and acetylation, linked to fatty acid molecules (C_8-C_{20}) (Figure 7-b). Their structure may vary with the producing microorganism and the substrates used (GEYS; SOETAERT; VAN BOGAERT, 2014;). MEL have an oily appearance and according to the number of acetyl groups and their locations in the mannose, they are characterized as MEL-A (diacetylated in O-4 and O-6), MEL-B (monoacetylated in O-6), MEL-C (monoacetylated at O-4), MEL-D (deacetylated) (FAN et al., 2014) and MEL-A2 (triacetylated (O-2, O-4 and O-6) (MORITA et al., 2011; ALIMADADI et al., 2018). The number of acyl groups in mannose and/or erythritol, and the length of the fatty acid chain, with its levels of saturation, may contribute to differences in several homologues of MEL (YU et al., 2015; NIU et al., 2017). In this way, a detailed characterization of all structures derived from MEL is essential to identify their structure-function relationship and potential application. An MEL variant, with greater hydrophilicity, was identified with the presence of a mannitol - instead of an erythritol residue in its structure (BECK et al., 2019).

MEL are well described for their self-assembling properties (sponge, cube and lamellar) that are influenced by carbohydrate stereochemistry (RODRIGUES, 2015). These structures can form giant vesicles or nanostructures that can be applied as models in the cellular and molecular study-processes (KITAMOTO et al., 2009). MEL production is commonly described

from fungi that belong to the Ustilaginales order (SPOECKNER et al., 1999). Other species, such as *Sporisorium* sp. aff. *sorghi* (ALIMADADI et al., 2018), *Pseudozyma* spp. (ANDRADE et al., 2017) and *Schizonella melanogramma* (MEL-A and -B) (DEML et al., 1980) are also described as MEL producers. As far as we known, the highest MEL (165 gL^{-1}) production was obtained in batch-fed cultivation of *Pseudozyma aphidis* (RAU et al., 2005).

The elucidation of MEL synthesis pathways showed conserved mechanisms, in which a cluster of five conserved genes, including an acetyltransferase gene (mat1), a member of the family of the main facilitators mmf1, two acyltransferases (mac1 and mac2) and a glycosyl transferase gene (emt1) are present (HEWALD et al., 2006).

The MEL produced by *Ustilago hordei* differ from those secreted by *Ustilago maydis*, as they are mostly mono-acetylated and contain a different mixture of acyl groups, although the MEL gene clusters of both species show a high degree of synergy (52%) and similarity (75%) to the respective genetic products (DEINZER et al., 2019). These differences result from different catalytic activities of mat1 and mac1 acylation in the mannosylerythritol fraction at the C2 position, while mac2 promotes acylation at the C3 position. This indicates that the elucidation of these enzymes with different substrate specificities (TAKAHASHI et al., 2012) may enable a specific MEL production (SAIKA et al., 2018a).

The production of MEL-D (deacetylated) corresponds to a small fraction compared to other MEL congeners produced during cultivation. As far as it is known, there are no reports of mainly deacetylated MEL production by microorganisms (SAIKA et al., 2018a). However, alternative strategies are possible by enzymatic treatment of MEL-B with lipases (FUKUOKA et al., 2011) and genetic modification. The "interruption" of the mat1 gene in *Pseudozyma hubeiensis* (MEL-C producer) favours the production of MEL-D (KONISHI; MAKINO, 2018).

MEL-A produced by the endophytic fungus *Ceriporia lacerate* are potential adjuncts in environmental bioremediation, and these biomolecules showed a decrease in surface tension (31 mNm-1) and emulsifying activity in hydrocarbon contaminants (78.5%), soybeans (76.1%) and vacuum pump oil (77.4%) (NIU et al., 2017). MEL-B and MEL-C can be applied in the formulation of biopesticides, as they are effective in altering the hydrophobicity of solid surfaces and inhibit germination of *Magnaporthe grisea* (rice blast) conidia (YOSHIDA et al., 2015).

MEL have excellent interfacial properties and pharmacological actions in drug administration (ARUTCHELVI et al., 2008), for this purpose MEL-A may be superior to MEL-B/C (FAN et al., 2014). The formulation of fungal liposomes coated with chitosan modified with MEL-A can be considered as a promising delivery system, with enhanced antioxidant

effects for bioactive compounds (WU et al., 2019). MEL-A have cytostatic activity to trigger apoptosis of B16 cells in vitro (FAN et al., 2016) and can dramatically increase gene transfection via membrane fusion (INOH et al., 2004). In biomedical applications, MEL produced by *Ustilago maydis* CGMCC 5,203 were used for the synthesis of gold nanoparticles (AuNPs) and demonstrated cytotoxic potential against HepG2 cells (BAKUR et al., 2019).

MEL-A and B also showed antimicrobial activity against gram-positive and gram-negative bacteria (KITAMOTO; ISODA; NAKAHARA, 2002) while MEL-A showed antibacterial activity against strains of *Micrococcus luteus* and *S. aureus* (FDA209P) and MEL-D against *Enterococcus faecalis* and *E. faecium* vancomycin resistant (VRE) strains (NASHIDA et al., 2018). In addition, MEL can be used as additives to improve the functional properties of proteins and formulation of edible films for food. These surfactant-protein interactions in food systems ensure consistency, foam capacity and adequate food emulsifying activity (PIOTROWSKI; LEWANDOWSKA; WOJCIECHOWSKI, 2012). In this sense, MEL addition for active films formulation based on starch provided greater flexibility, permeability to water vapor and hydrophilicity, favouring its use as an edible coating for food and biomedical devices (MANIGLIA et al., 2019).

MEL are also promising molecules for personal care products. *Pseudozyma antarctica* NBRC 10736 MEL has been patented as a substitute for skin moisturizers, such as ceramide (KITAGAWA et al., 2008). MEL-C produced by *Pseudozyma hubeiensis* have antioxidant effects and superior protective activity (30.3%) against oxidative stress in human skin cells compared to arbutin (13%), sunscreen and antioxidants (TAKAHASHI et al., 2012). In addition, MEL can be used as anti-melanogenic agents for effective skin lightening, as they significantly decrease the melanin content in primary normal human melanocytes (BAE et al., 2019).

2.3 TREHALOSE LIPIDS AND XYLOLIPIDS

Trehalose lipids (TL) consist of a trehalose disaccharide linked by an ester bond to long-chain fatty acids (Figure 7-c). Only *Fusarium fujikuroi* was reported to be a TL producer with reducing surface tension (20.08 mNm^{-1}). This filamentous fungus was isolated from soil samples contaminated with hydrocarbons (REIS et al., 2018). TL are present as trehalose dimicolate in bacteria such as mycobacteria, *Corynebacterium*, *Nocardia* and *Rhodococcus* (MORITA et al., 2016). Most TL are found in these prokaryotes cell walls (FRANZETTI et al., 2010; HUBERT et al., 2012), which can make it difficult to extract. In addition, most of these

strains can exhibit slow growth and may be pathogenic. Consequently, TL still arouse less industrial interest via biotechnological production (OTZEN, 2017).

Xylolipids (XLs) on the other hand are composed of xylose monomers linked to fatty acids (Figure 7-d) and are secreted (at a reported 7.48gL^{-1}) by *Pichia caribbica* when grown in xylose-rich media. In addition, XLs reduce the surface tension to 35.9 mNm^{-1} with a CMC of 1 mgL^{-1} and have some antibacterial activity against *S. aureus* (JOSHI-NAVARE; SINGH; PRABHUNE, 2014).

2.4 CELLOBIOSE LIPIDS

Cellobiose lipids (CL), also known as ustilagic acids, consist of cellobiose linked to the terminal hydroxyl group of a fatty acid (Figure 7-e) (BÖLKER; BASSE; SCHIRAWSKI, 2008). Under nitrogen starvation, the fungus *U. maydis* can produce CL and mannosylerythritol lipids in varying concentrations up to 30 gL^{-1}, depending on the carbon source (SPOECKNER et al., 1999). The studies of this class of biosurfactant are focused on two points. The first, is the structural characterization of the hydrophobic portion, and the second is its antimicrobial potential suggested by the involvement of the cytoplasmic membrane, by the inhibition of some enzymes, and by structural changes in the wall of sensitive microorganisms (PUCHKOV et al., 2002).

Ustilagomycetic yeasts of the genus *Pseudozyma* are commonly reported as producers of CL. Good examples are *P. fusiformata* (KULAKOVSKAYA et al., 2005), *P. aphidis* and *P. hubeiensis* (MORITA et al., 2013). The CL excreted by *P. flocculosa*, named flocculosine, are well known for their phytopathogenic action against powdery mildew (CHENG et al., 2003), yeasts and gram-positive bacteria commonly associated with human infections. The CL excreted by *Sympodiomycopsis paphiopedili* act against *Cryptococcus terreus* (45 µg mL^{-1}) and *Candida albicans* (160 µg mL^{-1}) (KULAKOVSKAYA et al., 2004) while those produced by *Trichosporon porosum* act against *Candida albicans* (0.2 mM) and *Filobasidiella neoformans* (0.03 mM) (KULAKOVSKAYA et al., 2010).

The CL produced by *Cryptococcus humicola* are additives for the formulation of colloids applied in the food and cosmetics industries (IMURA et al., 2014). Sodium salts improves the aqueous solubility of CLs and favours the formation of gels in alcoholic solution (ethanol or 1,3-butanediol) (IMURA et al., 2012; 2014). CL from *C. humicola*, when added to poly (lactic acid) films, modifies the surface properties increasing, for example, its wettability (FUKUOKA et al., 2018).

2.5 POLYOL LIPIDS

Polyol Lipids (PL) are considered to be an exclusive class of fungal biosurfactants. The main difference of the PL from other glycolipids is the replacement of the hydrophilic portion by a polyol (typically D-mannitol or D-arabitol) linked to an acetylated (R) -3-hydroxy fatty acid (GARAY et al. 2017a). This class of biosurfactants comprises two groups of molecules, the Liamocins (LM), produced by *Aureobasidium pullulans* (Figure 7-f) (PRICE et al., 2013), and the Polyol Fatty Acid Esters (PEFA), reported in *Rhodotorula* spp. (Figure 7-g) (CAJKA et al., 2016).

Several structures of LM are produced by *Aureobasidium melanogenum* (SAIKA et al., 2020). The LM are only acylated in the polyol portion, while the PEFA can present different acetylation degrees in the polyol, in addition to an acetylation in the fatty acid portion (GARAY et al., 2017a). Additionally, differences are found in the degree of acetylations in the PEFA depending on the producing fungus, which directly changes the surface properties of the molecule (LYMAN et al., 2018).

Regarding the physico-chemical properties, PL densities are higher than water, which favours the recovery of molecules. LM are commonly described as an oily compound, while PEFA have a viscous characteristic (GARAY et al., 2018). The small portions of the molecule can be modified by polyols supplementation in culture medium (PRICE et al., 2017). Different LM congeners with different polyol groups have antibacterial activity against strains of *Streptococcus* spp. (PRICE et al., 2017).

In general, LM have a potential as a new antimicrobial agent group, particularly in prophylactic applications in which a broad spectrum antibiotic may not be the best option (LEATHERS et al., 2018). LM inhibits the formation of oral biofilms of *S. mutans*, *S. sobrinus* (LEATHERS et al., 2019) and *S. suis* mainly by rupturing the pathogen's cell membrane (BISCHOFF et al., 2018).

Limited information are reported on PEFA applicability. The ability to reduce surface tension and its emulsifying capacity are contrasting, but offer promising prospects for therapeutic and environmental applications (GUERFALI et al., 2019; WANG et al., 2019a). The PEFA produced by *Rhodotorula paludigena* have a high capacity to reduce surface tension ($33.84 mNm^{-1}$) at low CMC ($13.18 mgL^{-1}$) and they have a superior capacity than Tween 80 to emulsify diesel (77%) (WANG et al., 2019a). However, the PEFAs produced by *R. babjevea* reduce surface tension to a lesser extent and have a high capacity to emulsify diesel (70%) (GUERFALI et al., 2019).

2.6 LIPOPEPTIDES

Lipopeptides (LPP) are composed of a portion of fatty acids with different degrees of branching and oxidation, linked to linear or circular oligopeptides. The peptide varies in number and type of amino acids (GEYS; SOETAERT; VAN BOGAERT, 2014). Some fungi-produce LPP with active surface properties as described in Table 3. Fusarium sp. BS-8 produces LPP which reduced surface tension (32 mNm^{-1}) at low CMC ($\geqslant$ 1.2 gL^{-1}) (QAZI et al., 2014). LPP produced by Mucor circinelloides also reduce the surface tension (26 mNm^{-1}) and the viscosity of motor oil by 98.25%, contributing to potential applications in microbial enhanced oil recovery (MARQUES et al., 2019). The cationic lipoprotein produced by *Yarrowia lipolytica* MTCC 9520 was applied as a stabilizing agent in the synthesis of silver nanoparticles, whose action was to prevent the formation of aggregates and facilitate nanoparticles production (RADHA et al., 2020).

The LPP denoted scopularides produced by *Scopulariopsis brevicaulis* have activity against several tumour cell lines, i.e., anticancer activity, even at low concentrations (10 $\mu g\ mL^{-1}$) (YU et al., 2008). Other LPP called eniatiatins are secreted by *Fusarium*, *Verticillium* and *Halosarpheia*. Eniatiatins are suggested for the treatment and prevention of atherosclerosis and hypercholesterolemia, since they inhibit the enzyme acyl-CoA:cholesterol acyltransferase and alter the concentration of intracellular ions, interrupting cellular metabolism (SY-CORDERO; PEARCE; OBERLIES, 2012).

Among other possible applications, those produced by *Candida lipolytica* UCP 0988 do not present toxicity against the germination of different vegetable seeds such as *Brassica oleracea*, *Solanum gilo* and *Lactuca sativa* (RUFINO et al., 2014), favouring germination in bioremediation soils containing traces of biosurfactants or enhancing water entry for seed germination (SANTOS et al., 2017b). In addition, these LPP do not present toxicity to the development of *Artemia salina* (RUFINO et al., 2014), a species of aquatic brine shrimp, enabling the application for remediation of aquatic environments (SANTOS et al., 2017b).

2.7 HYDROPHOBINS

Hydrophobins are globular proteins, with low molecular weight (<20 kDa) and primary sequence of about 100-150 amino acids and have eight cysteine residues that form four intramolecular disulfide bonds, stabilizing their tertiary structure and promoting surfactant and emulsifying activity (KULKARNI; NENE; JOSHI, 2017; DOKOUHAKI et al., 2021).

Hydrophobins are classified into two classes (I and II) according to their assembly characteristics at hydrophobic-hydrophilic interfaces, solubility and hydropathy. Class I hydrophobins form structures similar to amyloid fibrils, termed rodlet layers with a β-sheet conformation and are soluble only in strong acids, on the other hand, class II hydrophobins form regular crystalline structures with a random spiral conformation and can be easily dissolved with organic solvents or detergents (LINDER, 2009; BERGER; SALLADA, 2019). Some of these proteins may exhibit glycosylation (LINDER, 2009; DOKOUHAKI et al., 2021). Although some hydrophobins can form oligomers as a strategy to increase their solubility in solution, others remain monomeric even at high concentrations (VIGUERAS et al., 2014; SUNDE et al., 2017).

Class I hydrophobins were isolated from *Schizophyllum commune* (SC3) and class II hydrophobins from *Trichoderma reesei* (HFBI and HFBII) (LINDER, 2009; KULKARNI; NENE; JOSHI, 2017), as well as several ascomycetes and basidiomycetes secrete hydrophobins in their cell wall, which has an ecological role related to local environment modification (SUNDE et al., 2017; DOKOUHAKI et al., 2021). It is believed that these hydrophobins assist hyphae to grow from a liquid phase into air by covering the surface of aerial hyphae with a hydrophobic layer. Likewise, spore dispersal, adhesion, pathogenesis and breaking surface tension have been linked to hydrophobins (SUNDE et al., 2017; DOKOUHAKI et al., 2021). From a biotechnological perspective, due to their high surface activity and non-immunogenicity, hydrophobins have a versatility of applications as agents for solubilization and delivery of hydrophobic drugs, emulsifying agents for food, protein purification tags, tools for protein and cell immobilization, coatings for biomaterials, biosensors and, biomineralization templates (AIMANIANDA et al., 2009; BERGER; SALLADA, 2019; DOKOUHAKI et al., 2021). Although there is a growing potential to manipulate hydrophobin variants for specific applications by protein engineering and heterologous expression in host cells, the insertion of these molecules into commercial applications is still limited due to low productivity, additional steps for recovery and post-transcriptional modifications of the surfactant protein (BERGER; SALLADA, 2019; DOKOUHAKI et al., 2021).

3 FUNGI PRODUCING BIOSURFACTANTS: BIOPROSPECTING AND ISOLATION SOURCES

Fungi have ubiquitous habitat which can influence the production of different metabolites. The microbial ecology involved in the production of biosurfactants can indicate

possible sites of isolation and/or application of the molecules obtained from these organisms. Several theories were proposed to explain the evolution behind microbial biosurfactant production. The first involves emulsification and solubilization of hydrophobic substrates to create a microenvironment favourable to the growth and production of the organisms (VAN HAMME; SINGH; WARD, 2006). The second, involves the adhesion-release of the cell to surfaces: when associated with the walls, they can regulate the properties of the cell surface, increasing the permeability of the membrane, for example. In this way, they use the property of active surface to detach themselves from unfavourable environments or in the search for new habitats with greater availability of nutrients (CAMEOTRA et al., 2010). The third, presenting antibiotic activity as a defence mechanism against possible competitors for substrates (VAN BOGAERT et al., 2007). Fourthly, as an energy reserve, since they can enter the catabolic process as a possible source of energy during periods of nutrient limitation (MIMEE et al., 2009; SILVA et al., 2021a) and finally as protection for the microorganism against environments with high osmotic pressure, ionic imbalances and as protection against freeze/thaw cycles (SELBMANN et al., 2002).

The isolation of fungi dependents on accessing environments where biosurfactant-producing strains are thriving. Although cultivable microorganisms represent less than 1% of the diversity of known microbial species (ARAÚJO et al., 2020), the bioprospecting of fungal strains by culture-dependent technique has enabled the discovery of new biosurfactant-producing strains (SILVA et al., 2021a) as well as novel biosurfactant structures (KIM; LEE; YUN, 2015). Biosurfactant-producing fungi were isolated from hydrocarbon/petroleum contaminated soil samples (YALÇIN; ERGIN-TEPEBAŞI; UYAR, 2018), plant structures such as roots (ELDIN; KAMEL; HOSSAM, 2019), stems (SILVA et al., 2021a), leaves (SILVA et al., 2021a), cones (ADNAN et al., 2018), flowers (KIM; LEE; YUN, 2015), fruits (DERGUINE-MECHERI et al., 2018); and animals such as porifers (KIRAN et al., 2009) and arthropods (MOUSAVI; BEHESHTI-MAAL; MASSAH, 2015).

On the other hand, the search for biosurfactant-producing fungi can also be carried out through approaches that exploit extreme environments (BUENO et al., 2019) and cultivation-independent techniques (metagenomics), as total environmental DNA can be examined at sequence level or be function-oriented (JACKSON et al., 2015; ARAÚJO et al., 2020). Functional metagenomics identifies genes or pathways in an environmental clone library by their functional activity (in this case, produce biosurfactants), providing an advantage over sequence-based screening, principally due to the fact that no prior knowledge of the gene sequence for target activity is required (WILLIAMS; TRINDADE, 2017). Likewise,

metagenomics coupled with next-generation sequencing has enabled wider access to the diversity and function of many microorganisms in nature (KODZIUS; GOJOBORI, 2015). In addition, genetic information from non-culturable micro-organisms can be exploited for enhanced biosurfactant development (ARAÚJO et al., 2020). However, there are few published studies that employed metagenomics to prospect for biosurfactant-producing microorganisms (THIES et al., 2016). The main limitation for metagenomic biosurfactant discovery may be due to the complex regulatory mechanisms involved in its production (JACKSON et al., 2015).

3.1 BIOSURFACTANTS IN COLD ENVIRONMENTS

Although extremophilic microorganisms are potential producers of biosurfactants, as they can excrete them for their colonization in adverse environment, biotechnological production from these microorganisms is virtually unexplored (SCHULTZ; ROSADO, 2020). The bioprospecting of cold-adapted microorganisms has promoted the discovery of previously undescribed bacteria for biosurfactants production (TRUDGEON et al., 2020), however, studies focused on fungal strains remain scarce.

Filamentous fungi isolated from Antarctic soils such as *Thelebolus* sp. (MUKHOPADHYAY et al., 2014) and *Phoma herbarum* CCFEE 5080 produced EPS at 1.94 and 13.6 gL^{-1}, respectively, that may act as necessary protection to microclimatic conditions, characterized by low temperatures, high thermal fluctuations and repeated freeze-thaw cycles (SELBMANN et al., 2002). *Mortierella* sp., isolated from arctic soils contaminated by hydrocarbons are suspected to produce biosurfactants (HUGHES; BRIDGE; CLARK, 2007), since new biomolecules (Malpininas; Malpibaldinas) with emulsifying properties were produced by zygomycetes such as *Mortierella alpina* ATCC32222 and DH187 (BALDEWEG et al., 2019). In addition, extremophilic yeast *Moesziomyces antarcticus* (previously classified as *Candida antarctica*) produced biosurfactants such as mannosylerythritol lipids (PERFUMO et al., 2018) and diacylmannosylerythritol (KITAMOTO et al., 2001), the latter interfered with ice paste formation as they have an anti-agglomeration effect and a high ice packing factor (TRUDGEON et al., 2020). Among 68 yeasts isolated from Antarctic soils, only 11 produced biosurfactants, with *Candida glaebosa* as the most prominent (BUENO et al., 2019). Recently, other yeasts isolated from Antarctic marine and terrestrial environments such as *Meyerozyma guilliermondii* L21, *Cryptococcus victoriae* L92 and *Leucosporidium scotti* L120 have been reported as biosurfactant producers (CORREA et al., 2020).

Research on biosurfactants produced by microorganisms in cold environments establishes a turning point for bioprocess work carried out at low temperatures, without the need for heating, and action on surfaces with low temperatures (CHEN; JIANG, 2018; PERFUMO et al., 2018). Cold-adapted microorganisms are able to produce biosurfactants at low Krafft temperature (Figure 6-g), i.e., it allows their solubilization, micelle formation and application even at low temperatures (LU et al., 2018). Thus, these biosurfactants could be used as ingredients in antifreeze additives, cold detergents and to improve flow properties at freezing temperatures in biodiesel production (PERFUMO et al., 2018). However, the difficulty to mimic extreme conditions *in vitro* that allows the synthesis of the bioproduct is still a challenge to be overcome (SCHULTZ; ROSADO, 2020).

4 SCREENING OF BIOSURFACTANT PRODUCING FUNGI: METHODOLOGIES AND CHARACTERIZATION

The presence of biosurfactants in culture media can be assessed by a various of qualitative and/or quantitative methods that have different accuracy (TWIGG et al., 2021). Generally, the screening tests depend on the analysis of microbial metabolites produced by fungal strain during or after cultivation (PESSÔA et al., 2019). These tests are based on direct measurement of surface tension, indirect methods of surface activity, emulsification index and/or cellular hydrophobicity (WALTER; SYLDATK; HAUSMANN, 2010). The use of single techniques for screening can exclude strains that other techniques may have been able to detect (TWIGG et al., 2021). The desired strains are those that produce biosurfactants with the lowest CMC values and with highest surface tension reduction or emulsification activity. In this regard, the screening tests must be developed and established considering the addition of inducing substrates under different processing conditions (PESSÔA et al., 2019). Methodologies are constantly being improved to screen high numbers of isolates and correlate the findings with available data (KIRAN; THOMAS; SELVIN, 2010; OJHA et al., 2019), as well as, for screening clones from metagenomic libraries (THIES et al., 2016). Molecular techniques could be applied to screen strains with single gene clusters and islands of inherent production capacity, which would also facilitate the process and identification of similar gene sequences in other strains (ONWOSI et al., 2021).

After selecting the producing strain, the fungal strain must be essentially identified from their genetic sequencing through the region of the Internal Transcribed Spacer (ITS) (YALÇIN; ERGIN-TEPEBAŞI; UYAR, 2018; TWIGG et al., 2021). The results should be

compared with similar strains of the type of different species within the genus through multiple sequence alignment, ensuring unambiguous identification of the inoculum (TWIGG et al., 2021).

4.1 SURFACE TENSION MEASUREMENT

In the last twenty years, measuring the surface/interfacial tension of culture supernatants and/or filtrates has been the main semi-quantitative parameter used to confirm the presence of biosurfactants (MERCADÉ et al., 1996; ZADEH; MOGHIMI; HAMEDI, 2018). This methodology is sensitive, since the reduction in surface/interfacial tension is quantified until reaching the CMC, due to the saturation of this reduction, i.e., the numerical value obtained in the analysis only indicates if the microbial culture is producing biosurfactants or not. Therefore, it cannot be considered a quantitative parameter (MARCHANT; BANAT, 2012b). The positive criteria for biosurfactants production is the reduction of the surface tension to less than or equal to 35 mNm^{-1} (MULLIGAN, 2005).

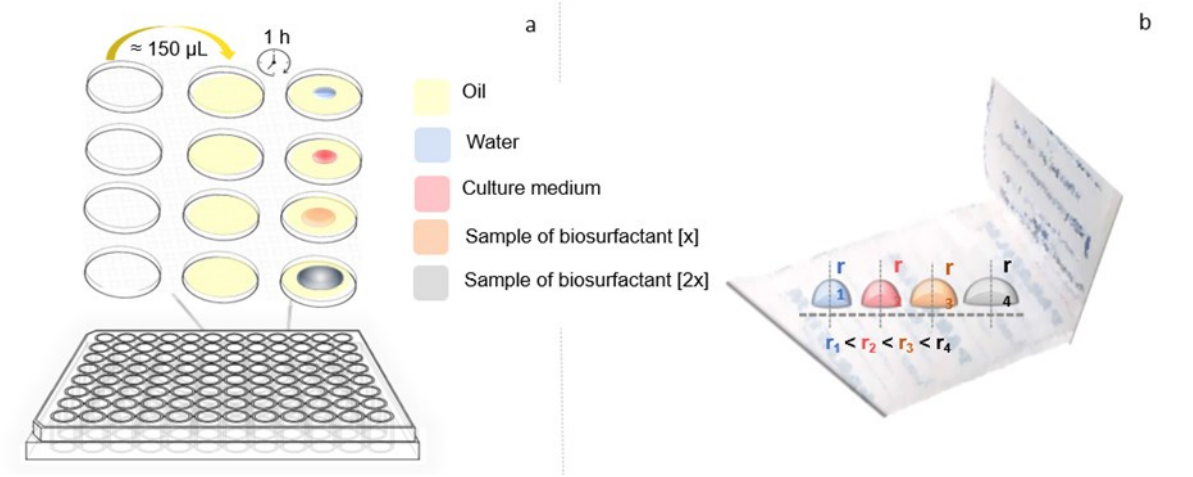
The surface or interfacial tension can be measured by several methods (du Nouy Ring, Wilhelmy Plate, Spinning Drop, Pendent Drop), with the du Nouy Ring method being the most used. This determination is made by a tensiometer and/or goniometer (DU NOÜY, 1925; EBNESAJJAD, 2011). The main advantage of this method is accuracy and the absence of false-negative results (BODOUR; MILLER-MAIER, 1998; ONG; WU, 2018). This technique requires specialized equipment and the analysis can be time-consuming, since simultaneous measurements are not possible and it requires an interval between each sample preparation (washing, wire buckling/platinum plate) (YOUSSEF et al., 2004; WALTER; SYLDATK; HAUSMANN, 2010).

4.2 DROP COLLAPSE TEST

The drop collapse test is based on the collapse of a drop when in contact with a hydrophobic surface. There is a directly proportional correlation between the drop diameter and the surfactant concentration, also indicating a semi-quantitative parameter for the presence of biosurfactants (JAIN et al., 1991; BODOUR; MILLER-MAIER, 1998). This assay can be carried out in 96-well microplates, which indicates high-throughput screening (Figure 8-a) (MOHANRAM; JAGTAP; KUMAR, 2016; WOŹNIAK-KARCZEWSKA et al., 2017). An adaptation of this method is the use of an hydrophobic surface of the commercial product

parafilm-M™ (Parafilm-M Test) to facilitate visualization (Figure 8-b) (KUIPER et al., 2004). Some authors have used methylene blue to stain samples to improve the visualization, as it does not influence in the drops shape (KUIPER et al., 2004; QIAO; SHAO, 2010).

Figure 8 – Drop collapse test/Parafilm-M



Caption: Collapse drop test occurs with the addition of oil and samples (3:1 v/v) to the wells of the microplate. First, oil is added to form a hydrophobic surface. Afterwards, drops of samples containing biosurfactants will collapse on contact with this surface as shown in a, and Parafilm-M test in b, where rn=1,2,3,4 = drop radius and n = analyzed sample.
Source: Elaborated by the author (2021).

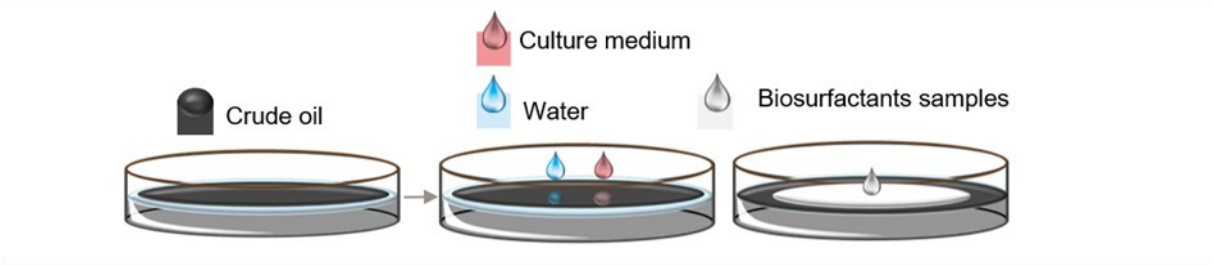
The drop collapse/parafilm-M test is accurate in distinguishing between producing and non-producing strains, with few false-negatives. This test is a quick and simple test that does not require specialized equipment and can be performed with a small volume of sample (YOUSSEF et al., 2004; ADNAN et al., 2018).

4.3 OIL SPREADING

The oil spreading test is based on the principle of decreasing the interfacial/superficial tension between the water and oil phases (YOUSSEF et al., 2004; ARCHANA et al., 2019). If a biosurfactant is present, there will be a destabilization in the intermolecular forces of the water/oil interface with the active surface molecule, displacing the volume of the oil on the surface (Figure 9) (YOUSSEF et al., 2004). As the repulsion between the oil and water molecules is reduced, the oil is displaced in proportion to the biosurfactant concentration. Thus, a semi-quantitative parameter is provided for the selection of strains with the greatest production potential (YELA et al., 2016). This methodology is considered sensitive, reliable,

fast, and easy to perform. However, large volumes of waste are generated (KIRAN et al., 2009; SILVA et al., 2014; DERGUINE-MECHERI et al., 2018).

Figure 9 – Oil spreading test

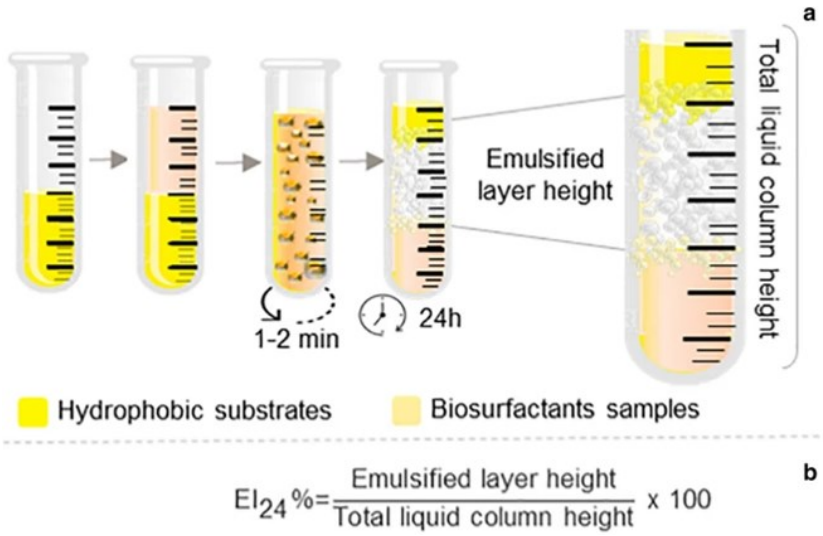


Caption: The oil spread test occurs with the addition of water and a smaller volume of oil to form a thin hydrophobic layer. Then, the test sample is added over the oil layer and provides the displacement of the area on the oil surface.
Source: Elaborated by the author (2021).

4.4 EMULSIFICATION INDEX

The emulsification activity was initially proposed through spectrophotometric analysis that require sophisticated equipment (CIRIGLIANO; CARMAN, 1984; MOHANRAM; JAGTAP; KUMAR, 2016). However, the Emulsification Index (EI24) is a simpler, effective and commonly used method that consists of measuring the ratio between the emulsified volume and the total volume of the analysed sample (Figure 10) (COOPER; GOLDENBERG, 1987). Both methodologies are widely used (VELIOGLU; UREK, 2016; SHAH et al., 2017). The EI24 is easy to perform and is considered as an appropriate indicator for strain screening, but a large volume of waste is produced (UZOIGWE et al., 2015).

Figure 10 – Emulsification index



Caption: EI_{24} test: addition of equal volumes (1:1) of hydrophobic substrates and samples containing biosurfactants in graduated tubes. Subsequently, the mixture is stirred at high speed, being maintained in the absence of disturbance for phase separation and stability of the formed emulsion (a). The emulsification percentage value can be measured according to b.
Source: Elaborated by the author (2021).

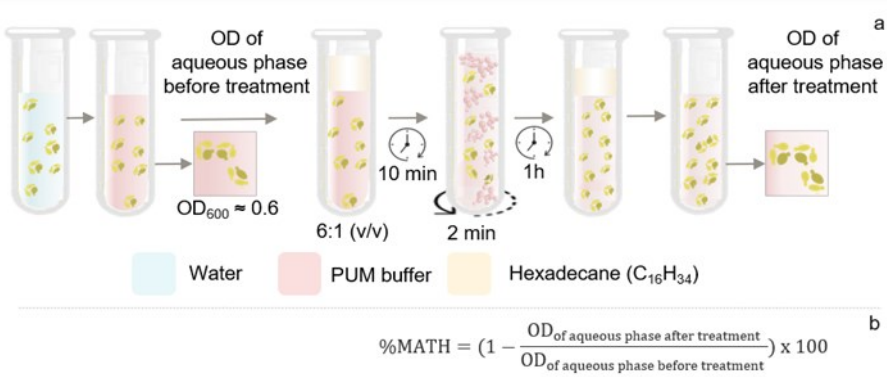
The EI_{24} shows the molecule capacity in forming stable emulsions between different immiscible phases i.e., this index acts independently of the effect of surface tension of the medium to prevent drop coalescence (HABA et al., 2000). Biosurfactants contained in the samples, upon contact with hydrophobic substrates, will reach an equilibrium phase between oil/water/biosurfactant system and promote solubilization to form emulsions (SATPUTE et al., 2010; YELA et al., 2016). This functional property has been used to assess the biosurfactant ability to form and stabilize emulsions on different hydrophobic substrates, organic solvents and environmental conditions (BANAT et al., 2010; PELE et al., 2019; PESSÔA et al., 2019). The emulsions stability is influenced by the compaction and size of the globules. Emulsions with low compaction and large globules generally show reduced stability (DESAI; BANAT, 1997). An important characteristic in the emulsion stabilization is the adsorption of the emulsifier at the liquid-liquid interface (SÁNCHEZ-VÁZQUEZ et al., 2018).

The ability of a molecule to form stable emulsions is not always associated with the ability to reduce surface tension. It is suggested that strains producing biosurfactants are also producers of bioemulsifiers, but not the other way around (YOUSSEF et al., 2004; VARJANI; UPASANI, 2017). Therefore, the emulsification index provides a perspective on the production of bioemulsifiers by the selected isolates.

4.5 CELL ADHESION TO HYDROCARBON HYDROPHOBICITY ANALYSIS

The Microbial Adhesion to Hydrocarbon (MATH) test is based on the cell surface hydrophobicity (ROSENBERG; GUTNICK; ROSENBERG, 1980). There is a direct correlation between the cell surface hydrophobicity and its adhesion in hydrocarbon droplets, which favours emulsification and assimilation of hydrophobic substrates (Figure 11) (EL-HANAFY et al., 2017; AL-HAWASH et al., 2018c). This test measures the partition of cells between the aqueous and hydrophobic phases. The fungus surface is considered hydrophobic when more than 50% of the cells migrate from the buffer (PUM - phosphate, urea, magnesium) to the hexadecane. On the other hand, it is considered hydrophilic when more than 50% of the cells remain in the buffer (KAUR et al., 2017).

Figure 11 – Cell adhesion to hydrocarbon hydrophobicity analysis



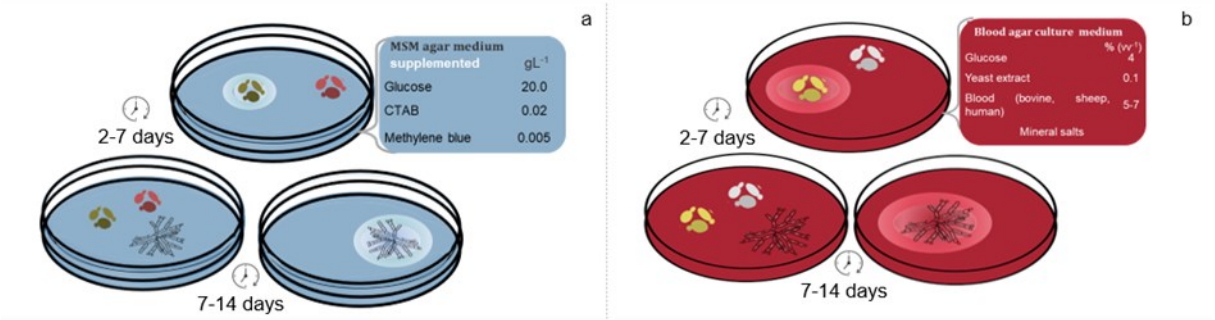
Caption: The microbial adhesion to hydrocarbon test is initiated by washing fungal cells to remove contaminants, followed by a resuspension in buffer solution (PUM) until reaching a standardized optical density (DO). Thereafter, the hydrocarbon is added, and the mixture is stirred. After complete separation of phases, the organic phase is removed, and the optical density is measured again (a). The percentage value of microbial cell adhesion to hydrocarbons is obtained as in b.
Source: Elaborated by the author (2021).

Few reports were found in literature for superficial hydrophobicity of filamentous fungi (AL-HAWASH et al., 2019). Therefore, it is necessary to search for new effective methodologies to improve on existing measurements of cellular hydrophobicity of filamentous fungi which is affected by differentiated mycelial growth, cultivation conditions and the physiological state of strains (KULKARNI; NENE; JOSHI, 2017). Sampling for the MATH may cause hyphae fragmentation, phase separation, possibly changes in hydrophobicity affecting standardization and resulting in incorrect measurements (SMITS et al., 2003; CHAU et al., 2009).

4.6 METHYLENE CTAB-BLUE AGAR PLATE METHOD AND BLOOD HAEMOLYSIS

Methylene Blue Agar-CTAB method is selective for extracellular anionic biosurfactants, since halos formed in the culture are the result of insoluble salts produced between the anionic biosurfactant and the cationic detergent Cetyltrimethylammonium Bromide (CTAB) (SIEGMUND; WAGNER, 1991). This test is qualitative, considered a primary screening study, easy to perform and to view responses (Figure 12-a) (MNIF; GHRIBI, 2015; ADNAN et al., 2018; SPINA et al., 2018; ELDIN; KAMEL; HOSSAM, 2019).

Figure 12 – Methylene CTAB-Blue agar plate method and blood haemolysis



Caption: Methylene blue-CTAB agar test (a) and blood hemolysis test (b). Biosurfactant-producing strains produce a halo when grown in a solid medium of defined composition. The cultivation period for obtaining the results varies according to culture strain.
Source: Elaborated by the author (2021).

The CTAB assay is widely reported in the literature with consistent results for screening bacteria and yeasts. However, the CTAB method presents limitations for filamentous fungi, as it may underestimate positive results due to the spread of mycelial growth, production of metabolites involved in the dye degradation and dye absorption (KABBOUT; TAHA, 2014; SPINA et al., 2018).

The blood haemolysis test was developed for the selection of bacteria producing biosurfactants (MULLIGAN; COOPER, 1984). However, it has recently been used to prospect for filamentous fungi and yeasts producing biosurfactants (Figure 12-b) (ELDIN; KAMEL; HOSSAM, 2019; MARCELINO et al., 2019; FERREIRA et al., 2020). The haemolysis results from the lysis of erythrocytes due to disintegration of cell membranes due to activities of some surfactant (DEHGHAN-NOUDEH; HOUSAINDOKHT; BAZZAZ, 2005; MARCELINO et al., 2019).

The limitations of the blood haemolysis test are related to difficulties in maintaining the culture conditions in the blood agar media that would favour the production of

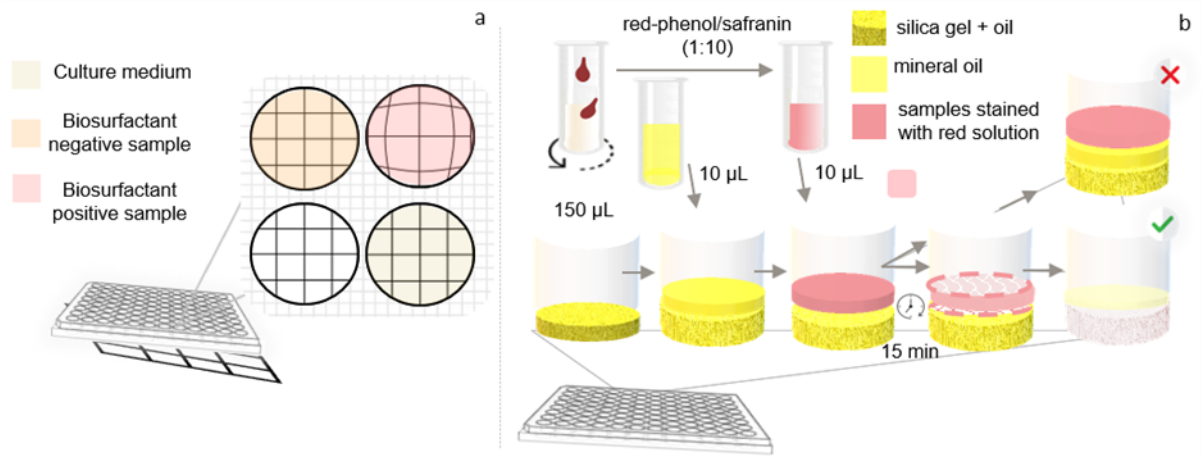
biosurfactants in addition to possible interference of microbial haemolysins with the results (MOUSAVI; BEHESHTI-MAAL; MASSAH, 2015). Low biosurfactant concentration produced can also present weak diffusion in the solid medium and favour the non-formation of halos (WALTER; SYLDATK; HAUSMANN, 2010). Some results of blood haemolysis tests for fungi and yeasts have been shown to be false-negatives, as they have shown negative results for haemolysis while other positive results are obtained for different tests of surface activity and/or emulsification indexes (MOUSAVI; BEHESHTI-MAAL; MASSAH, 2015; GARG; PRIYANKA; CHATTERJEE, 2018; ELDIN; KAMEL; HOSSAM, 2019). Therefore, due to the variability of the results, other screening tests must be associated with blood haemolysis to corroborate biosurfactant production.

4.7 HIGH-THROUGHPUT SCREENING, MICROPLATE AND PENETRATION ASSAYS

The application of two or more methods for preliminary confirmation of biosurfactant producer strains takes longer time, as does the interval between inoculation and production of biosurfactants. High-throughput screening involves the development and execution of rapid methods applied to a large number of isolates. Currently, there are only few screening procedures for biosurfactant detection considered as "high yielding". Microplate and penetration assays are carried out in 96-well microplates, which increases the number of tested strains and require lower sample volumes (WALTER; SYLDATK; HAUSMANN, 2010; MOHANRAM; JAGTAP; KUMAR, 2016; RIZZO et al., 2018).

The samples analysed by microplate test are visually inspected. The test provides a qualitative result for biosurfactants presence due to the optical distortion promoted instantly in the image. If there are biosurfactants in its composition, the image formed on the microplate is modified (Figure 13-a) (CHEN; BAKER; DARTON, 2007; MNIF; GHRIBI, 2015). On the other hand, the penetration test is colorimetric and involves two insoluble phases. The direct contact between these phases changes the analysed sample’s colour in the presence of biosurfactants, because it allows for the interaction between phases (Figure 13-b) (WALTER; SYLDATK; HAUSMANN, 2010).

Figure 13 – High-throughput screening, microplate and penetration assays



Caption: Microplate assay is initiated by placing a grid paper under a microplate. The culture medium/water in a microplate well is considered to have a flat surface. A sample with biosurfactant will have a change at the well edge and the fluid surface will become concave, and consequently distorts the image (a). The penetration test occurs by adding a hydrophobic paste to the wells of the microplate, which is covered with mineral oil. Then, the sample is stained with red solution and added to the hydrophobic surface. The presence of biosurfactants is detected through its influence on the rupture of the barrier (interface) of the oil layer in the paste, which favors the entry of silica into the hydrophilic phase, and the upper phase changes the color from light red to cloudy white (b).
Source: Elaborated by the author (2021).

5 NEW PRODUCTION TECHNOLOGIES

Biosurfactants are considered extracellular or cell membrane-associated secondary metabolites and have an ecological role similar to antibiotics and pigments that interact in membrane regulation (ZAKY et al., 2014; MOUSAVI; BEHESHTI-MAAL; MASSAH, 2015; JADHAV; PRATAP; KALE, 2019). However, some authors report that biosurfactant biosynthesis is associated with microbial growth when hydrophobic substrates are used, which indicates that these primary metabolites may also facilitate the absorption of substrates for fungi development (AL-HAWASH et al., 2018c; YALÇIN; ERGIN-TEPEBAŞI; UYAR, 2018; BALAN; GANESH KUMAR; JAYALAKSHMI, 2019). Fungi have a more rigid and complex cell wall composition, i.e., they are more resistant to high concentrations of biosurfactants during cultivation, while bacteria have more sensitive cell structures (MORITA et al., 2007; MARCELINO et al., 2019).

In most cases, fungal biosurfactants are produced by submerged cultures conducted in shaking flasks and/or bioreactors (ADAMCZAK; BEDNARSKI, 2000; COLLA et al., 2010). Solid state production is also promising (BANAT et al., 2021) A recent study showed 39% of published research articles on biosurfactant production through solid cultivation are fungal, among which SL represent 14% and hydrophobins 2% of the types produced (BANAT et al.,

2021). Solid state cultivation promoted the production of hydrophobins by *Purpureocillium lilacinum* (34.8 mNm^{-1} and 1.3 mg of protein g^{-1}), which did not occur in submerged culture (VIGUERAS et al., 2014). Moreover, a 107% increase in yield was achieved in solid culture with complex substrate (sesame oil cake) compared to submerged culture using YMG medium for production of hydrophobins from *Pleurotus ostreatus* (KULKARNI; NENE; JOSHI, 2020a).

Most studies on cultivation in solid medium for biosurfactant production are conducted in complex residues (agro-industrial) (KULKARNI; NENE; JOSHI, 2020a), which can make extraction difficult due to the greater diversity of interaction (product-medium and medium-solvent), maintaining residual impurities in the recovered extract (BANAT et al., 2021). In addition, solid-state cultivation scale presents challenges to be overcome on an industrial scale, such as the design of bioreactors that allow automated control and monitoring of parameters of the cultivation process (KRIEGER; CAMILIOS NETO; MITCHELL, 2010; JIMÉNEZ-PEÑALVER et al., 2016), as well as minimizing heterogeneity in terms of heat and mass transfers (BANAT et al., 2021). However, solid state cultivations avoid possible problems associated with foaming or high viscosity commonly reported in submerged cultures (SINGH; PATIL; RALE, 2019).

6 NUTRITIONAL PARAMETERS AND MULTIPLE METABOLITES

In general, culture medium is mainly composed of a carbon and nitrogen source plus macro and micronutrients (JIMOH; LIN, 2019), as well as, its price corresponds to about 10 to 30% out of total cost of the product (BANAT et al., 2014). Thus, culture medium composition may be considered the main cost reduction strategy in a bioprocess (JIMOH; LIN, 2019). Likewise, the cultivation systems with multiple metabolite production have been reported as an effective approach to improve process profitability due to the use of more efficient types of bioreactors and substrates (GUERFALI et al., 2019; WANG et al., 2019a).

Simultaneous production of biosurfactants and other bioproducts, using an integrated platform concept, can also be an economic strategy (GUERFALI et al., 2019). Garay et al. (2017b) reported the production of PEFA and intracellular triacylglycerols (TG) by *Rhodotorula paludigena* (20.9 gL^{-1} PEFA and 8.8 gL^{-1} TG) and *Rhodotorula babjevae* (11.2 gL^{-1} PEFA and 18.5 gL^{-1} TG). In optimized culture conditions, *R. paludigena* produced the highest yield of PEFA (48.5 gL^{-1}) and intracellular lipids (16.9 gL^{-1}) ever reported. The lipid sources produced are substrates for the biofuel production industry (WANG et al., 2019a).

In other cases, biosurfactant production is also associated with the excretion of hydrolytic enzymes such as lipases, proteases and amylases (MARTINHO et al., 2019; KRELING et al., 2020b). The fungus *Pleurotus sajor-caju* simultaneously produces biosurfactants (4.01 gL^{-1}) and ligninolytic enzymes (laccase, manganese peroxidase and lignin peroxidase) in solid state cultivation with sunflower seed husks as substrate (VELIOGLU; UREK, 2014). In addition, *P. antarctica* PYCC 5048 produces MEL and xylanolitic enzymes from the direct conversion of xylan in a single step, without the need for enzymatic supplementation (FARIA et al., 2015).

An unidentified yeast isolated from fresh rhizosphere samples of healthy planted crops have lipolytic activity and produce biosurfactants when grown on hydrophobic substrates (ELDIN; KAMEL; HOSSAM, 2019). Lipases and biosurfactants may act in synergism to favour the bioavailability of these substrates from the hydrolysis of complex biopolymers for cell growth (PERFUMO et al., 2018). If additional steps are necessary for the recovery of the produced co-products, harvesting two products (lipases and biosurfactants) in a single bioprocess can be a promising strategy to improve overall process profitability.

6.1 CARBON SOURCES

The carbon sources initially provided and utilized in the culture medium interfere in different metabolic pathways for biosurfactant production (JIMOH et al., 2021). The combination of energy sources, such as carbohydrates and lipids in culture medium, has been commonly reported (SOUZA et al., 2018a; YU et al., 2021). In this context, several sources of carbon can be used in culture media such as glucose, sucrose (BRUMANO et al., 2017), xylose (FARIA et al., 2015), inulin (WANG et al., 2019a), cassava wastewater (ANDRADE et al., 2017), cane molasses (ALMEIDA et al., 2017), soy molasses (SOLAIMAN et al., 2004), glycerol (SILVA et al., 2020) and hydrolyzates from restaurant food waste (KAUR et al., 2019). Additionally, lipid sources, such as refined vegetable oils and residual food frying oils (MADDIKERI; GOGATE; PANDIT, 2015; FERNANDES et al., 2020; FERREIRA et al., 2020; YU et al., 2021), wastewater from the oil industry (SILVA et al., 2014; SOUZA et al., 2017), motor oil (YALÇIN; ERGIN-TEPEBAŞI; UYAR, 2018) and animal fat (SANTOS et al., 2014; RADHA et al., 2020), have also been used. In addition, the carbon source supply directly influenced the chemical composition and surfactant properties of sophorolipids produced when *S. bombicola* was grown in biodiesel or rapeseed oil, with biodiesel a new esterified

sophorolipid structure was produced with lower CMC value and surface tension (KIM et al., 2020).

In general, non-residual and non-traditional substrates showed low productivity values for sophorolipid production by *S. bombicola* even when supplemented with glucose. As examples, petroselinic acid (positional isomer of oleic acid) (0.24 $gL^{-1}h^{-1}$) (DELBEKE et al., 2016); horse oil (composed of linoleic, palmitoleic fatty acids and unsaturated) (0.40 $gL^{-1}h^{-1}$) (MAENG et al., 2018); castor oil (*Ricinus*) (0.20 $gL^{-1}h^{-1}$) (BAJAJ; ANNAPURE, 2015); jatropha oil (*Jatropha curcas*) (0.03 $gL^{-1}h^{-1}$), karanja oil (*Pongamia*) (0.03 $gL^{-1}h^{-1}$) and neem oil (*Azadirachta*) (0.01 $gL^{-1}h^{-1}$) (WADEKAR et al., 2012). This is probably due to the composition of fatty acids and also the presence of inhibitory compounds in these substrates (WADEKAR et al., 2012).

Lignocellulosic hydrolyzates have been used to produce MEL and sophorolipids, although the presence of sugar dehydration, such as furfural and hydroxyl-methyl furfural had an inhibitory effect on the production (SANTOS et al., 2018; MARCELINO et al., 2019). The hydrolyzates detoxification processes can partially remove those inhibitors (MARCELINO et al., 2019; YU et al., 2021), since the yield of sophorolipids was doubled when hydrolysate from detoxified corn straw and oleic acid were used as carbon sources for *S. bombicola* (YU et al., 2021).

A variety of agro-industrial by-products are reported for the production of biosurfactants through solid-state cultivation, such as oil cakes from the oil refining industry with the addition of beet molasses (JIMÉNEZ-PEÑALVER et al., 2016), soybean bran, husk, rice straw and bran (COLLA et al., 2010; CASTIGLIONI et al., 2014), wheat bran, soybean seeds, peanuts (PAREKH; PANDIT, 2012), grape residues and potato skins (VELIOGLU; UREK, 2015). In this context, substrates for solid-state cultivation, as digestate from biowaste supplemented with glucose and fats (0.02 g g^{-1}) and stearic acid (0.211 g g^{-1}) showed low yield for sophorolipids production (JIMÉNEZ-PEÑALVER et al., 2018; CERDA et al., 2019).

6.2 NITROGEN SOURCES

Different sources of inorganic and organic nitrogen can influence biosurfactants synthesis. The corn-steep liquor (CAS 66071-94-1), yeast extract and urea are organic sources, as well as nitrate and ammonium salts are inorganic sources commonly used (SOUZA et al., 2018b; LIU et al., 2019) Normally, during cell growth excess carbon and nitrogen sources is suggested, followed by depletion of the nitrogen source (DOLMAN et al., 2019; SAUR et al.,

2019). The C/N ratio can interfere with biosurfactant production, so it is recommended to maintain a high C/N ratio during cultivation, observing a range of 16:1 to 18:1 (DESAI; BANAT, 1997; JAHAN et al., 2020).

The physico-chemical properties of biosurfactant can be modified by providing different nitrogen sources. Inorganic nitrogen sources were more suitable than organic sources for the production of sophorolipids, this latter promoted preferably lactonic and low polarity sophorolipids (MA et al., 2011), as well as the substitution of peptone for tryptone in the culture medium reduced the emulsification index of the biosurfactants produced by Aspergillus flavus (ISHAQ et al., 2015).

6.3 TRACE ELEMENTS

Although natural by-products contain several nutrients due to their complex composition, the amounts of nutrients are suboptimal (ANDRADE et al., 2018). Supplementation with amino acids (BALDEWEG et al., 2019) and micronutrients such as magnesium, phosphorus, sodium, copper and manganese can meet these nutritional requirements and favour biosurfactant production (FAN et al., 2014; NIU et al., 2017). Optimizing the concentration of nutrients (phosphate salts, sulphates and chlorides) in the culture medium by using response surface methodology increased MEL production at 61% by *Ceriporia lacerate* (NIU et al., 2017). Culture media containing peptone, copper and manganese salts provided greater production of MEL-A homologues by *P. aphidis* ZJUDM34 compared to media without these microelements (FAN et al., 2014). Metallic ions can influence the selective production of different sophorolipids by *Wickerhamiella domercqiae*, since lactonic congeners were mainly produced in the presence of Mg^{2+} ions, while Fe^{2+} ions promoted the production of acidic sophorolipids, as well as Cu^{+2} ions favoured maximum productivity (0.89 $gL^{-1}h^{-1}$) (CHEN et al., 2014b). The synthesis of sophorolipids by *S. bombicola* was increased by approximately 17% due to the addition of Mg^{2+}, Mn^{2+}, Cu^{2+} and principally Fe^{+2} to a specific concentration, decreasing slowly with additional increase of ion concentration (YU et al., 2021).

7 STRATEGIES FOR INCREASING YIELD AND PRODUCTIVITY

During biosurfactant production, cell aging, depletion of nutrients or the accumulation of product and/or by-products can inhibit cell activity and reduce biosurfactant production rate (JIANG et al., 2020). Process yield, chemical structures and physico-chemical properties vary

according to substrates composition, producing-strains and cultivation system (batch, fed-batch, semi-continuous) (SILVA et al., 2014; ZHANG et al., 2018). In addition, changes in cultivation parameters and conditions such as volume of inoculum and absence of agitation resulted in product with different emulsifying properties, surface activity and yield by *Mucor hiemalis* (FERREIRA et al., 2020). Table 4 shows a survey of the main components of the culture medium, parameters process and the kinetic values related to fungal biosurfactant production in agitated flasks, bench-top and pilot scale bioreactors.

Table 4 – Survey of fungal biosurfactants production parameters found in the literature

Producer microorganism	Substrates (C/N)	Working volume (L)	Mixing (rpm)	Aeration (vvm)	pH/ Temperature (°C)	Volumetric productivity ($gL^{-1}h^{-1}$)	Maximum yield (gL^{-1})	Surface tension ($mN\ m^{-1}$)	Reference
Candida lipolytica	animal fat (5%) and corn-steep liquor (2.5%)	50.0	200	ND	5.3/28	0.30	40.00	25.00	Santos et al. (2017b)
Candida tropicalis UCP0996	frying oil (2.5%), corn liquor (2.5%) and cane molasses (2.5%)	25.0	200	1.0	5.5/28	0.06	7.36	27.48	Almeida et al. (2017)
Candida sphaerica	ground-nut oil refinery residue (9%) and corn-steep liquor (9%)	20.0	150	ND	5.3/27	0.15	21.00	27.00	Luna et al. (2015)
Candida antarctica	soybean oil (80 gL^{-1}) and yeast extract (1 gL^{-1})	2.0	200	1.0	ND	0.19	28.00	35.00	Adamczak; Bednarski (2000)
Starmerella bombicola	glucose (10%), sunflower acid oil (10%), yeast extract (4 gL^{-1}) and urea (1 gL^{-1})	2.0	550	1.0	3.5/30	0.27	51.50	35.50	Jadhav; Pratap; Kale (2019)
Starmerella bombicola	glucose (100 gL^{-1}) and corn-steep liquor (10 gL^{-1})	2.5	800	1.0	3.9/25	1.55	342.00	ND	Liu et al. (2019)
Pseudozyma tsukubaensis	cassava wastewater	3.0	100/150	0.4 and 0.8	ND	0.02	1.26	26.00	Andrade et al. (2017)
Rhodotorula paludigena	glucose (150 gL^{-1}) and yeast extract (1.5 gL^{-1})	4.0	ND	1.0	6.5/27	0.12	20.90	ND	Garay et al. (2017b)
Aureobasidium pullulans	sucrose (50 gL^{-1}), peptone (0.6 gL^{-1}) and yeast extract (0.4 gL^{-1})	0.7	300-1200	0.5	6.5-3.5/30	0.09	15.00	ND	Saur et al. (2019)
Cultivation of filamentous fungi in shake flasks									
Rhizopus arrhizus	crude glycerol (3%) and corn-steep liquor (5%)		150	ND	5.5/28	0.02	1.74	28.80	Pele et al. (2019)
Fusarium sp. BS-8	sucrose (40 gL^{-1}) and yeast extract (0.8 gL^{-1})		150	ND	7.0/30	0.01	2.43	ND	Qazi et al. (2014)
Mucor hiemalis	soybean oil waste (5%); sodium glutamate (1%)		150	ND	ND/28	0.08	7.73	32.00	Ferreira et al. (2020)
Aspergillus niger	banana stalk powder (5,75g), yeast extract (1 gL^{-1}) and peptone (3 gL^{-1})		ND	ND	7.0/35	0.03	5.50	ND	Asgher et al. (2020)
Cunninghamella echinulata	soybean oil waste (2%) and corn-steep liquor (8%)		150	ND	5.5/28	0.04	5.18	31.70	Souza et al. (2018b)

As a rule of thumb, 50 gL^{-1} is the minimum acceptable titre for any basic chemical produced and can be higher in many cases. Productivity below 2.0 $gL^{-1}h^{-1}$ is generally unmarketable due to the high capital costs (VAN DIEN, 2013). According to Table 4, only strains of *S. bombicola* show productivity and yield close to these titres, and the other strains under these conditions showed lower productivity. Filamentous fungi normally require long periods to reach maximum production and show lower yield compared to yeasts.

Studies showed that high biosurfactants yields can be achieved through fed-batch bioreactor cultivation with online variables control (dissolved oxygen concentration, pH and temperature, etc.) and also for variables available offline (concentration of specific medium components, substrate, etc.) (BAJAJ; ANNAPURE, 2015; OCHOA, 2019). Changes in flow rates according to the feeding favour productivity and final yield (OCHOA, 2019). Taking as an example the production of sophorolipids from *S. bombicola*, pulse feeding with biodiesel and glucose increased productivity to 1.55 $gL^{-1}h^{-1}$ compared to 0.40 $gL^{-1}h^{-1}$ when grown in shake flasks (KIM et al., 2020) and a productivity 1.45 $gL^{-1}h^{-1}$ was achieved by continuous feeding of hydrophobic substrate and with intermittent pulses of glucose (LIU et al., 2019). In addition, an increase in yield (14%) was achieved by propagating under fed batch conditions while exposing to ultrasonic waves, which is due to the phonophoretic effect that increases the permeability of the cell membrane and favours the absorption and conversion of nutrients (MADDIKERI; GOGATE; PANDIT, 2015). Higher productivity (1.59 $gL^{-1}h^{-1}$) was achieved through semi-continuous cultivation with integrated two-stage separation processes compared to fed batch production (1.25 $gL^{-1}h^{-1}$) (ZHANG et al., 2018).

The inoculum is typically produced using pure cultures in media not necessarily the same as that used for production (STANBURY; WHITAKER; HALL, 2017a). Bioprocesses for filamentous fungi are usually started with fungal spores which develop into hyphae branches and hyphal extensions to form structures called “pellets” (POSCH; HERWIG; SPADIUT, 2013). The amount of inoculum supplied is about 3 to 10% of the working volume of the bioreactor, and it is expected that a larger volume can minimize the lag phase and produce a larger amount of biomass, which reduces the process time, consequently, the productivity of the bioreactor is increased (STANBURY; WHITAKER; HALL, 2017a). Pre-treatments applied during inoculum preparation, such as application of an electric field after the development of *Aspergillus brasiliensis* spores in an electrochemical bioreactor, allowed the increase in production of bioemulsifier by 19.5% (SÁNCHEZ-VÁZQUEZ et al., 2018). This was due to the fact that electric current promotes changes in the cell membrane and induces gene

expression associated with the hydrophobin proteins that facilitates substrate uptake (SÁNCHEZ-VÁZQUEZ et al., 2018).

The ideal pH reported for biosurfactant production is slightly acid, preferably between 5-7 (Table 4). In some bioprocesses, the pH of the medium strongly influences biosurfactant production and the transport of various components across the cell membrane (SAUR et al., 2019; BANAT et al., 2021). The pH should be kept stable to avoid cell lysis, however change in its value may trigger biosurfactant production, its value however is usually slightly reduced by the end of the cultivation (SANTOS et al., 2017b; SAUR et al., 2019). A strain of *A. pullulans* produced liamocins (15 gL^{-1}) only after decreasing the pH of the culture broth from 6.0 to 3.5 (SAUR et al., 2019). In addition, low pH prevents microbial contamination of the culture medium and favours its maintenance through longer fermentation times (VAN BOGAERT et al., 2007).

Biosurfactant production processes are typically aerobic, requiring high rates of aeration and mixing during submerged cultivation. To enhance oxygen transfer rate in sophorolipids production, a 2:1 height/diameter ratio of the bioreactors is recommended (BAJAJ; ANNAPURE, 2015; HALL, 2018). However, there are no studies related to fungal biosurfactant production with chemical engineering parameters, such as oxygen transfer coefficient (kLa). The effect of aeration rates has been less investigated as most studies are focused on the characterization and application of the molecules and not necessarily on the effects of aeration. Normally, dissolved oxygen is controlled between 25-50% saturation (ADAMCZAK; BEDNARSKI, 2000; LIU et al., 2019). High rates of aeration can increase the formation of foam, which hinders the recovery of the biosurfactant and decreases its yield (MARTI et al., 2014; BRUMANO et al., 2017).

The mixing of the culture medium ensures a more homogenized system in submerged cultures, its application in solid cultures using filamentous fungi is limited due to the high shear stress (BAJPAI, 2020). There are few reports related to the effect of mixing speed on biosurfactant production. Intermittent mixing of the solid medium cultivated with *S. bombicola* for SL production increased substrate bioavailability and process yield (0.235 g/g) (JIMÉNEZ-PEÑALVER et al., 2016), possibly through minimizing mass/heat transfer problems. In submerged cultures for biosurfactants production carried out in agitation flasks, the mixture is performed by orbital agitation. Stirring speeds between 150-800 rpm have been commonly reported for fungal biosurfactant production (SANTOS et al., 2014; DERGUINE-MECHERI et al., 2018; LIU et al., 2019), speeds of about 150 rpm are usual for filamentous strains to maintain cellular integrity and speeds above 200 rpm are easily achieved in cultures of agitated

bioreactors (ADNAN et al., 2018; PELE et al., 2019). High stirring speeds are required when there is an increase in viscosity concomitant with biosurfactant production, which limits oxygen transfer (LIU et al., 2019).

The optimum culture temperature for both yeast and filamentous fungi is typically within the mesophilic range of 25-30 °C (Table 4). This condition is advantageous for the industry since it does not increase costs related to the cooling stages of the culture medium (SANTOS et al., 2017b). However, the thermophilic fungi *Fusarium fujikuroi* produces trehalose lipids with lower surface tension (20.08 mNm^{-1}) at 47 °C (REIS et al., 2018) while psychotolerant yeasts isolated from cold environments produce biosurfactants at 20 °C (BUENO et al., 2019).

Moisture is an important parameter in solid-state cultivation, as it influences microbial growth rate on the substrates and determines the process yield. Fungal cultures require water activity around 0.5-0.6 (BANAT et al., 2021). The ideal value for moisture depends on parameters such as size, porosity and composition of the substrate, as well as on the characteristics of the strains. Values between 45% and 75% have been reported for SL production from *S. bombicola* (JIMÉNEZ-PEÑALVER et al., 2016; CERDA et al., 2019).

7.1 EXPERIMENTAL DESIGN AND RESPONSE SURFACE METHODOLOGY

Statistical experimental designs allow the verification of the influence of multiple variables, individually or in relation to each other, and reduce the number of assays carried out. This method can indicate the most significant variables of the process and subsequently allow for optimizations (ANTONY, 2014).

The individual study of each independent variables (pH, agitation, nutrients, inoculum size) that have an effect on biosurfactant production can be a first strategy for optimization (LEATHERS et al., 2018). Alternatively, when more than five independent variables are investigated, the Placket-Burman model can be used to assess variables that influence the system. This approach allows for optimizations in the fractional or complete experimental designs (STANBURY; WHITAKER; HALL, 2017b; LEATHERS et al., 2018). The use of fractional experimental designs for example showed that yeast extract (2 gL^{-1}) and wastewater from the olive oil industries [1.5% (w/w)] provided similar biosurfactant production by *Aureobasidium thailandense* as that of lauric acid ester (MENESES et al., 2017).

The literature states that different parameters and components of the culture medium, when optimized by statistical models (response surface methodology), result in 1.65 to 4 times

higher productivity compared to non-optimized media (GEETHA; BANAT; JOSHI, 2018). After optimizing the amount of substrate (waste cooking oil) and inoculum size through a central composite design for example, the yield of MEL by *P. aphidis* ZJUDM34 was 6-fold higher (61.50 gL^{-1}) compared to the non-optimized medium (10.25 gL^{-1}) (NIU et al., 2019).

7.2 GENETIC AND METABOLIC ENGINEERING, AN EMERGING ALTERNATIVE

An alternative to increase productivity and the development of new surfactants is through genetic improvements of strains (ABDEL-MAWGOUD; STEPHANOPOULOS, 2018; JIMOH; LIN, 2019). Genetic material can be modified by random mutation or by metabolic engineering (KODZIUS; GOJOBORI, 2015). Although it is conceivable that random mutation from techniques such as exposure to UV radiation, chemical components and base analogues has enabled the selection of mutant strains for biosurfactant production (JIMOH et al., 2021), only a single study was published on the use of mutagenic agent in fungi. It was reported that after exposed to the chemical mutagen (ethidium bromide) *Aspergillus niger* increased its yield of biosurfactant production by 43% (ASGHER et al., 2020).

Since 2014, the US 8,911,982 B2 patent granted the company Evonik the right to produce sophorolipids from genetically modified *S. bombicola* through the insertion and deletion of genes that overexpress enzymes involved in the synthesis of those compounds (SCHAFFER; WESSEL; THIESSENHUSEN, 2014). The majority of the production was directed to acidic sophorolipids (138 gL^{-1}) through the deletion of the lactone esterase gene (*Δsble*) in *S. bombicola*, restricting biosynthesis of the lactonic forms (BACCILE et al., 2017). Another example is mono-acylated and deacetylated MEL-D production by the deletion of an acyltransferase gene (*PtMAC2*) in *Pseudozyma tsukubaensis*. This gene recombination allowed the production of new mannosylerythritol lipid derivatives, maximizing their applicability in the industry (SAIKA et al., 2018b).

The production of new structural variants of biosurfactants expands their application. A good example is the Bolaform (sophorosides), a compound that consists of a long hydrophobic spacer with hydrophilic groups at both ends. These changes increase the molecule's solubility in water and the formation of micelles, thus improving the physico-chemical properties of the bioactive agent (SOETAERT; VAN BOGAERT; ROELANTS, 2013; VAN RENTERGHEM et al., 2018). The deletion of the acetyltransferase (*Δat*) and lactonase genes (*Δlac*) and the insertion of a second glycosyltransferase gene (*Δugtbl*) in *S. bombicola* promoted Bolaform product production (SOETAERT; VAN BOGAERT; ROELANTS, 2013).

In this context, the deletion of the putative alcohol oxidase gene fao1 from *S. bombicola* (*ΔatΔsbleΔfao1*) allowed the supply of fatty alcohols instead of fatty acids to the culture medium, favouring the production of Bolaform biosurfactant ($20gL^{-1}$) (VAN RENTERGHEM et al., 2018).

Although the heterologous production of fungal biosurfactants in bacteria is an interesting alternative, since it is easier and faster, the production of hydrophobins in bacterial host presents very low yields (10 to 100 mg L^{-1}) and require additional steps for inclusion body purification such as denaturation/renaturation to recover the biosurfactant (BERGER; SALLADA, 2019). The yield in heterologous production of hydrophobin (330 mgL^{-1}) in *Pichia pastoris* can be increased by 30-fold through an increase of 3 copies of the overexpressed gene chaperone protein Ero1p and a 10-fold increase with the overexpression of the gene KAR2 encoding the endoplasmic reticulum resident chaperone protein (SALLADA; HARKINS; BERGER, 2019). The expression of hydrolytic enzymes, such as lipases, in biosurfactants producer fungi is a strategy for petroleum derivatives consumption. A 1.7-fold increase in MEL-B yield in a recombinant strain of *P. tsukubaensi* was achieved after the insertion of the lipase gene *PaLIPAp* (*PaLIPA*) from *P. antarctica* T-34 (SAIKA et al., 2017). In addition, *Saccharomyces cerevisiae* were also used as an expression vector to produce rhamnolipids (a bacterial biosurfactants) through the insertion of genes from *Pseudomonas aeruginosa* (BAHIA et al., 2018) and sophorolipids from the expression of the glycosyltransferase (*gtf-1*) gene from *C. bombicola* (SOLAIMAN et al., 2014). This approach promoted glycosylation of lipids and increased the spectrum for use of substrates (including sterols) (SOLAIMAN et al., 2014).

Metabolic flow analysis between wild and mutant strains of *S. bombicola* showed that an inadequate supply of intracellular acetyl-CoA in the mutant strain, as well as citrate metabolism, can negatively affect sophorolipids biosynthesis, since it is related to fatty acid metabolism (YANG et al., 2019). However, the regulatory mechanisms that determine genomic analysis and attempts to induce the overproduction of biosurfactants can only be explored in a restricted way due to the wide structural variety of these molecules and biosynthesis pathways involved in production (JACKSON et al., 2015). The application of advanced tools in genomics, transcriptomics, proteomics and metabolomics to elucidate complete biosynthetic pathways and their regulation in biosurfactant producing strains, improves our understanding of the bottle necks in synthesis pathways of some metabolites, diverting most of the energy to the main metabolic pathways involved in the production of biosurfactant (ONWOSI et al., 2021).

8 BIOSURFACTANTS DOWNSTREAM PROCESSING

8.1 RECOVERY AND PURIFICATION

The structural diversity of biosurfactants and their unknown thermodynamic data present additional challenges in relation to their recovery. Different molecular characteristics, such as location, ionic charge and solubility, significantly influence their recovery and purification (WEBER et al., 2012; JIMOH; LIN, 2019). In general, downstream processing depends on biosurfactant itself and the purity required for application.

Biosurfactants associated with fungal cell membrane (or intracellular) require cell lysis or sonication to be released (GUSMÃO et al., 2010). Application of ultrasound in biosurfactant recovery produced by *Fusarium proliferatum* increased the yield by 30% before extraction with a solvent (BHARDWAJ; CAMEOTRA; CHOPRA, 2015). On the other hand, when biosurfactants are already excreted in the medium, the fungal biomass can be separated by filtration and/or centrifugation, which allows better conditions for recovery of the bioproduct (FERREIRA et al., 2020).

The recovery of extracellular biosurfactants includes acid precipitation with hydrochloric acid (MARCELINO et al., 2017) followed by solvent extraction (ethyl acetate, acetone, ethanol) to remove lipid contaminants or residual carbohydrate compounds (HUBERT et al., 2012; SOUZA et al., 2018b). The economic advantage of this process is the possibility to recover the solvent. An MEL recovery system (94% efficient) was developed by combining the following solvents: methanol/water/n-hexane (pH 2) (SHEN et al., 2019).

The separation processes with membranes such as microfiltration and ultrafiltration are used to recover biosurfactants (DHAR et al., 2021). Ultrafiltration processes with high molecular weight membranes (100 kDa) facilitate the recovery and increase biosurfactants purity, which can be extended with a cross-flow filtration unit. At concentrations above CMC, biosurfactants increase their molecular weight due to the property of self-aggregation, forming micelles that favours retention in the membrane (ANDRADE et al., 2017).

Several techniques, in particular foam fractionation, gravity separation and membrane separation, have been shown to be effective in the recovery of biosurfactants, since these techniques can become useful to avoid problems caused by product accumulation in the medium (DOLMAN et al., 2019). Furthermore, the integrated production and recovery of sophorolipids can be industrially promising, since it allowed the extension of the process time

(480h) with 93% efficiency in six separation cycles, reducing the probability of contamination and avoiding the addition of chemicals (WANG et al., 2020a).

The production and integrated gravitational separation of biosurfactants allow continuous recovery of sophorolipids (86%-280 g) of higher or lower density than the culture medium, while medium broth and cells are recirculated. This system provides reductions of up to 11% in the volume of the bioreactor (DOLMAN et al., 2017). An integrated system for the production and separation of sophorolipids by gravity in a bioreactor with double ventilation tubes and double sieve plates has been proposed (ZHANG et al., 2018). The two-stage recovery system improved the purity of the sophorolipids by 23.3%, allowing the recycling of substrates and yeast cells, minimizing inhibition processes and increasing substrate rate consumption (ZHANG et al., 2018).

Foam fractionation is another integrated technology that enable the continuous recovery of extracellular hydrophobins (70%) from the culture medium in a recirculation system, as a possibility to minimize the uncontrolled foaming in bioreactors, while recovering the enriched biosurfactant produced (WINTERBURN; RUSSELL; MARTIN, 2011). However, these approaches are largely inefficient and are not yet feasible for industrial applications. For example, foam fractionation is only suitable for the separation of biosurfactants at very low concentrations and the membrane separation is limited by incrustations on the membrane (CHEN; CHEN; JUANG, 2008; ANDRADE et al., 2017; SHEN et al., 2019).

Other techniques can also be applied such as adsorption chromatography (FERNANDES et al., 2020), lyophilization (BALAN; GANESH KUMAR; JAYALAKSHMI, 2019) and crystallization (YANG et al., 2012; WANG et al., 2020b) which provides greater purity and stability for storage of the molecule. It is important to note that downstream costs related to biosurfactant production can represent up to 80% out of total cost (NAJMI et al., 2018; JAUREGI; KOURMENTZA, 2019), but purification steps can be reduced depending on the purity required for biosurfactant application.

8.2 STRUCTURAL AND IONIC CHARACTERIZATION

Biosurfactants characterization is an important step in experimental studies, as it allows the discovery of new chemical structures and to analyse the influence of the substrate on its structure (BALAN; GANESH KUMAR; JAYALAKSHMI, 2019; KIM et al., 2020), as well as elucidating structures with accuracy which helps determining potential applications.

Only higher resolution techniques, such as spectroscopy and spectrometry, can correctly determine the structures of various biosurfactant congeners.

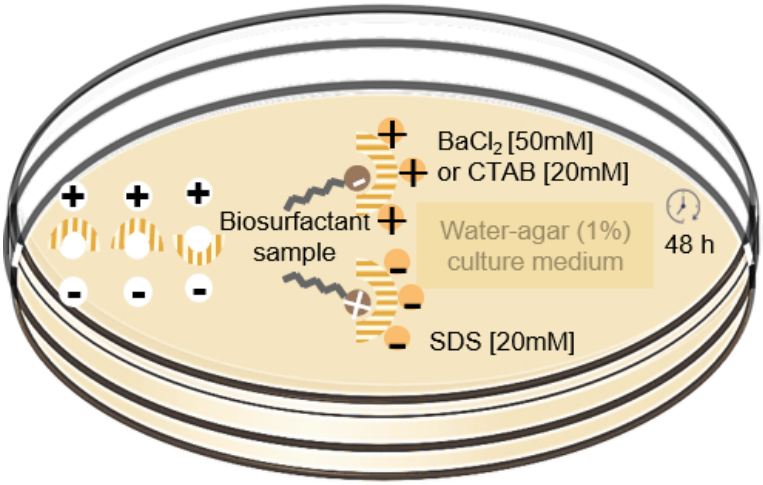
The combination of Thin Layer Chromatography (TLC) with Fourier Transform Infrared (FTIR) spectroscopy can enable a partial characterization of biosurfactants (DERGUINE-MECHERI et al., 2018). FTIR clarifies the different basic functional groups (alkyls, carbonyls and esters) of biosurfactants based on molecular vibrations at specific wavelengths (GARTSHORE; LIM; COOPER, 2000). A series of Nuclear Magnetic Resonance (NMR) spectroscopy experiments enable the accurate identification of each functional group, as well as the position of the bonds in the carbohydrate and lipid molecules. This technique can differentiate the structural isomers of the same molecule and analyse the purity of the sample (SMYTH et al., 2010).

The Mass Spectrometry (EM) provides molecular weight of the biosurfactant, i.e., the technique requires extremely purified biosurfactants (BALAN; GANESH KUMAR; JAYALAKSHMI, 2019; WANG et al., 2019a). The molecular mass obtained by this procedure can be combined with the results provided by Gas Chromatography (GC) to determine the side chain compositions of the fatty acids present in the biosurfactant (SILVA et al., 2010), a technique commonly called Mass Spectrometry coupled to Gas Chromatography (GC-MS) (ANDRADE et al., 2018). On the other hand, the hydrophilic portion is commonly characterized by High Performance Liquid Chromatography (HPLC) (KIM et al., 2020).

Matrix-Assisted Laser Desorption Ionization Time of Light Mass Spectrometry – (MALDI-TOF-MS) is a technique highly sensitive due to its smooth ionization capacity and suitability for high molecular weight biosurfactants (polar and non-polar) (SMYTH et al., 2010). The use of MALDI-TOF-MS remain restricted due to its high costs (JIMOH; LIN, 2019) and, when associated with NMR and GC-MS and /or with HPTLC and GC, it is effective in distinguishing individual variants of MEL for example (ANDRADE et al., 2017; BECK et al., 2019).

The double diffusion agar test is a simple technique to determine the ionic charge of biosurfactants (Figure 14), as reported by several authors (SILVA et al., 2010; RUFINO et al., 2014; MARQUES et al., 2019). The qualitative result is obtained through visual inspection and validated by the electrokinetic technique (zeta potential), where the surface charge of the molecule is determined by measuring the repulsion between the hydrophilic parts of the biosurfactant (AMARAL et al., 2006; ANDRADE et al., 2018; SOUZA et al., 2018b).

Figure 14 – Double diffusion agar test



Caption: The double diffusion agar test occurs with the addition of biosurfactant samples in wells in a row regularly spaced against another row of wells filled with a known cationic or anionic element. The appearance of precipitation lines (48h) between the wells indicates the ionic character of the biosurfactants.
Source: Elaborated by the author (2021).

9 BIOTECHNOLOGICAL APPLICATIONS IN COMMERCIAL PRODUCT FORMULATION

Several companies already sell products containing SL and MEL in their composition. The Asian company Allied Carbon Solutions Co., Ltd (Japan) is a reference in the production and commercialization of SL on a large scale (≈ 1000L). Other Asian companies such as Toyobo and Saraya Co. (Japan), Kanebo Cosmetics Inc (Japan) and MG Intobio Co., Ltd (South Korea) market personal care products containing MEL and SL. In Europe, the German companies Evonik, Ecover and Henkel produce and/or market SL and MEL, respectively, in detergents and cleaning products. Likewise, the companies Givaudan SA (France) and Holiferm Technology (UK) produce and apply SL in cosmetic formulations.

The formulation of personal care products such as toothpastes and mouthwashes containing biosurfactants were proposed using produced from *S. bombicola* (FARIAS et al., 2019; RESENDE et al., 2019). The toothpastes also contained chitosan extracted from Mucorales and showed no toxicity, as well as, showed desirable characteristics, such as pH around 9, foaming ability (63-95%), and inhibition of cell viability of the cariogenic bacteria Streptococcus mutans (RESENDE et al., 2019).

Biosurfactants produced by *C. lipolytica* and *C. bombicola* together with potassium sorbate (chemical preservative) were active ingredients in product formulations for hydrophobic pollutant remediation (FREITAS et al., 2016; SANTOS et al., 2017b). These

formulations maintained their surfactant properties and stability for 90 days at sufficient level to ensure their application as a dispersant (FREITAS et al., 2016; SANTOS et al., 2017b). The minimum shelf life required for commercialization is typically 120 days (FREITAS et al., 2016; SANTOS et al., 2017b). An alternative for increasing biosurfactants shelf life is drying by atomization. The absence of water promotes greater stability and preservation, although it increases costs (BARCELOS et al., 2014). The storage of liquid/powder sophorolipids is usually carried out in polyethylene packaging such as bags, bottles, drums and containers, these biosurfactants have pale yellow to brown coloration.

Biosurfactants produced by *Candida utilis* were tested for possible uses in the food sector to formulate salad dressings and mayonnaise, this ingredient provided physicochemical stability in temperature and acidity variations during food processing (CAMPOS et al., 2015; CAMPOS; STAMFORD; SARUBBO, 2019). Detergents containing biosurfactant produced from *Cunninghamella echinulata* has also been tested for use in the chemical-textile industry, as they promoted cleaning and removal of motor oil (86%) in cotton fabric, maintaining the stability of the structural integrity of the fibre (ANDRADE et al., 2018). The use of biosurfactants in biomedical, therapeutic and pharmaceutical application have been recently reviewed highlighting potential applications in cleaning handwash formulations and plastic and fabric surface coating agents useful for combating microbial infections (CERESA et al., 2020).

Some tests for potential fungal biosurfactant uses in applied to public health applications have also been tested. Biosurfactants produced from *Scheffersomyces stipites* (MARCELINO et al., 2017) and *Wickerhamomyces anomalus* (FERNANDES et al., 2020) promoted the death of larval exoskeletons of the *Aedes aegypti* mosquito after 12h and 24h, respectively, showing their potential in larvicidal formulations against dengue arbovirus vector. The commercial formulations reported here for the several applications did not show toxic effects, except for larvicidal activity (not analyzed).

10 CONCLUSIONS

Nature provides enormous possibilities to isolate biosurfactant-producing fungi that colonize diverse environments. The fungal bioprospecting for biosurfactant production has allowed the discovery of strains not yet described in the literature, as well as new molecular structures with surfactant action. However, bioprospecting based on metagenomic data still represents an untapped potential. Likewise, there is still a gap in relation to molecular methods intended for the screening of biosurfactant-producing organisms.

Economic issues and low process productivity are the main challenges to overcome fungal biosurfactant large-scale production. Although several energy sources are promising for biosurfactant production, the economic viability to use waste streams can only be obtained with careful analysis between cost minimization and low yield due to the formation of inhibitory compounds. Changes in carbon/nitrogen sources and recombinant strains can produce biosurfactants with new and different chemical structures that ensure better physicochemical properties for wider applications. Statistical and bioprocess tools applied to this biomolecule can result in achieving higher yields/productivity as well as better overall operational processes.

Sophorolipids and mannosylerythritol lipids are the main fungal biosurfactants investigated studied through process development to applications, both molecules are yeast products. Filamentous fungi biosurfactants have received less attention and were more focused on isolation and bioprospection, which allowed the identification of new species, some of which are able to produce trehalose lipids (not yet described for eukaryotes) and lipopeptides (previously only observed in yeast). Few other studies with filamentous are restricted to the influence and optimization of nutritional conditions, production in association with other metabolites, and applications for environmental remediation, textile and cleaning. In general, fungal biosurfactants have wide applicability and have already been formulated as ingredients for several commercial products, which probably opens new windows for future studies aimed at their inclusion in various sectors of different market segments.

CAPÍTULO III

Caracterização morfológica e molecular de fungos isolados de solos contaminados com hidrocarbonetos

1 INTRODUÇÃO

A contaminação ambiental com hidrocarbonetos de petróleo é um problema mundial que causa danos a todos seres vivos, pois o petróleo é composto por classes de hidrocarbonetos (ramificados ou não) como alifáticos, aromáticos, policíclicos aromáticos (HAP), resinas e asfaltenos que apresentam diferentes solubilidade, volatilidade e toxicidade (VARJANI, 2017; MAGALHÃES et al., 2022).

A biorremediação microbiana de hidrocarbonetos tem sido uma alternativa que utiliza o metabolismo de bactérias, fungos ou microalgas para descontaminação ambiental (QUINTELLA; MATA; LIMA, 2019). Alguns fungos possuem plasticidade morfológica e vias metabólicas moduladas para produção de metabólitos (enzimas, biosurfactantes, pigmentos) que permitem sua colonização em solos contaminados com hidrocarbonetos (DA SILVA et al., 2023). Em outras palavras, o uso de fungos autóctones de solos contaminados com hidrocarbonetos possivelmente pode apresentar vantagens em processos de bioaugmentação de solos contaminados. Este fato é devido os fungos possuírem mecanismos fisiológicos adaptados e apropriados que os permitem tolerar e resistir a toxicidade de hidrocarbonetos, bem como para solubilizar e/ou degradar tal contaminante (MEDAURA et al., 2021; REYES-CÉSAR et al., 2014).

Por exemplo, fungos filamentosos podem penetrar suas hifas no solo para interagir com hidrocarbonetos por meio da excreção de biosurfactantes e/ou enzimas para facilitar a assimilação/degradação de contaminantes como sua fonte nutricional (HARMS; SCHLOSSER; WICK, 2011; SINGH et al., 2020). No entanto, foi reportado que a ecologia microbiana de solos contaminados é influenciada pelo tipo e tempo de exposição do hidrocarboneto, e pela profundidade da camada de solo (AL-OTIBI; AL-ZAHRANI; MARRAIKI, 2023; SPINI et al., 2018).

Embora microbiomas fúngicos e seu potencial biotecnológico tenham sido revelados por metagenômica (HAQUE et al., 2022), somente o isolamento de fungos (via técnicas dependentes de cultura) permite a obtenção de linhagens que podem ser utilizadas como inóculo em bioprocessos (SIMISTER et al., 2015; YALÇIN; ERGIN-TEPEBAŞ; UYAR, 2018). Técnicas clássicas de microbiologia têm sido usadas para isolar fungos de solos; por exemplo, a diluição seriada e espalhamento em placas contendo meio de cultura sólido e antibióticos específicos (FAYEULLE et al., 2019; BUENO et al., 2019). Além disso, o enriquecimento de solos com hidrocarbonetos tem sido uma estratégia para isolar fungos cultiváveis que possivelmente são capazes de assimilar/degradar o hidrocarboneto, além de estimular o

desenvolvimento de linhagens devido ao fornecimento de outros micronutrientes no meio de enriquecimento (COVINO et al., 2015; AL-HAWASH et al., 2018).

No entanto, isolados fúngicos antes de serem usados em bioprocessos devem ser submetidos apropriadamente a um protocolo preliminar para garantir viabilidade, disponibilidade e elegibilidade da linhagem isolada (SMITH, 2012). Este protocolo pós-isolamento inclui técnicas de preservação a longo prazo, armazenamento em Coleções de Cultura, e identificação por meio de características morfológicas e técnicas de biologia molecular (geralmente sequenciamento da região ITS) (SCHMIDT; CHRISTENSEN; JOHNSEN, 2010; QAZI et al., 2014; BUENO et al., 2019). Dentre os métodos de preservação de linhagens em longo prazo, a criopreservação de células fúngicas a -80 °C é uma estratégia eficaz para manutenção de isolados, usando glicerol (20%) como agente criopreservante (BHARDWAJ; CAMEOTRA; CHOPRA, 2015; SILVA et al., 2014).

Pesquisa de campo tem sido realizada em onze unidades experimentais de solos ou águas subterrâneas contaminadas com hidrocarbonetos de petróleo, etanol ou biodiesel; as quais estão localizadas no Núcleo Ressacada de Pesquisa em Meio Ambiente (REMA) da Universidade Federal de Santa Catarina (UFSC). Diferentes técnicas físicas, químicas e/ou biológicas de remediação foram implementadas nestas unidades experimentais, tais como: atenuação natural monitorada e acelerada; oxidação química; barreira permeável de biocarvão; e bioestimulação de bactérias autóctones com adição de nitrato, sulfato, ferro e acetato (RAMOS et al., 2014; CORSEUIL et al., 2015; RAMOS et al., 2016; MÜLLER et al., 2017; MARTINS, 2022).

Estudos sobre o microbioma de algumas unidades experimentais do REMA/UFSC foram majoritariamente realizados via técnicas independente de cultura, e reportaram a presença de bactérias do gênero *Desulfitobacterium* and *Geobacter* (RAMOS et al., 2014; MÜLLER et al., 2017). Recentemente, o primeiro estudo foi realizado (via técnica dependente de cultura) sobre a comunidade microbiana que ali prospera (KERBER, 2020). Este reportou o isolamento de 12 linhagens bacterianas de água subterrânea contaminada (com diesel e biodiesel de soja), e que o isolado *Bacillus* sp. é um promissor inóculo para produção de biosurfactantes (KERBER, 2020). Até onde sabemos, em relação a comunidade fúngica, estas unidades experimentais permanecem inexploradas. Este fato é atribuído ao primeiro estudo realizado não ter isolado (em cultura pura) colônias fúngicas, ele apenas avaliou o crescimento de fungos (via contagem de unidades formadoras de colônias) em barreira permeável de biocarvão durante a remediação de solo contaminado (com diesel e biodiesel de palma) (MARTINS, 2022).

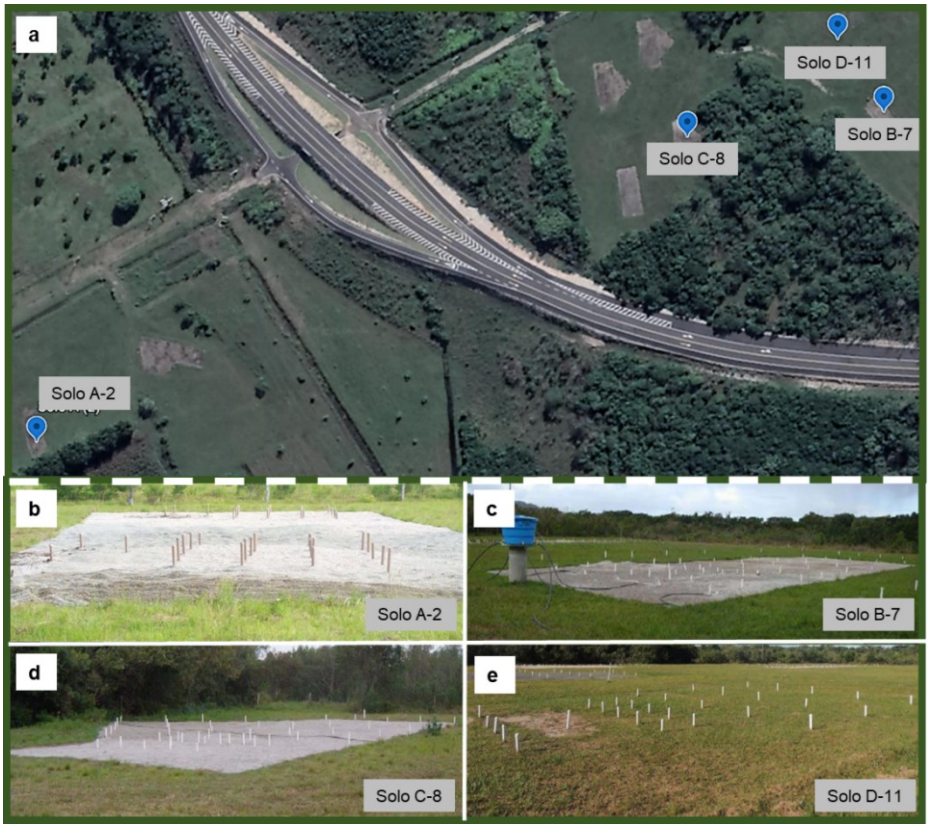
Em suma, é presumível sobre a influência de fatores bióticos e abióticos na diversidade de fungos cultiváveis, bem como sobre a importância de explorar a biodiversidade a partir do isolamento de fungos cultiváveis para armazená-los em coleção de cultura e implementá-los em processos biotecnológicos (por exemplo, em micorremediação). Portanto, é hipotético-dedutivo que a especiação de fungos cultiváveis dos solos (do REMA/UFSC) variam de acordo com o contaminante ou biotratamento no qual ele está submetido. Neste contexto, esta pesquisa objetivou isolar e identificar fungos cultiváveis de solos contaminados (do REMA) com diferentes contaminantes e biotratamentos para obter uma coleção de cultura como fonte de inóculos para bioprocessos.

2 MATERIAL E MÉTODOS

2.1 AMOSTRAGEM

Amostras de solo de quatro unidades experimentais A-2, B-7, C-8 e D-11 localizadas no REMA/UFSC foram coletadas a uma profundidade de 1,4 m, em 14 de janeiro de 2020 (Figura 15). As informações sobre a coordenada geográfica, fonte de contaminação, data de liberação de contaminante, e biotratamento que estão sendo realizados nestas unidades experimentais, são apresentadas na Tabela 5. As amostras coletadas foram armazenadas em tubos esterilizados de polipropileno Falcon (de 50 mL) com tampa de mesmo material e em baixa temperatura (5 °C) até o isolamento que ocorreu no mesmo dia.

Figura 15 – Aerofotogrametria da Fazenda Experimental Ressacada localizada no REMA/UFSC (a) com destaque das unidades de solos contaminados A-2 (b), B-7 (c), C-8 (d) e D-11 (e)



Fonte: Elaborado pelo autor (2022).

Tabela 5 – Amostragem de solos contaminados em processos de biorremediação

Área de coleta		Fonte de contaminação	Volume liberado (L); Data de liberação	Biotratamento	Coordenadas geográficas
A	2	Diesel (100%)	20; 03/2001	Atenuação natural monitorada	27° 40' 54.3" S 48° 32' 12.2"W
B	7	Gasolina (90%) e etanol (10%)	100; 10/2009	Biorremediação ativa com sulfato	27° 40' 49.4" S 48° 31' 58.1"W
C	8	Diesel (80%) e biodiesel de soja (20%)	100; 07/2010	Biorremediação ativa com acetato de amônia	27° 40' 49.8" S 48° 32' 01.4"W
D	11	Biodiesel de palma (100%)	100; 08/2013	Biorremediação ativa com MgO_2 Fe_2O_2	27° 40' 48.3" S 48° 31' 59.3"W

2.2 ISOLAMENTO FÚNGICO

O isolamento fúngico foi realizado por meio de técnicas clássicas de microbiologia (semeadura em superfície). Uma amostra de 10 g de solo contaminado foi homogeneizada em frascos Erlenmeyer contendo 90 mL de solução esterilizada de NaCl (0,85%) usando agitador orbital (130 rpm, 28 ° C, 30 min). 100 µL de diluições (10^{-2}, 10^{-3}, 10^{-4} e 10^{-5}) da amostra foram espalhadas em placas de Petri contendo: (1) em meio de cultura batata dextrose ágar (BDA); ou (2) meio Martin, composto de (em g•L^{-1}) glicose (10), KH_2PO_4 (1,0), $MgSO_4$•$7H_2O$ (0,5), peptona (5,0), rosa bengala (0,03), e ágar (20,0). O corante Rosa Bengala ($C_{20}H_2Cl_4I_4O_5$) impede o rápido crescimento radial de possíveis linhagens fúngicas que poderiam inibir o aparecimento daquelas de crescimento mais lento (FAYEULLE et al., 2019; OGAKI et al., 2020). Os antibacterianos (em µg mL^{-1}) tianfenicol (50) (Sigma – Aldrich®), estreptomicina (30) e penicilina G (30) (Gibco®) foram adicionados em ambos em meios de cultura. As placas de Petri foram armazenadas (sob ausência de luz) em estufa a 28°C. O crescimento de fungos filamentosos e leveduras foi monitorado até o trigésimo dia. O experimento foi realizado em triplicata. As colônias morfologicamente distintas foram subcultivadas em meio fresco até a obtenção de cultura pura (FAYEULLE et al., 2019).

2.3 ENRIQUECIMENTO DE SOLO

A bioestimulação da microbiota de solos contaminados foi realizada por meio de enriquecimento em cultura líquida com hidrocarbonetos como única fonte de carbono (KULSHRESTHA; KUMARI, 2011; AL-HAWASH et al., 2018). Dez gramas de solo contaminado (Tabela 5) foram homogeneizados em frasco Erlenmeyer 250 mL contendo 90 mL de meio Czapek-Dox modificado, composto (em g•L^{-1}) de $NaNO_3$ (2,0), K_2HPO_4 (1,0), $MgSO_4$ * $7H_2O$ (0,5), KCl (0,5), $FeSO_4$ (0,01); e óleo diesel ou borra de petróleo não esterilizados (ambos fornecidos por Petrobras) na concentração de 1% (v/v), usando agitador orbital (130 rpm, 28˚C, 15 dias) para novo isolamento de linhagens com morfologia diferentes daquelas previamente isoladas, conforme descrito no tópico anterior. Dez mililitros das culturas enriquecidas (1%) foram novamente adicionados em meio Czapek-Dox modificado, porém com adição de óleo diesel ou borra de petróleo na concentração de 5% para realização dos mesmos procedimentos anteriores. Após isso realizou-se o isolamento conforme descrito no item anterior.

2.4 PRESERVAÇÃO E MANUTENÇÃO DE LINHAGENS FÚNGICAS

Uma porção ativa de isolado fúngico (aproximadamente 1 cm^2) crescido em meio BDA foi criopreservada a -80 ° C em glicerol (20%). Os isolados fúngicos foram adicionados à Coleção de Cultura Microbiana do Laboratório de Microrganismos e Processos Biotecnológicos (LAMPB) da Universidade Federal de Santa Catarina (UFSC). A preservação em médio prazo das linhagens foi mantida em ágar inclinado a 5°C; e em curto prazo foram realizadas subculturas repetidas em meio fresco de BDA (SCHMIDT; CHRISTENSEN; JOHNSEN, 2010; SMITH et al, 2013).

2.5 IDENTIFICAÇÃO MORFOLÓGICA

A caracterização macromorfológica foi realizada a partir de características intrínsecas da colônia, considerando a coloração, tipo de micélio e borda, produção de pigmentos e aspectos particulares de cada isolado. A caracterização micromorfológica foi realizada por meio da técnica de microcultivo (GOMES et al., 2010). A câmara de microcultivo foi realizada em placa de Petri (90 x 15mm) contendo papel filtro (em sua superfície interna) sob 2 lâminas de vidro e 2 lamínulas para serem previamente embaladas em papel Kraft e esterilizadas em autoclave (121 °C, 30 min). Meio de cultura BDA foram cortados em cubos (1 cm^2) com uma lâmina de bisturi esterilizado, e dois cubos foram colocados sobre a lâmina (contida na câmara) usando uma alça de platina esterilizada. O isolado fúngico foi inoculado nas faces laterais do cubo e uma lamínula foi colocada sobre a face superior. O papel de filtro foi embebido com 1000 µL de água destilada esterilizada para manter a umidade atmosférica. A câmara de microcultivo foi mantida em temperatura ambiente (22 ± 2 °C) por 7 ou 14 dias, de acordo com o crescimento e esporulação da linhagem. A preparação das lâminas foi realizada por meio do gotejamento de 15 µL de lactofenol com azul de algodão (para linhagens hialinas) ou sem azul de algodão (para linhagens demáceas) para fixação das lamínulas contendo as estruturas reprodutivas, as quais foram examinadas por microscopia óptica (100x) conforme descrito por Kern e Blevins (1999).

2.6 IDENTIFICAÇÃO MOLECULAR

Oito isolados fúngicos foram selecionados aleatoriamente para caracterização molecular via Reação em Cadeia da Polimerase (PCR) por sequenciamento Sanger ou por tecnologia MiSeq Illumina (MEDAURA et al., 2021; ROLLEMBERG et al., 2022).

Por um lado, o DNA genômico dos isolados AF38D, AF41D, AF42D e AF64PD foi extraído usando o kit Power Soil DNA (MoBiol Laboratories, Solana Beach, CA), de acordo com o protocolo do fabricante. O DNA extraído foi avaliado em relação a sua concentração e qualidade (A_{260}/A_{280}) usando um espectrofotômetro Nanodrop 1000 (Thermo Scientific, EUA) e armazenados a -20 °C para uso posterior (BENGUENAB; CHIBANI, 2020). O espaçador transcrito interno (ITS) de DNAr dos isolados fúngicos, foi amplificado usando o primer direto ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3') e primer reverso ITS4 (5'-CTTGGTCATTTAGAGGAAGTAA-3') (WHITE et al., 1990). A PCR (para um volume final de 25 µL) consistiu em $MgCl_2$ (1 µL, 50 mM); dNTPs (0,2 µL, 100 mM); primer ITS1-F e ITS4-B (ambos 0,13 µL, 20 µM); BSA (1µL, 15 mM), Taq Polimerase (0,20 µL, 5 $U \bullet \mu L^{-1}$); e DNA genômico do isolado (2 µL). O volume de DNA foi substituído por água destilada como controle negativo para verificar a ausência de contaminação. Os amplicons foram obtidos usando termociclador Mastercycler® (Eppendorf, EUA), programado por 3 min a 94°C (hotstart); 35 ciclos por 45 s a 94°C, 45 s a 53°C e 60 s a 72°C; e uma extensão final de 10 minutos a 72°C (VISAGIE et al., 2014). Os produtos de PCR foram corados com GelRed, visualizados em gel de agarose 1% preparado com tampão TAE 1X (90 mM Tris-acetato e 2 mM EDTA, pH 8,0) a 60V, e documentados usando transiluminador ChemiDoc™ (Alpha Innotech, EUA) (LAWRENCE; NOURI; TROUILLAS, 2019). Os produtos de PCR foram purificados por precipitação solvente/sal usando etanol, isopropanol e cloreto de sódio (GREEN; SAMBROOK, 2017); e após foram quantificados via NanoDrop, antes do armazenamento a -20 °C. Amostras purificadas contendo acima ou igual a 30 ng de DNA foram enviadas para empresa Macrogen (Coréia do Sul) para sequenciamento via Sanger.

Por outro lado, devido não ter sido possível extrair o DNA dos isolados AF10D, AF52DD, AF48D e AF99PD usando o kit supracitado, esta extração foi realizada pela empresa Neoprospecta Microbiome Technologies (Floriánopolis, Brasil). O DNA extraído foi submetido ao sequenciamento de alto rendimento da região ITS-1. Fragmentos miceliais destes isolados foram transferidos assepticamente para um microtubo (fornecido pela empresa) contendo solução tampão (Neosample X) e a codificação prévia do isolado, os quais foram armazenados em embalagem apropriada (fornecida pela empresa) e enviados para extração de DNA genômico seguindo um protocolo do proprietário. Os amplicons foram obtidos usando os primers ITS1 (5'-GAACCWGCGGARGGATCA-3') e ITS2 (3'-

GCTGCGTTCTTCATCGATGC-5') (WHITE et al., 1990; SCHMIDT et al., 2013), e sequenciados usando o sistema de sequenciamento MiSeq, com 300 ciclos e adaptador single-end. As sequências foram analisadas sobre sua qualidade Phred usando o pipeline Sentinel por meio do programa FastQC v.0.11.8 (ROLLEMBERG et al., 2022).

O uso desta

2.7 ANÁLISE FILOGENÉTICA

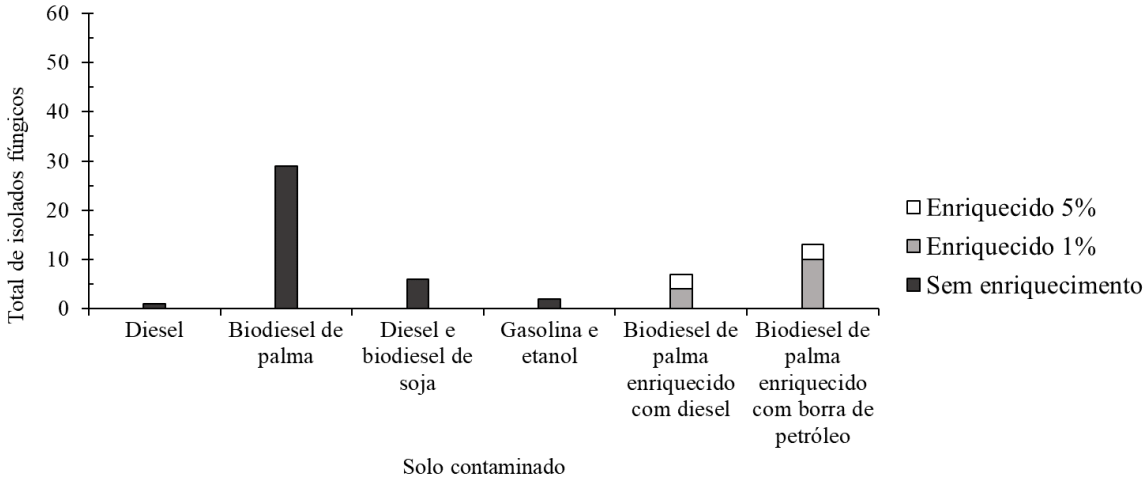
As sequências nucleotídicas dos isolados foram comparadas para estimar a simililaridade com sequencias de genes ITS disponíveis no banco de dados do NCBI (Centro Nacional de Informações sobre Biotecnologia) - GenBank usando o programa de alinhamento BLASTn (https://www.ncbi.nlm.nih.gov/blast). As sequências que apresentaram cobertura, valor de E = 0 e identidade ≥ 85%, foram selecionadas para identificação em nível de gênero. Árvores filogenéticas foram construídas pelo método de Maximum Likelihood, com o modelo Tamura-Nei (TAMURA; NEI, 1993) e 1000 bootstrap de repetições, usando o software MEGA v6 (Molecular Evolutionary Genetics Analysis). As sequências de nucleotídeos utilizadas neste estudo foram obtidas/submetidas ao GenBank (Apêndice A – Tabela S1).

3 RESULTADOS

3.1 ISOLADOS FÚNGICOS

Uma coleção de 58 fungos cultiváveis (55 filamentosos e 3 leveduras unicelulares) foi isolada de solos (do REMA/UFSC) contaminados com etanol, gasolina, diesel /ou biodiesel em processo de biorremediação (Apêndice B, Tabela S2) usando o meio BDA (n= 35) ou Ágar Martin (n= 23) (Figura 16).

Figura 16 – Distribuição de número de isolados de solos contaminados do REMA/UFSC em função do contaminante



Fonte: Elaborado pelo autor (2022).

Trinta e oito fungos foram isolados de solos contaminados sem a técnica de enriquecimento, sendo que 1, 2, 6 e 29 destes foram isolados de solos contaminados com diesel, gasolina e etanol, biodiesel de soja e diesel, e biodiesel de palma respectivamente.

Nenhum fungo morfologicamente distinto dos isolados de solo sem enriquecimento, foi isolado das amostras de solos contaminados com diesel; gasolina e etanol; biodiesel de soja e diesel; quando enriquecidos com óleo diesel ou borra de petróleo. Vinte fungos filamentosos morfologicamente distintos foram isolados do solo contaminados com biodiesel de palma quando enriquecidos com diesel ou borra de petróleo, sendo que sete fungos foram a partir do enriquecimento com diesel 1% (n= 4) e 5% (n= 3), enquanto treze foram com borra de petróleo 1% (n= 10) e 5% (n= 3).

Quarenta e cinco microrganismos unicelulares foram isolados e embora os meios BDA e Martin continham antibacterianos com amplo espectro de ação; somente 3 destes foram identificados como leveduras por meio de microscopia e coloração Gram, uma vez que as células de levedura se apresentaram maiores em tamanho do que as bacterianas, blastoconídios e se coram roxo escuro na coloração de Gram. Todos isolados fúngicos foram armazenados e criopreservados (glicerol 20%, -80 °C) de acordo com as Diretrizes de Melhores Práticas, que são recomendadas pela Organização para Cooperação e Desenvolvimento Econômico (OCDE) para centros de recursos microbianos (SMITH, 2012).

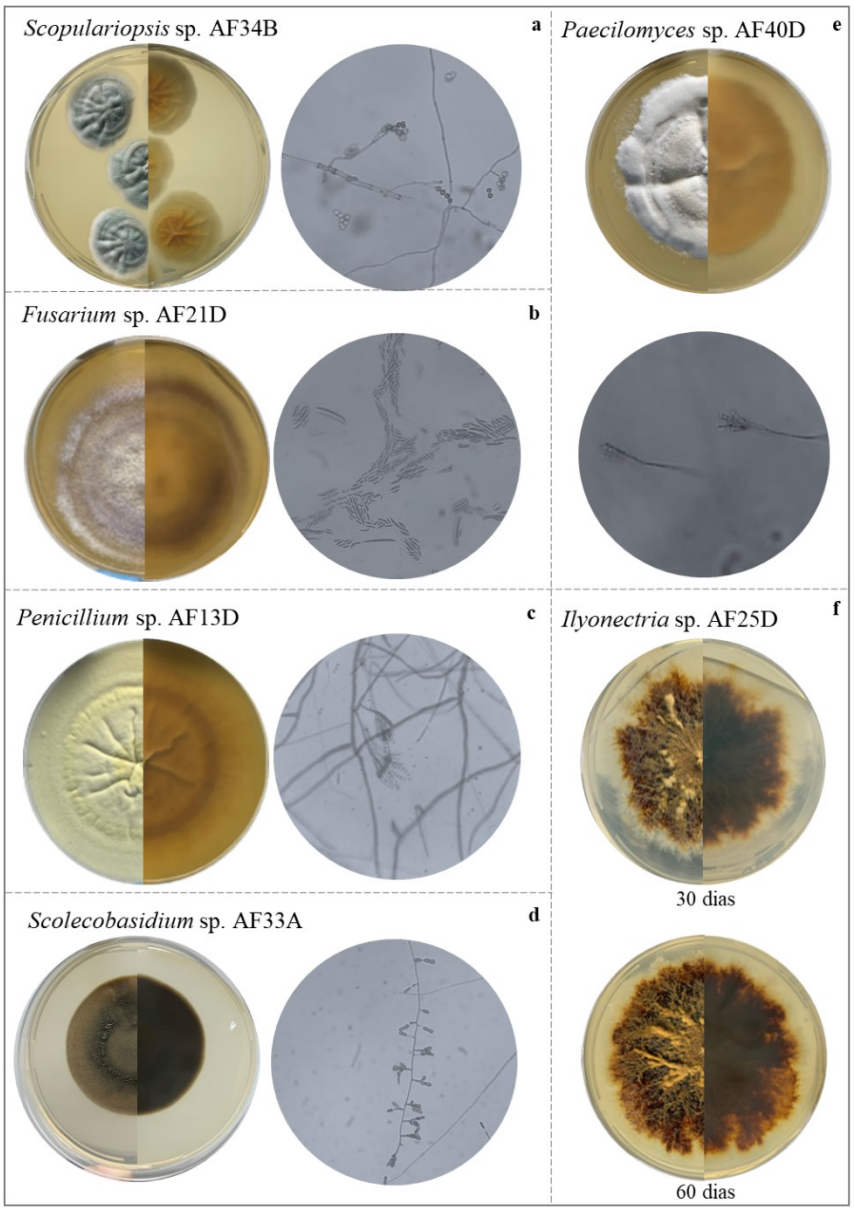
3.2 IDENTIFICAÇÃO FÚNGICA

Cinquenta e quatro fungos filamentosos foram identificados pela micromorfologia de suas estruturas aderidas em lâmina de vidro; os quais foram classificados em cincos gêneros: *Scopulariopsis* (n= 1), *Scolecobasidium* (n= 1), *Paecilomyces* (n= 4), *Penicillium* (n= 8) e *Fusarium* (n=40) (Apêndice B, Tabela S2). O isolado filamentoso AF25D não apresentou microestruturas reprodutivas, embora suas características macromorfológicas corroboram com a identificação pertencente ao gênero *Ilyonectria*, tais como: coloração no centro laranja-marrom-escuro e zona externa amarelo-escuro; hifas aéreas feltradas, esparsas, ligeiramente elevadas e desniveladas; bordas irregulares com extremidades lobuladas; e reverso com cor semelhante ao anverso e sem sulcos (LAWRENCE; NOURI; TROUILLAS, 2019). A Figura 17 apresenta um isolado de cada gênero obtido, *Scopulariopsis*, *Fusarium*, *Penicillium*, *Scolecobasidium, Paecilomyces* e *Ilyonectria* e sua respectiva microestrutura (exceto de *Ilyonectria*); enquanto a Figura 18 sumariza a distribuição numérica destes isolados em função do tipo de contaminante, enriquecimento e meio de cultura usado.

Aproximadamente 70% das linhagens são pertencentes ao gênero *Fusarium*. Estas foram isoladas de todos os solos enriquecidos ou não, usando meio BDA ou Martin; exceto em solo contaminado com diesel que apenas permitiu isolar (em BDA) a linhagem *Scolecobasidium* sp. AF33A. O enriquecimento com diesel e borra de petróleo em solos contaminados com biodiesel de palma, permitiu isolar somente linhagens de *Fusarium* e *Penicillium*. As três leveduras, oito linhagens de *Penicillium,* e *Ilyonectria* sp. AF25D foram isolados de solo contaminado com biodiesel de palma, enquanto aquelas de *Paecilomyces* foram isoladas de biodiesel de soja e diesel, e biodiesel de palma. A linhagem *Scopulariopsis* sp. AF34B foi isolada de solo contaminado com gasolina e etanol.

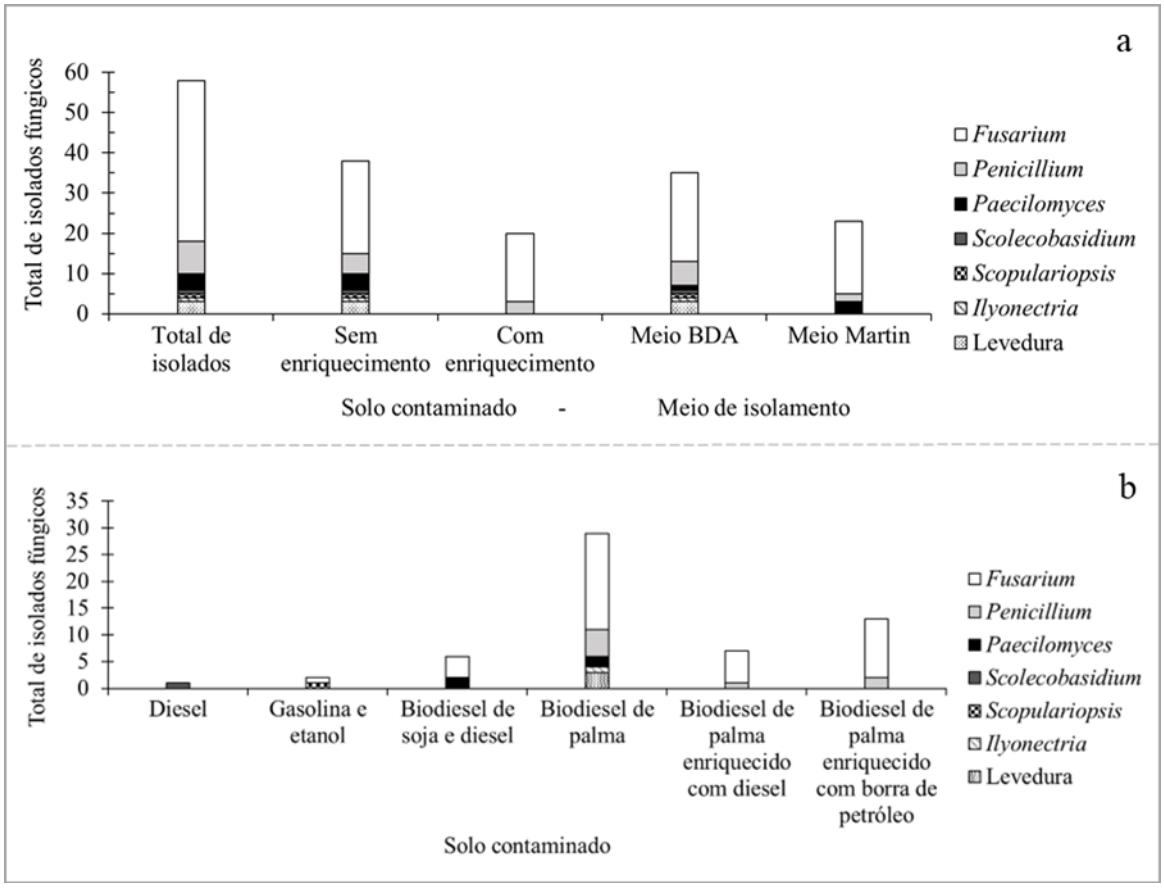
Os isolados pertencentes ao gênero *Fusarium* apresentaram características morfológicas com variações contrastantes em sua macro e micromorfologia, e elevado nível de setorização que induz a mudanças espontâneas na morfologia destas linhagens (Apêndice C, Figura S3). Estes isolados apresentaram coloração branca, rosa, violeta e alaranjado; hifas aéreas; micélio cotonoso e lanoso; bordas regulares e irregulares; crescimento rápido; alguns com excreção de pigmentos; e reverso sem sulcos. A presença de macroconídios com 1 a 4 septos, microconídios em formato falciforme/oval, e clamidósporos esféricos (terminal e/ou intercarlado) corroboram para identificação micromorfológica dos isolados de *Fusarium* sp. (Apêndice D, Tabela S4).

Figura 17 – Isolados fúngicos do gênero *Scopulariopsis* (a), *Fusarium* (b), *Penicillium* (c), *Scolecobasidium* (d), *Paecilomyces* (e) e *Ilyonectria* (f) crescidos em meio BDA em 25°C por 21 dias (direita, vista anverso; esquerda, vista reversa) e suas respectivas microestruturas (em 100x) são mostrados em a, b, c, d, e; respectivamente



Fonte: Elaborado pelo autor (2022).

Figura 18 – Distribuição do número de isolados (de solo contaminado) pertencentes aos gêneros identificados em função do tipo de contaminante, enriquecimento e meio de cultura usado



Fonte: Elaborado pelo autor (2022).

Os isolados AF13D, AF17D, AF38D, AF41D, AF42D, AF64PD, AF78DD e AF98PD apresentaram características morfológicas semelhantes a linhagens de *Penicillium*, embora tais características divergiam entre cada isolado (Apêndice E, Figura S5). Estes isolados apresentaram hifas aéreas com mudança de coloração (branca, creme e verde), bordas irregulares e regulares, setorização, e reverso com sulcos. A presença de conidióforo monoverticilado e/ou biverticilado, métulas abundantes com formação de fiálides curtas que suportam conídios globosos e dispostos em cadeia, são as principais características micromorfológicas que classificam estes isolados como linhagens de *Penicillium* sp.

As linhagens de *Paecilomyces* (AF46C, AF47C, AF40D, AF45D) apresentaram pequenas diferenças macromorfológicas relacionados ao tipo de hifas aéreas e bordas, presença de setorização, coloração (branca e creme), e reverso com sulcos (Apêndice E, Figura S5). Estes isolados apresentaram hifas septadas, conidióforos ramificados no topo com fiálides longas contendo conídios elipsóides, que corroboram para identificação de *Paecilomyces* sp.

O isolado AF33A foi identificado como pertencente ao gênero *Scolecobasidium* e apresentou coloração cinzento-escuro-oliva, micélio aéreo curto, borda regular, crescimento lento (aparecimento da colônia após 20 dias), colônia largamente aderida ao meio de cultura, e reverso marrom escuro sem sulcos (Figura 17). A análise microscópica de *Scolecobasidium* sp. AF33A revelou a presença de longas hifas septadas que dão origem a conidióforos curtos contendo conídios em forma de foguete.

O isolado AF34B apresentou características morfológicas semelhantes a linhagem de *Scopulariopsis*, tais como micélio aéreo e aveludado, borda irregular com alteração de cor na extremidade, e reverso com sulcos (Figura 17). A micromorfologia de *Scopulariopsis* sp. AF34B apresentou largas hifas septadas, conidióforos com longas e curtas fiálides contendo conídios globosos e extrudidos (borda filiforme) dispostos em cadeia.

A identificação em nível de espécie dos isolados não foi definida e não é recomendada que seja realizada somente por meio de características morfológicas (O'BRIEN et al., 2008; SILVA et al., 2023). Este fato é devido a possíveis resultados inconsistentes serem alcançados, uma vez que é sabido sobre a variação da macromorfologia fúngica (cor, forma de micélio, tipo de borda, exsudato) de acordo com as condições nutricionais e operacionais disponibilizadas para o crescimento microbiano (VISAGIE et al., 2014). Além disso, embora a macromorfologia de isolados possam corroborar (com a literatura) para identificação fúngica, a micromorfologia pode diferir em alguns aspectos (FRISVAD; SAMSON, 2004; O'BRIEN et al., 2008). A identificação morfológica foi complementada usando métodos moleculares para garantir melhor acurácia na identificação fúngica, uma vez que nem sempre é possível distinguir a especiação da linhagem somente por características morfológicas (FRISVAD; SAMSON, 2004; VISAGIE et al., 2014).

Oito linhagens selecionadas aleatoriamente dentro dos gêneros identificados tiveram regiões ITS do gene do DNA ribossômico sequenciadas. As sequências de nucleotídeos obtidas foram analisadas por meio da ferramenta BLASTn do banco de dados genômicos NCBI e foram sequencialmente submetidas ao GenBank. O resultado desta identificação foi sumarizado na Tabela 6.

Tabela 6 – Identificação de isolados fúngicos por micromorfologia e sequenciamento da região ITS

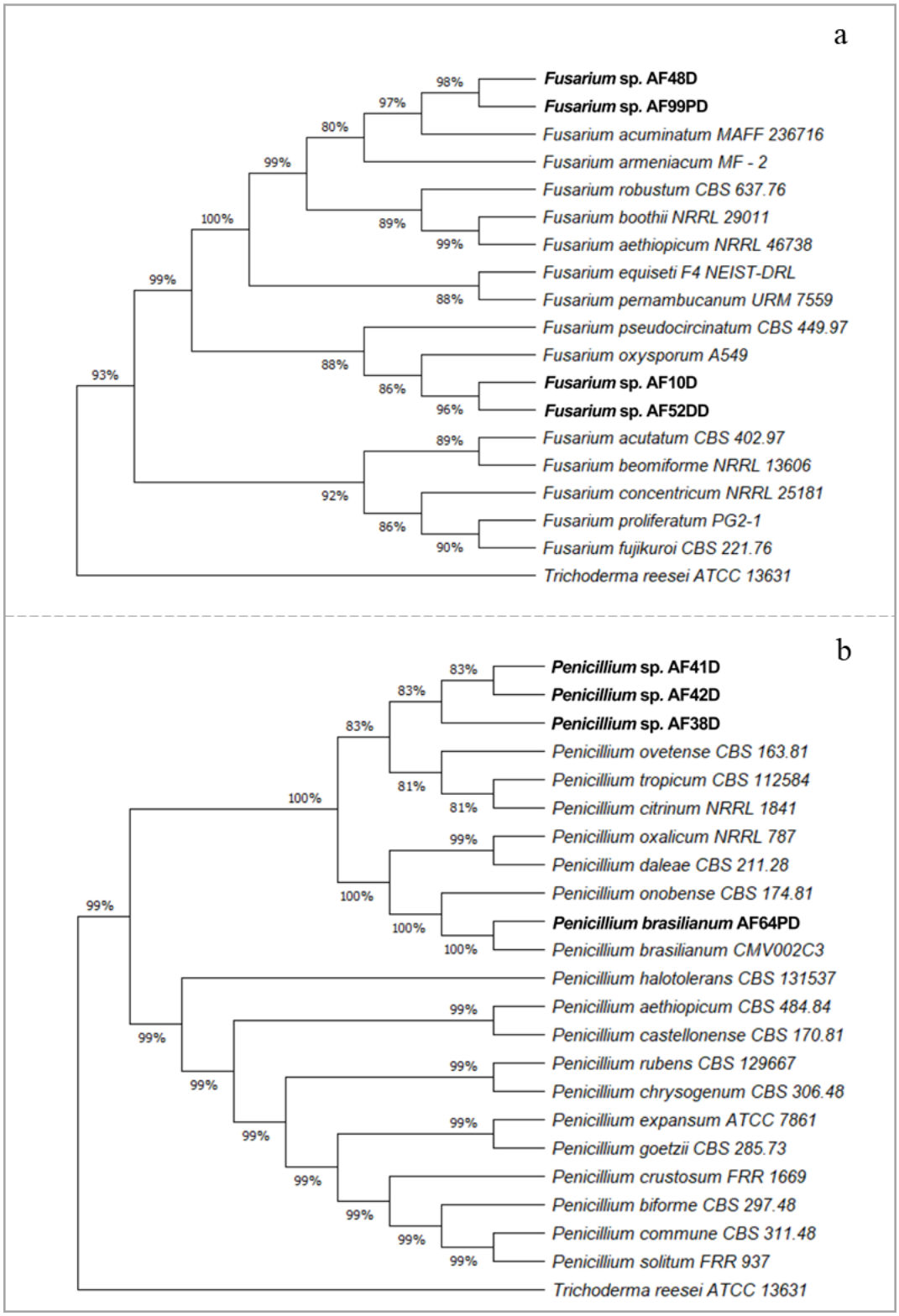
Linhagem	Fonte – solo contaminado com	Identificação micromorfologia	Microrganismo próximo	Número Genbank	E-value	Iden-tidade (%)	Número Genbank Linhagem
AF10D	Biodiesel de palma	*Fusarium* sp.	*Fusarium oxysporum*	MN959989.1	0.0	92	OQ282829

AF38D	Biodiesel de palma	*Penicillium* sp.	*Penicillium ovetense*	KC411680.1	1E-48	88	OQ282834
AF41D	Biodiesel de palma	*Penicillium* sp.	*Penicillium ovetense*	KC411680.1	1E-48	88	OQ282835
AF42D	Biodiesel de palma	*Penicillium* sp.	*Penicillium ovetense*	KC411680.1	1E-48	88	OQ282836
AF48D	Biodiesel de palma	*Fusarium* sp.	*Fusarium acuminatum*	KC477845.1	0.0	90	OQ282830
AF52DD	Biodiesel de palma, e enriquecido com óleo diesel	*Fusarium* sp.	*Fusarium oxysporum*	KT876659.1	0.0	89	OQ282831
AF64PD	Biodiesel de palma, e enriquecido com borra de petróleo	*Penicillium* sp.	*Penicillium brasilianum*	MK450675.1	2E-102	100	OQ282833
AF99PD	Biodiesel de palma, e enriquecido com borra de petróleo	*Fusarium* sp.	*Fusarium acuminatum*	JN624894.1	0.0	90	OQ282832

Os isolados fúngicos AF10D, AF48D, AF52DD e AF99PD foram previamente identificadas como linhagens de *Fusarium*, e o sequenciamento de DNA ribossômico corroborou com a identificação micromorfológica, embora não foi possível inferir a espécie, uma vez que a identificação de *Fusarium* é polifilética. A árvore filogenética, construída com linhagens de referência de *Fusarium* (Apêndice A, Tabela S1), mostrou que cada dupla das linhagens AF48D e AF99PD, e AF10D e AF52DD agruparam-se em clados diferentes, porém em um mesmo ramo com 98% e 96% de máxima verossimilhança, respectivamente (Figura 19). O alinhamento Blast revelou que os isolados *Fusarium* sp. AF48D e *Fusarium* sp. AF99PD apresentaram 90% de similaridade com a linhagem *Fusarium acuminatum* (Código acesso Genbank KC477845.1 e JN624894.1), enquanto os isolados *Fusarium* sp. AF10D e *Fusarium* sp. AF52DD apresentaram respectivamente 92% e 89% de similaridade com *Fusarium oxysporum* (Código acesso Genbank MN959989.1 e KT876659.1). As sequências ITS de *Fusarium* sp. AF10D, *Fusarium* sp. AF48D, *Fusarium* sp. AF52DD e *Fusarium* sp. AF99PD foram submetidas ao Genbank com códigos de acesso OQ282829, OQ282830, OQ282831 e OQ282832, respectivamente.

Os isolados AF38D, AF41D, AF42D e AF64PD foram previamente identificados morfologicamente como linhagens de *Penicillium*, e o sequenciamento da região ITS corroborou com esta identificação, embora somente seja possível sugerir identificação da linhagem AF64PD em nível de espécie. A árvore filogenética, construída com linhagens de referência de *Penicillium* (Apêndice A, Tabela S1), mostrou que AF41D e AF42PD agruparam-se em um mesmo ramo, pertencente ao clado de AF38D com 83% de máxima verossimilhança; enquanto AF64PD foi agrupado ao clado de *P. brasilianum* com alto valor de bootstrap igual a 100.

Figura 19 – Árvores filogenéticas de espécies *Fusarium* (a) e *Penicillium* (b) construídas pelo método Maximum-Likelihood com bootstrap de 1000 repetições, usando sequências ITS implementadas em software MEGA v6. Linhagens em negrito indicam isolados deste estudo. *Trichoderma reesei* ATCC 13631 foi utilizado como grupo externo (raiz da árvore)



Fonte: Elaborado pelo autor (2022).

O alinhamento blast revelou que os isolados *Penicillium* sp. AF38D, *Penicillium* sp. AF41D apresentaram 88% de similaridade com a linhagem *Penicillium ovetense* (Código acesso Genbank KC411680.1)*;* e o isolado AF64PD pertence a espécie *P. brasilianum (*Código acesso Genbank MK450675.1)*.* As sequências ITS de *Penicillium* sp. AF38D, *Penicillium* sp. AF41D, *Penicillium* sp. AF42D e *P. brasilianum* AF64PD foram submetidas ao Genbank com códigos de acesso OQ282834, OQ282835, OQ282836 e OQ282833, respectivamente.

4 DISCUSSÃO

Neste capítulo hipotetizou se os solos contaminados (do REMA) submetidos a processos de remediação (atenuação natural e bioestimulação), poderia ser um habitat propício (ainda inexplorado) para isolar fungos cultiváveis, os quais são pertencentes a gêneros potencialmente promissores a remediação de hidrocarbonetos.

Os meios de cultura BDA e Ágar Martin são comumente utilizados para isolamento de fungos do solo devido sua composição rica em macro- e micronutrientes (OGAKI et al., 2020; BARON et al., 2021), e neste trabalho permitiram isolar 55 fungos filamentosos e 3 leveduras. O critério para seleção destes isolados foi baseado nas diferenças macromorfológicas apresentadas pelas linhagens crescidas em tais meios. Este fato torna subjetiva esta seleção devido a possibilidade de exclusão de linhagens que apresentam alterações morfológicas somente após um maior tempo de incubação, e consequentemente, elas também não foram isoladas devido a sobreposição (em placa) com outras linhagens de crescimento rápido.

É sabido que muitos fungos não estão adaptados ao crescimento em meio de culturas ricos em nutrientes, ou eles não esporulam em meios de culturas artificiais. A limitação de nutrientes (pela diluição do meio) é uma abordagem que poderia ter sido realizada para ampliar a possibilidade de isolamento de outras linhagens (COLLADO et al., 2007; FERRARI; ZHANG; VAN DORST, 2011). Foi reportado a população fúngica isolada de solos contaminados (ou não) em meio contendo baixa concentração de nutrientes foi significativamente diferente daquela isolada em meio rico em nutrientes (FERRARI; ZHANG; VAN DORST, 2011).

O número e ecologia dos isolados fúngicos variou em relação ao tipo de contaminante liberado no solo conforme a seguinte sequência: biodiesel de palma (n= 29) > biodiesel de soja e diesel (n= 6) > gasolina e etanol (n= 2) > diesel (n= 1). O maior número de linhagens isoladas de solos contaminados com biodiesel de palma pode ser especulado devido este contaminante ser mais facilmente assimilado pela microbiota autóctone. Este resultado corrobora com o relato

que areia de rio, quando contaminada artificialmente com biodiesel de peixe, permitiu um crescimento mais rápido e visível de espécimes fúngicas em comparação com aquela contaminada com diesel, a qual não apresentou crescimento fúngico (HOREL; SCHIEWER, 2020). A sequência supracitada é inversa quando se considera a data de liberação do contaminante no solo em relação ao número de isolados. A partir deste fato, presume-se que o maior tempo de exposição do solo ao contaminante (por exemplo, diesel por 20 anos) influenciou negativamente a capacidade de fungos cultiváveis serem isolados em comparação ao solo com menor tempo de exposição (por exemplo, biodiesel de palma por 7 anos) (Tabela 5). É presumível que outros fatores podem também influenciar a ecologia (abundância, diversidade e riqueza) dos fungos cultiváveis destes solos, tais como: o volume de contaminante liberado (por exemplo, somente a liberação de diesel foi de 20 L, os demais contaminantes foram 100 L); e o tipo de tratamento e composto químico fornecido para bioestimulação no qual o solo está submetido (Tabela 5).

Neste estudo foi utilizado óleo diesel e borra de petróleo (em concentração 1% e 5%) como única fonte de carbono na composição do meio líquido Czapek-Dox para enriquecimento do solo. Somente o enriquecimento de solo contaminado com biodiesel de palma permitiu o isolamento de linhagens morfologicamente distintas daquelas previamente isoladas de solos não enriquecidos, e o aumento de 1 para 5% na concentração de diesel ou borra de petróleo não aumentou o número destas linhagens. Este fato indica que provavelmente outras linhagens fúngicas (de mesmo ou outro solo) podem ter sido desprezadas no isolamento, e corrobora para o menor número de isolados (n= 20) quando comparado com solos não enriquecidos (n= 38). No entanto, foi reportado que o aumento da concentração de petróleo de 1 para 5% durante o enriquecimento de solos, promoveu um efeito adverso na diversidade de fungos em dezoito semanas (HOREL; SCHIEWER, 2020).

Comumente camada superficiais (até 15 cm) de solo são coletadas para isolar fungos cultiváveis (MAO; GUAN, 2016; BENGUENAB; CHIBANI, 2020) ao contrário deste estudo no qual a coleta de solo ocorreu em profundidade de 1,4 m. É válido reportar que a profundidade de camada de solo influencia diretamente a comunidade microbiana que ali prospera, uma vez que aumentando a profundidade diminui a disponibilidade de matéria orgânica e oxigênio, o que impacta a diversidade, abundância e riqueza de espécies (SPINI et al., 2018). Neste contexto, é presumível que as linhagens aqui isoladas podem ter metabolismo microaerófilo/anaeróbico, assim como *Exophiala oligosperma* 29R-2-F02 isolada de sedimentos marinhos coletados a 2,5 km de profundidade (ZAIN UL ARIFEEN et al., 2022); e

que estes isolados ao decompor matéria orgânica em condições anaeróbicas (alta profundidade) podem produzir H_2 para alimentar metanogênicos autotróficos (DRAKE et al., 2021).

A identificação polifásica de isolados fúngicos é a melhor alternativa para alcançar resultados confiáveis sobre a especiação da linhagem (FRISVAD; SAMSON, 2004). Todos isolados fúngicos obtidos são pertencentes ao filo Ascomycota, e estão inclusos dentro dos gêneros *Fusarium*, *Penicillium*, *Paecilomyces*, *Scopulariopsis*, *Scolecobasidium* e *Ilyonectria*. Espécies destes gêneros são comumente reportadas como autóctones de solos contaminados com hidrocarbonetos (AJELLO; MCGINNIS; CAMPER; 1977; ARANDA et al., 2017; GAŁĄZKA et al., 2020; AL-ZABAN; ALHARBI; MAHMOUD, 2021; HAMAD et al., 2021; KUMAR et al., 2023). Algumas espécies destes gêneros são cosmopolitas e/ou patogênicas (vegetal, animal e/ou humano); por exemplo*: F. oxysporum*, *Penicillium marneffei*, *Paecilomyces variotii*, *Scopulariopsis brevicaulis*, *Scolecobasidium humicola* e *Ilyonectria robusta* (AJELLO; MCGINNIS; CAMPER; 1977; LI et al., 2011; MAO; GUAN, 2016; MARCHAND et al., 2017; LAWRENCE; NOURI; TROUILLAS; 2019; KUMAR et al., 2023).

A partir da identificação macro- e micromorfológica permitiu a segregação inicial dos isolados em morfogrupos para melhor direcionar as próximas tomadas de decisão em pesquisas (por exemplo, selecionar fungos produtores de pigmentos ou descartar possíveis patógenos). É especulado que os isolados de *Fusarium*, *Penicillium* e *Paecilomyces* apresentaram mutações espontâneas observadas pela setorização em placas, provavelmente devido serem autóctones de solos contendo contaminantes tóxicos e mecanismos de resistência/tolerância de cada isolado (REYES-CÉSAR et al., 2014). Recomenda-se a caracterização das leveduras isoladas por meio de testes sobre sua capacidade de fermentar açúcares, crescimento em diferentes tipos de fontes de carbono e nitrogênio, e macromorfologia (SHETAIA et al., 2016; DERGUINE-MECHERI et al., 2018).

A identificação molecular de isolados fúngicos é uma etapa recomendada para garantir a elegibilidade da linhagem, e biossegurança de seu uso em bioprocessos (SILVA et al., 2023), além de ser uma alternativa para fungos que tem esporulação lenta ou ausente (por exemplo, isolado *Ilyonectria* sp. AF25D). A região ITS do DNAr é difundida como o universal marcador molecular para identificação de isolados fúngicos (WHITE et al., 1990). A identificação molecular via sequenciamento ITS dos isolados selecionados (n = 8) corroborou com a prévia identificação morfológica destas linhagens. Os isolados *Fusarium* sp. apresentaram maior similaridade com *F. acuminatum* e *F. oxysporum* que são potenciais patógenos vegetais, enquanto os isolados *Penicillium* sp. apresentaram maior similaridade com o fitopatógeno *P.*

ovetense (também denominado como *P. phoeniceum*) (FRISVAD; SAMSON; STOLK, 1990; VALDEZ et al., 2009; WANG et al., 2015; MARCHAND et al., 2017).

Dentre os isolados obtidos neste estudo, a maioria são linhagens de *Fusarium* provavelmente devido espécies deste gênero sobreviverem em diferentes camadas de solo durante décadas e/ou em condições anaeróbicas, o que está relacionado a alta resistência dos clamidósporos (STEINKELLNER; LANGER, 2004; SPINI et al., 2018; XIONG et al., 2017). O gênero *Fusarium* foi relatado (a partir de técnicas dependente e independente de cultura) como o mais abundante em solos contaminados e coletados a 1, 2 e 3 metros de profundidade quando enriquecidos com parafina, benzeno, pireno, fenantreno e naftaleno (SPINI et al., 2018).

O isolado AF64PD foi identificado em nível de espécie como *P. brasilianum,* uma linhagem autóctone de solos e plantas (endófito ou fitopatógeno) que pode produzir metabólitos com propriedades físico-químicas e biológicas interessantes para indústria alimentícia (por exemplo, enzimas hidrolíticas) e farmacêutica (por exemplo, antimicrobianos) (BAZIOLI et al., 2017).

Em suma, os gêneros *Fusarium* e *Penicillium* são polifiléticos e suas especiação geralmente é realizada pelo uso de combinação de diferentes marcadores moleculares (O'CALLAHAN et al., 2020; KHUNA et al., 2022). Neste sentido, é recomendado o uso de ITS (subunidade pequena do RNAr) combinado com alguns genes codificadores de proteínas, tais como β-tubulina (TUB2), calmodulina (CaM), fator de alongamento de tradução (TEF-1α), e RNA polimerase II (RPB1 e RPB2 da subunidade maior); para inferir a especiação dos isolados de *Fusarium* (STEINKELLNER; LANGER, 2004; KHUNA et al., 2022; SANTOS et al., 2022) e *Penicillum* (VISAGIE et al., 2014; GONÇALVES et al., 2019; O'CALLAHAN et al., 2020).

Em síntese, a literatura é expansiva sobre o uso de inóculos fúngicos pertencentes aos gêneros *Fusarium*, *Penicillium* e *Paecilomyces* em bioprocessos objetivados a remediação de hidrocarbonetos, enquanto os gêneros *Ilyonectria*, *Scopulariopsis* e *Scolecobasidium* foram pouco explorados (AL-OTIBI; AL-ZAHRANI; MARRAIKI, 2023; KUMAR et al., 2023; ; HAMAD et al., 2021; AL-HAWASH et al., 2018; GARZOLI et al., 2015). Por fim, a excreção de enzimas (hidrolíticas, oxidativas) e/ou biosurfactantes são os principais mecanismos envolvidos em bioprocessos de remediação de hidrocarbonetos a partir de espécies pertencentes a estes gêneros (GUPTA; PATHAK; RAVI, 2023; ARANDA et al., 2017; GARZOLI et al., 2015; SIMISTER et al., 2015).

5 CONCLUSÃO

Além de explorar a biodiversidade, o isolamento de fungos de solos tem contribuído para o desenvolvimento de bioprocessos. Aqui uma coleção de cultura contendo 58 fungos autóctones de solos REMA/UFSC (enriquecidos ou não) foi obtida. A identificação morfológica de 47 linhagens, e molecular de 8 linhagens via sequenciamento da região ITS revelou que estes isolados estão inclusos nos gêneros *Fusarium, Penicillium, Paecilomyces, Scolecobasidium, Ilyonectria* e *Scopulariopsis*; inclusive sugere-se que uma linhagem foi identificada a nível de espécie (*Penicillium brasilianum* AF64PD). O tipo de contaminante influenciou o isolamento de gêneros específicos, uma vez que as linhagens *Scolecobasidium* sp. AF33A e *Scopulariopsis* sp. AF34B foram isolados respectivamente em solos contaminados com diesel; e gasolina e etanol.

Algumas espécies de todos os gêneros supracitados já foram descritas como potencialmente patogênicas a humanos e/ou animais. Assim, é recomendado que seja realizada uma outra identificação molecular envolvendo o sequenciamento de outros marcadores (TUB2, CaM, TEF-1α, RPB1 e RPB2). Este fato é atribuído aos aspectos de biossegurança para uso destes isolados; embora comumente isto não seja realizado, uma vez diversas aplicações biotecnológicas tem sido reportadas a partir de inóculos fúngicos identificados somente a nível de gênero, e/ou isolados potencialmente patogênicos (QAZI et al., 2014; SHETAIA et al., 2016; AL-HAWASH et al., 2018; SENA et al., 2018). Por fim, os isolados deste estudo provavelmente podem ser usados como inóculos em futuros bioprocessos, uma vez que todos estão criopreservados de modo a garantir sua estabilidade genética e mesmas propriedades quando estavam prosperando na natureza.

CAPÍTULO IV

Bioprospecção de isolados fúngicos para produção de biosurfactantes: seleção de fontes de nitrogênio e perfil cinético

1 INTRODUÇÃO

Surfactantes são moléculas anfifílicas derivadas de petróleo que são comumente adicionadas em produtos de diversos mercados/indústrias e aplicações disponíveis (por exemplo: agroquímicos, construção civil, campo petrolífero, farmacêutica, limpeza, cuidado pessoal, alimentos, remediação ambiental) (DESAI; BANAT, 1997; JAHAN et al., 2020). Eles apresentam propriedades físico-químicas como atividade de superfície (redução da tensão superficial/interfacial), atividade emulsificante, alteração de solubilidade entre fases fluidas, e capacidade de automontagem (formação de micelas) (JAHAN et al., 2020; ONWOSI et al., 2021).

Alternativamente em substituição aos químicos, os biosurfactantes microbianos são promissoras moléculas que apresentam maior degradabilidade e menor toxicidade (BANAT et al., 2021; DESAI; BANAT, 1997). Biosurfactantes bacterianos tem sido mais explorados em comparação aos fúngicos (ELDIN; KAMEL; HOSSAM, 2019; SANTOS et al., 2017a). Os fungos produzem biosurfactantes com diversas estruturas químicas (por exemplo, soforolípidios, lipídios de manosileritritol, lipídios de celobiose, xilolipídios, lipídios de poliol) que apresentam diferentes potenciais para aplicações industriais e/ou ambientais (ALMEIDA et al., 2016; FARIAS et al., 2019; SILVA et al., 2021c). Biosurfactantes fúngicos podem apresentar relevantes propriedades biológicas (por exemplo, atividade antimicrobiana ou terapêutica) adicionadas as suas propriedades físico-químicas, as quais também podem ser superiores aos químicos (CHAPRÃO et al., 2015; ADNAN et al., 2018; GUERFALI et al., 2019). Entretanto, os fungos filamentosos têm sido menos explorados do que as leveduras para produção de biosurfactantes (SILVA et al., 2021c).

Neste contexto, os fungos são recursos biológicos que prosperam natureza e/ou estão registrados em Coleções de Culturas Microbianas; os quais tem sido explorados por meio de seu isolamento e bioprospecção para uso como inóculo em bioprocessos (BUENO et al., 2019; ELDIN; KAMEL; HOSSAM, 2019; MARTINHO et al., 2019). A bioprospecção de isolados fúngicos tem permitido a descoberta de novas linhagens produtoras de biosurfactantes (SILVA et al., 2019a); e/ou de estruturas de biosurfactantes não descritas anteriormente (JOSHI-NAVARE; SINGH; PRABHUNE, 2014; REIS et al., 2018). Em suma, a bioprospecção de isolados fúngicos de ambientes frios, solos contaminados e plantas de mangue tem revelado potenciais inóculos para produção de biosurfactantes, após eles terem sido triados por meio de técnicas de colapso da gota, índice de emulsificação, hidrofobicidade celular e tensiometria (YALÇIN; ERGIN-TEPEBAŞI; UYAR, 2018; BUENO et al., 2019; MARTINHO et al., 2019).

Em geral, o baixo rendimento ou produtividade de fungos filamentosos é um dos principais desafios a serem superados no bioprocessamento de biosurfactantes (SILVA et al., 2021c). Diversas tecnologias foram desenvolvidas para aumentar o rendimento, ou propor/testar melhores condições nutricionais ou operacionais para produção de biosurfactantes usando inóculo fúngico (ALMEIDA et al., 2017; SÁNCHEZ-VÁZQUEZ et al., 2018). Por exemplo, o estudo do comportamento cinético envolvido nesta produção, é uma alternativa para avaliar e implementar futuros bioprocessos com maior rentabilidade, uma vez que torna possível estimar o tempo necessário para alcançar maior rendimento.

É sabido que o rendimento, composição e propriedades físico-químicas de biosurfactantes fúngicos são influenciados de acordo com a disponibilidade e razão de fontes de carbono e nitrogênio (C/N) no meio de cultura (JOSHI-NAVARE; SINGH; PRABHUNE, 2014; ISHAQ et al., 2015; ALMEIDA et al., 2017). A formulação de meios de cultura contendo extrato de levedura (meio complexo) e/ou nitrato de sódio (meio quimicamente definidos) como fonte de nitrogênio para crescimento de fungos produtores de biosurfactantes, promoveu diferenças nas velocidades de crescimento, consumo de substrato e produção de biosurfactantes (BECK; ZIBEK, 2020).

Neste contexto, nossa hipótese é que isolados fúngicos de solos contaminados com hidrocarbonetos, solos antárticos e endofíticos de plantas de mangue produzem biosurfactantes, e o perfil cinético desta produção é influenciado pelo uso e concentração de extrato de levedura ou nitrato de sódio. Neste contexto, esta pesquisa objetivou prospectar fungos filamentosos (alguns inexplorados) dos ambientes supracitados para produção de biosurfactantes; e propor a formulação de três meios de cultura contendo tais fontes de nitrogênio em diferentes concentrações para avaliar a ação surfactante de linhagens selecionadas, e estimar o perfil cinético desta produção usando a tensiometria.

2 MATERIAL E MÉTODOS

2.1 ISOLADOS FÚNGICOS

Um total de 118 isolados de fungos filamentosos foram triados para biosurfactantes usando uma coleção de cultura de isolados fúngicos de solos contaminados com hidrocarbonetos (n=65) e solos antárticos (n=46), que é mantida no Laboratório de Microrganismos e Processos Biotecnológicos (LAMPB); e endofíticos de mangue da Ilha de Santa Catarina (n=7) mantidos na Coleção Steindel (BASTOS, 2022; CAVALCANTE, 2022).

Essas duas coleções de culturas estão localizadas no Departamento de Microbiologia, Imunologia e Parasitologia (MIP) da Universidade Federal de Santa Catarina (UFSC), Brasil. Os isolados são pertencentes aos gêneros *Fusarium* (n=40), *Penicillium* (n=18), *Pseudogymnoascus* (n=12), *Paecilomyces* (n=4), *Thelebolus* (n=3), *Aspergillus* (n=2), *Trichoderma* (n=2), *Cladosporium* (n=1), *Scolecobasidium* (n=1), *Scopulariopsis* (n=1), *Curvularia* (n=1), *Buergenerula* (n=1). Os isolados fúngicos estavam mantidos a -80°C em glicerol e foram reativados em placas contendo meio BDA, as quais foram incubadas a 25°C durante 3 a 10 dias.

2.2 TRIAGEM PRIMÁRIA: CONDIÇÕES DE CULTIVO E AMOSTRAGEM

As linhagens foram cultivadas em meio de cultura líquido para uma triagem prévia de biosurfactantes em amostras isentas de células (SILVA et al., 2021a). Três porções ativas das colônias fúngicas previamente crescidas em BDA foram transferidas (via alça de platina) para frascos Erlenmeyer de 250 mL contendo 50 mL de meio de cultura composto por ($g \bullet L^{-1}$) $C_6H_{12}O_6$ (40), extrato de levedura (0,5), KH_2PO_4 (0.2), $MgSO_4 \bullet 7H_2O$ (0.2), óleo de soja (0,5%); com 1 $mL \bullet L^{-1}$ de solução de oligoelementos contendo ($mg \bullet L^{-1}$) $FeSO_4 \bullet 7H_2O$ (0,63), $MnSO_4$ (0.01), $ZnSO_4$ (0,62) (PELE et al., 2019). O meio de cultura foi previamente esterilizado por autoclavagem (121° C, 15 min). Os cultivos fúngicos foram mantidos em agitador orbital (130 rpm, 25 °C) por 7 a 28 dias, e depois submetidos a centrifugação (10.000 rpm, 30 mL, 10 min) para que os sobrenadantes livres de células fossem usados como amostras em ensaios de triagem.

2.3 METODOLOGIAS DE TRIAGEM: ÍNDICE DE EMULSIFICAÇÃO, COLAPSO DA GOTA E MENSURAÇÃO DA TENSÃO SUPERFICIAL

A presença de biosurfactantes foi detectada em amostras por meio de técnicas como índice de emulsificação, colapso da gota e mensuração da tensão superficial (AL-HAWASH et al., 2018a; KUMAR et al., 2023).

Em tubo de vidro, 2 mL de amostra e 2 mL de óleo diesel comercial foram adicionados e submetidos sequencialmente a agitação vórtex por 2 min e condição estática por 24h. O índice de emulsificação foi calculado (em %) por meio da razão entre a altura da fase emulsionada e altura total da camada líquida, multiplicando por 100. O teste colapso da gota foi realizado por meio de gotejamento de 20 µL de amostras sobre a superfície hidrofóbica de Parafilm-M

(produto comercial) (YALÇIN; ERGIN-TEPEBAŞI; UYAR, 2018). A forma da gota foi observada após 1 min, o diâmetro (em cm) da gota foi mensurado e comparado (via subtração) com o da amostra controle. A medição da tensão superficial (em mNm^{-1}) foi realizada (em triplicata) por meio da metodologia gota pendente em goniômetro Ramé-Hart Instrument Co. (Modelo 250-F1) usando o programa DROPimage Advanced (REIS et al., 2018) para analisar 10 vezes (com intervalo de 1 s) o perfil de curvatura da gota pendente formada na extremidade da agulha. Entre cada sucção de amostras, a seringa foi lavada (3x) com água destilada. Em todos os ensaios de triagem, volumes iguais do surfactante docecil sulfato de sódio (SDS 1%) e meio de cultura esterilizado foram usados como controle positivo e negativo, respectivamente.

A porcentagem de Redução da Tensão Superficial (RTS) foi calculada para melhor estimar/especular o potencial de isolados fúngicos para produção de biosurfactantes, conforme a equação 1:

$$RTS\ (\%) = \frac{(TS_b - TS_c)}{TS_c} * 100 \quad (1)$$

Onde TS_b corresponde a tensão superficial do meio esterilizado; e TS_c corresponde a tensão superficial da amostra.

2.4 TRIAGEM SECUNDÁRIA: SELEÇÃO DE LINHAGENS, FORMULAÇÃO DE MEIO DE CULTURA E COMPORTAMENTO CINÉTICO

Dezesseis isolados foram selecionados (Tabela 7) para uma triagem secundária usando três meios de culturas contendo extrato de levedura ou $NaNO_3$ para avaliar a influência da fonte de nitrogênio no perfil cinético da RTS. Os critérios para esta seleção incluíram isolados que: (1) apresentaram maiores resultados de RTS na triagem primária; (2) são pertencentes a gêneros pouco explorados para biosurfactantes; (3) foram preferencialmente identificados via sequenciamento molecular ITS; ou (4) potencialmente não patógenos humanos e animais (por exemplo, o isolado *Scolecobasidium* sp. AF33A (PRENAFETA-BOLDÚ; HOOG; SUMMERBELL, 2019).

A composição do meio de cultura para produção de biosurfactantes não tem necessariamente a mesma para o crescimento de biomassa. O desenvolvimento apropriado e/ou preliminar do inóculo pode permitir melhor desempenho na produção de biosurfactantes (SILVA et al., 2021a).

Para crescimento de biomassa, um pré-inóculo foi preparado a partir da transferência de três porções ativa da colônia (aproximadamente 1 cm²) linhagem crescida em meio BDA (28 °C, 7 dias), para frascos Erlenmeyer de 250 mL contendo 50 mL de meio de cultura X ou Y ou N. Estes meios foram formulados a partir de alterações na composição proposta por Azin, Moghimi e Heidarytabar (2018) e Qazi et al. (2014). Os meios de cultura X, Y e N foram constituídos por ($g•L^{-1}$) glicose (10,0), KH_2PO_4 (3,0), $MgSO_4.7H_2O$ (3,0), $FeSO_4.7H_2O$ (0,3), $MnSO_4.H_2O$ (0,01); $ZnSO_4$ (0,01); e $CuSO_4$ (0,028). No entanto, os meios X e Y contém extrato de levedura em concentrações 0.05 e 5.0 $g•L^{-1}$, respectivamente; enquanto o meio N contém 5,0 $g•L^{-1}$ de $NaNO_3$. As culturas foram mantidas em agitador orbital (130 rpm, 25 °C, 7 dias) para produção do inóculo.

Para produção de biosurfactantes, um inóculo de 10% (v/v) foi transferido para frascos Erlenmeyer (250 mL) contendo 100 mL dos meios de cultura X, Y e N; porém para tal propósito, estes meios foram suplementados com o dobro de glicose (20 gL^{-1}) e óleo de soja comercial 0,5% (v/v). As culturas foram mantidas em agitador orbital (130 rpm, 25° C, 7 dias), e submetidas a centrifugação (10.000 rpm, 2 mL, 10 min) para amostragem de sobrenadantes isentos de células, a cada 24 h entre o intervalo de 72 h a 168 h. A presença de biosurfactantes foi mensurada pela tensão superficial das amostras, e estimada pelo RTS conforme realizado na triagem primária.

2.5 ANÁLISE ESTATÍSTICA

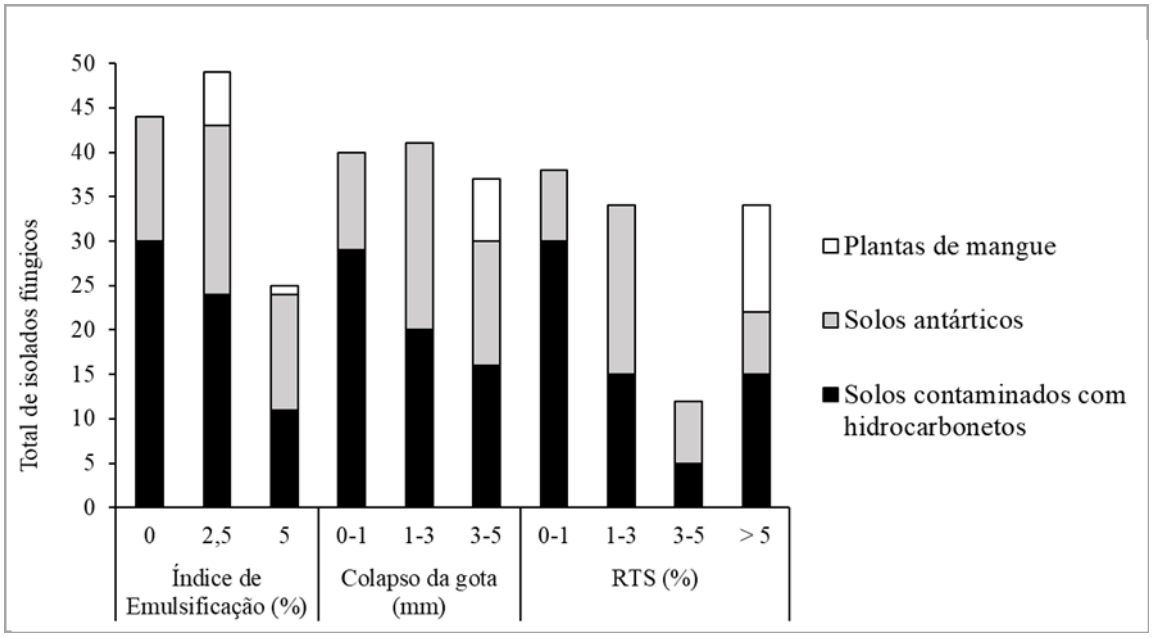
Os dados quantitativos obtidos da triagem primária foram submetidos a análise estatística descritiva por meio do coeficiente de correlação de Pearson para verificar a relação entre os resultados das variáveis dependentes, usando o programa Microsoft Office Excel 2016. As análises estatísticas dos resultados da triagem secundária foram realizadas usando o programa estatístico R (R CORE TEAM, 2022). Estes dados foram submetidos à análise de homogeneidade de variância pelo teste de Bartlett e ao teste de normalidade de resíduos (Shapiro-Wilk) para posterior análise de variância (ANOVA) pelo teste Scott-Knott com um intervalo de confiança a 5% de probabilidade de erro. Os gráficos, disposição dos erros padrão da média e as regressões polinomiais (2° ordem) também foram gerados por meio do programa R, usando os pacotes “ggplot2” (WICKHAM, 2016), “ggthemes” (ARNOLD, 2021), “RColorBrewer” (NEUWIRTH, 2022), “ggpmisc” (APHALO, 2017) e “AgroR” (SHIMIZU; MARUBAYASHI; GONCALVES, 2022).

3 RESULTADOS

3.1 TRIAGEM PRIMÁRIA: UMA COMBINAÇÃO DE TÉCNICAS

Um total de 118 fungos isolados de solos contaminados com hidrocarbonetos (n=65), solos antárticos (n=46) e plantas de mangue (n=7) foram triados para produção de biosurfactante, usando os testes índice de emulsificação, colapso da gota, e tensiometria (Apêndice F- Tabela S6). Isolados destes três ambientes apresentaram resultados positivos para triagem de biosurfactantes (Figura 20).

Figura 20 – Resultados da triagem primária em função da distribuição em números de isolados em relação as fontes de isolamento por cada método colapso da gota (COG), índice de emulsificação (IE) e redução da tensão superficial (RTS)



Fonte: Elaborado pelo autor (2023).

O teste colapso da gota/Parafilm-M avaliou indiretamente a redução da tensão superficial da cultura fúngica. A gota que continha biosurfactantes colapsou (ou seja, aumentou seu diâmetro ou deformou-se) quando interagiu com a superfície hidrofóbica de Parafilm-M (produto comercial), provavelmente devido à redução da tensão superficial nas interfaces liquido/sólido (YALÇIN; ERGIN-TEPEBAŞI; UYAR, 2018). O teste Parafilm-M permitiu classificar os isolados fúngicos em três níveis 0-1, 1-3 e 3-5 mm de diâmetros colapsados, contendo aproximadamente 40 isolados em cada. A deformação da gota de 5 mm foi alcançada

por 16 isolados fúngicos pertencentes aos gêneros *Penicillium* sp. P10R5, P10R6, P10R7, Seção Glabra e *P. brasilianum* AF64PD; *Aspergillus* sp. SC21P3 e Seção Flavi; *Pseudogymnoascus* sp. SC26P3 e SC23P3, *Trichoderma* sp. P05R2 e RASC1B10122, *Curvularia* sp. ILRR1A20047, e *Fusarium* sp. AF99PD. As linhagens codificadas NU30, ACF51 e ACF49 (todas isoladas de solos antárticos) também apresentaram colapso da gota de 5 mm (Apêndice F- Tabela S6).

O índice de emulsificação detectou a presença de biosurfactantes/bioemulsificantes após o cultivo fúngico, por meio da capacidade destas biomoléculas em emulsificar o composto hidrofóbico disponibilizado (ou seja, óleo diesel) (ELDIN; KAMEL; HOSSAM, 2019). Os maiores índices de emulsificação alcançados foram de 5% e 2,5% para 25 e 49 isolados, respectivamente (Figura 20). As linhagens que apresentaram índice de emulsificação de 5% são pertencentes aos gêneros *Penicillium* sp. SCUV02.P1, AF13D, P10R5, P10R6, P10R7 e *P. brasilianum* AF64PD, *Fusarium* sp. AF99PD, AF7D, AF27D e AF54DD, *Pseudogymnoascus* sp. SC26P3, NU04, SC10P3 e NU26, *Trichoderma* sp. P05R2 e RASC1B10122, *Ilyonectria* sp. AF25D e *Thelebolus* sp. SC29P3. As linhagens não identificadas ACF51, ACF54, NU03, ACF49, NU12 também apresentaram índice de emulsificação de 5% (Apêndice F- Tabela S6).

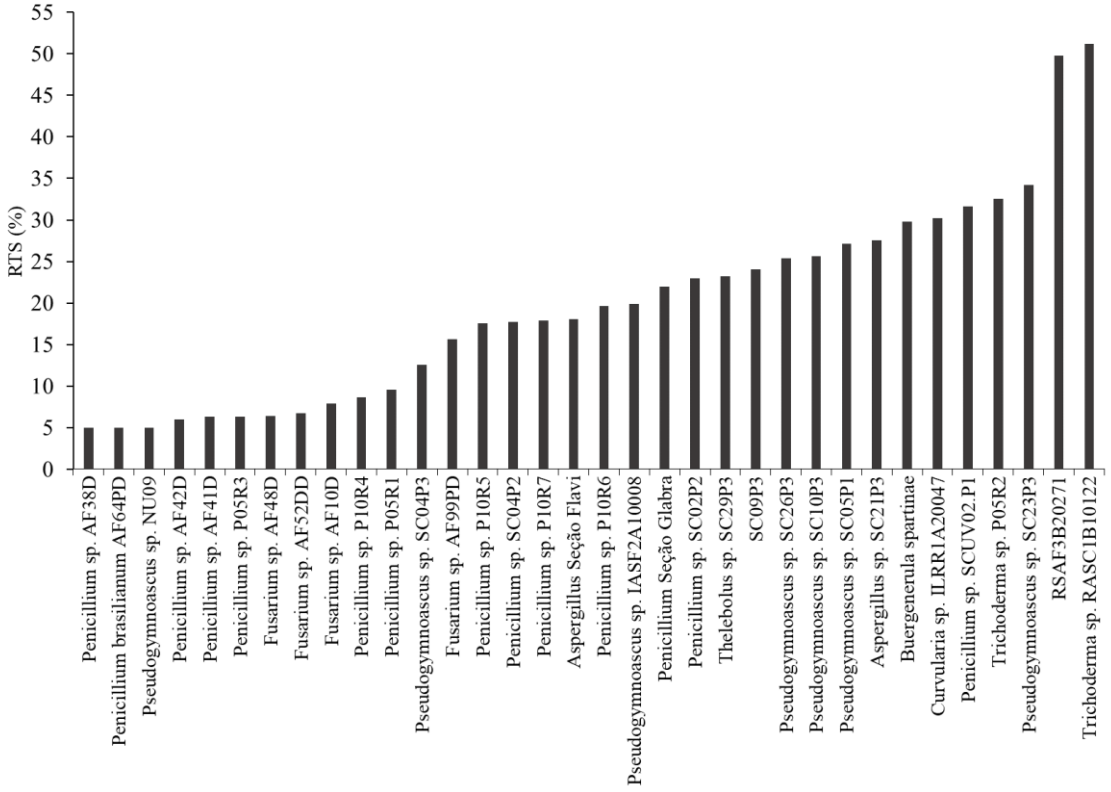
A tensiometria detectou a presença de biosurfactantes após o cultivo fúngico, por meio da capacidade destas moléculas em reduzir a tensão superficial do meio de cultura (AL-HAWASH et al., 2018a). A tensão superficial do meio de cultura esterilizado (controle) foi mensurada em 68 $mN \bullet m^{-1}$. A RTS foi o parâmetro usado para segregar as linhagens, uma vez que o aumento do valor de RTS pode indicar "alta ação surfactante" (ELDIN; KAMEL; HOSSAM, 2019).

O isolados *Paecilomyces* sp. AF45D, *Cladosporium* sp. NU07, *Scolecobasidium* sp. 33A e *Scopulariopsis* sp. 34B tiveram a RTS inferior a 4%. As duas primeiras linhagens supracitadas alcançaram índice de emulsificação de 2,5%, e colapso da gota de 4 e 2 mm, respectivamente (Apêndice F- Tabela S6).

Dentre os 34 isolados que apresentaram o potencial RTS superior a 5%, somente 6 apresentaram RTS superior a 30%, o que representa uma tensão superficial inferior a 47,60 $mN \bullet m^{-1}$ (Figura 21). Os principais isolados de cada gênero triado que apresentaram maior RTS foram *Trichoderma* sp. RASC1B10122 (51,2% - 33,1 $mN \bullet m^{-1}$), *Pseudogymnoascus* sp. SC23P3 (34,2% - 44,7 $mN \bullet m^{-1}$), *Penicillium* sp. SCUV02.P1 (31,6% - 46,4 $mN \bullet m^{-1}$), *Curvularia* sp. ILRR1A20047 (30,2% - 47,4 $mN \bullet m^{-1}$), *Buergenerula spartinae* (29,8% - 47,7 $mN \bullet m^{-1}$), *Aspergillus* sp. SC21P3 (27,5% - 49,3 $mN \bullet m^{-1}$), *Thelebolus* sp. SC29P3 (23,2% - 52,1 $mN \bullet m^{-1}$) e *Fusarium* sp. AF99PD (15,6% - 57,35 $mN \bullet m^{-1}$). As linhagens não identificadas

RSAF3B20271 e SC09P3 apresentaram RTS de 49,7% (34,1 mN•m^{-1}) e 24% (51,6 mN•m^{-1}), respectivamente (Figura 21).

Figura 21 – Resultado da triagem primária para isolados que apresentaram RTS ≥ 5%



Fonte: Elaborado pelo autor (2023).

Os maiores valores de RTS foram alcançados por linhagens isoladas de solos antárticos e endofíticos de mangue, embora um maior número de isolados de solos contaminados foram triados (Figura 20). Todos isolados de planta de mangue apresentaram RTS superior a 18%; ou colapso da gota de 3 a 5 mm (Apêndice F, Tabela S6). As linhagens *Trichoderma* sp. P05R2 e RASC1B10122, e *Pseudogymnoascus* sp. SC23P3 correlacionaram os três maiores resultados alcançados na triagem. Os resultados numéricos das três metodologias para triagem de biosurfactantes, apresentaram correlações de Pearson positiva variando entre 0,39-0,64 (Tabela 8), sendo a maior correlação entre o teste colapso da gota e índice de emulsificação, e a menor entre a tensiometria e índice de emulsificação.

Tabela 7 – Correlação de Pearson entre os testes de triagem

Teste	Índice de emulsificação	Colapso da gota	Tensiometria
Índice de emulsificação	-	0,64	0,39
Colapso da gota	-	-	0,57
Tensiometria	-	-	-

Fonte: Elaborado pelo autor (2023).

3.2 TRIAGEM SECUNDÁRIA: INFLUÊNCIA DA FONTE DE NITROGÊNIO E CINÉTICA DE PRODUÇÃO DE SURFACTANTE

Dezesseis isolados fúngicos foram selecionados para triagem secundária (Tabela 8), a qual foi realizada para avaliar a influência de uma fonte orgânica (extrato de levedura) ou inorgânica ($NaNO_3$) de nitrogênio para produção de biosurfactantes fúngicos.

Tabela 8 – Isolados fúngicos selecionados para triagem secundária

Isolado fúngico[a]	**Fonte de isolamento**	**Sequências ITS/ Genbank acesso**	**Referência**
Curvularia sp. ILRR1A20047	Planta de mangue	Ainda não submetido	Bastos, 2022
Buergenerula spartinae	Planta de mangue	OQ300436	Bastos, 2022
Trichoderma sp. P05R2	Solo contaminado com hidrocarbonetos	Ainda não submetido	Ainda não publicado
Trichoderma sp. RASC1B10122	Planta de mangue	Ainda não submetido	Bastos, 2022
Pseudogymnoascus sp. SC23P3[a]	Solo antártico	-	Cavalcante, 2022
Pseudogymnoascus sp. NU09	Solo antártico	OQ286516	Cavalcante, 2022
Penicillium sp. P10R6[a]	Solo contaminado com hidrocarbonetos	-	Ainda não publicado
Penicillium sp. AF42D	Solo contaminado com hidrocarbonetos	OQ282836	Capítulo IV
Penicillium sp. AF38D	Solo contaminado com hidrocarbonetos	OQ282834	Capítulo IV
Penicillium sp. AF41D	Solo contaminado com hidrocarbonetos	OQ282835	Capítulo IV
Penicillium brasilianum AF64PD	Solo contaminado com hidrocarbonetos	OQ282833	Capítulo IV
Penicillium sp. SCUV02.P1	Solo antártico	OP603793	Cavalcante, 2022
Fusarium sp. AF10D	Solo contaminado com hidrocarbonetos	OQ282829	Capítulo IV
Fusarium sp. AF48D	Solo contaminado com hidrocarbonetos	OQ282830	Capítulo IV
Fusarium sp. AF52DD	Solo contaminado com hidrocarbonetos	OQ282831	Capítulo IV
Fusarium sp. AF99PD	Solo contaminado com hidrocarbonetos	OQ282832	Capítulo IV

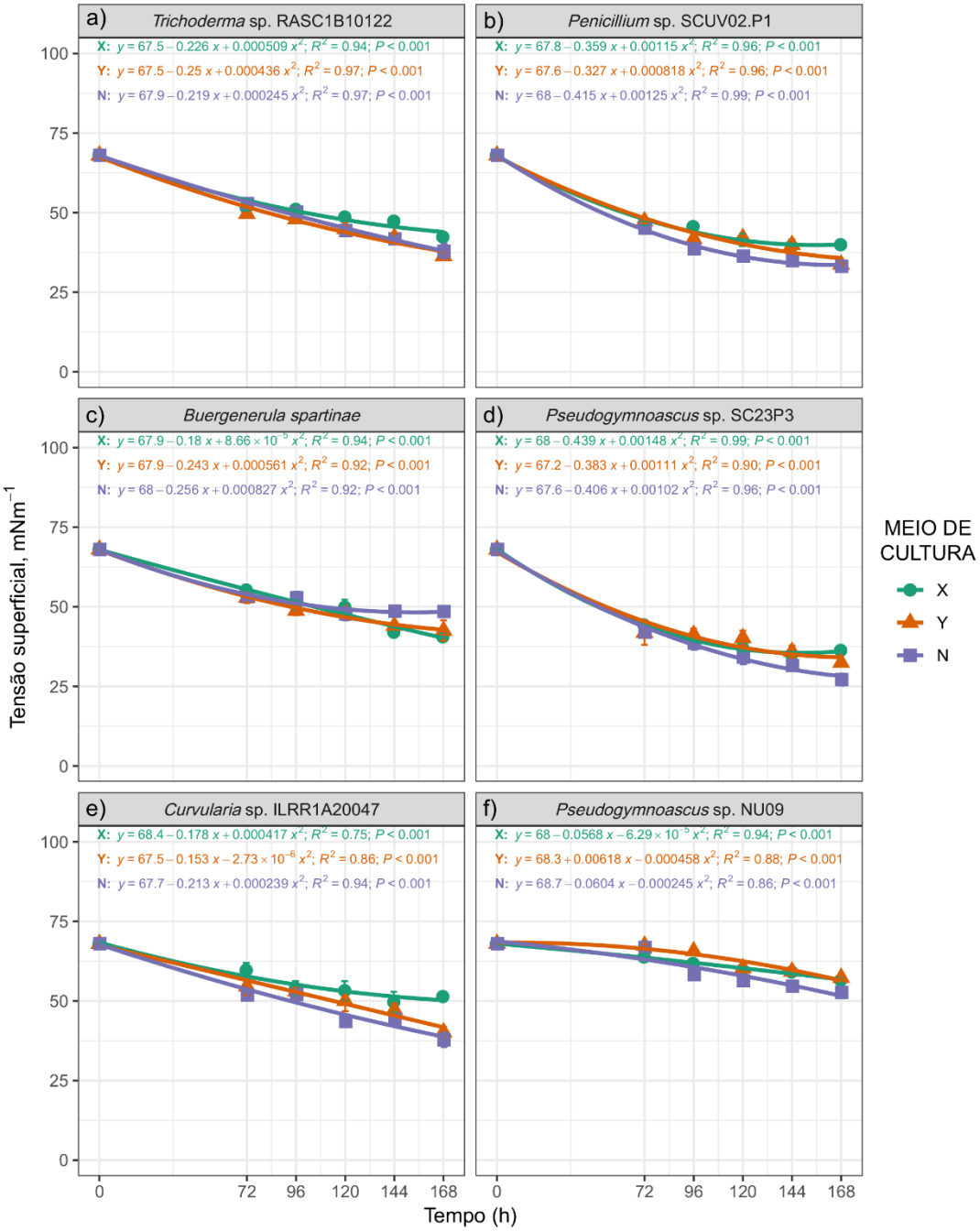
[a] identificado por macro- e micromorfologia.
Fonte: Elaborado pelo autor (2023).

A tensiometria foi usada para avaliar a ação surfactante da biomolécula produzidas, e para estimar o perfil cinético desta produção em 72 h, 96 h, 120 h, 144 h e 168h usando três meios de cultura contendo extrato de levedura (Meio X e Meio Y) ou $NaNO_3$ (Meio N) em concentração 0,5 g•L^{-1} (Meio X) ou 5 g•L^{-1} (Meio Y e N) (Figura 22, 23 e 24; Apêndice G e H, Tabela S7 e S8). A análise de variância (ANOVA) com teste Scott-Knott (95% de confiança) foi realizada para determinar a significância estatística dos fatores linhagem, meio de cultura e tempo nesta produção (Apêndice I, Tabela S9).

Em 72h, as linhagens *Trichoderma* sp. P05R2 (Fig. 22d), *Pseudogymnoascus* sp. SC23P3 (Fig. 23a), e *Penicillium* sp. SCUV02.P1 (Fig. 22b) apresentaram uma significativa redução da tensão superficial em relação ao início do cultivo, porém esta redução não diferiu significativamente para nenhum meio. Em geral, estes isolados alcançaram os menores valores de tensão superficial (em mN•m^{-1}) em meio X (36,2 - 46,4), Y (38,7 - 47,3) ou N (35,8 - 45,1) em 72h, embora a redução da tensão superficial da cultura de *Trichoderma* sp. P05R2 foi a menor e significativamente diferente de *Pseudogymnoascus* sp. SC23P3 e *Penicillium* sp. SCUV02.P1. A cultura de *Penicillium* sp. SCUV02.P1 não apresentou redução significativa da tensão superficial em meio X (41,4 mN•m^{-1}) a partir de 120h, e em meio N (34,9 mN•m^{-1}) a partir de 144h; enquanto a cultura *Trichoderma* sp. P05R2 usando meio X (28,9 mN•m^{-1}) e N (26,7 mN•m^{-1}) a partir de 144h.

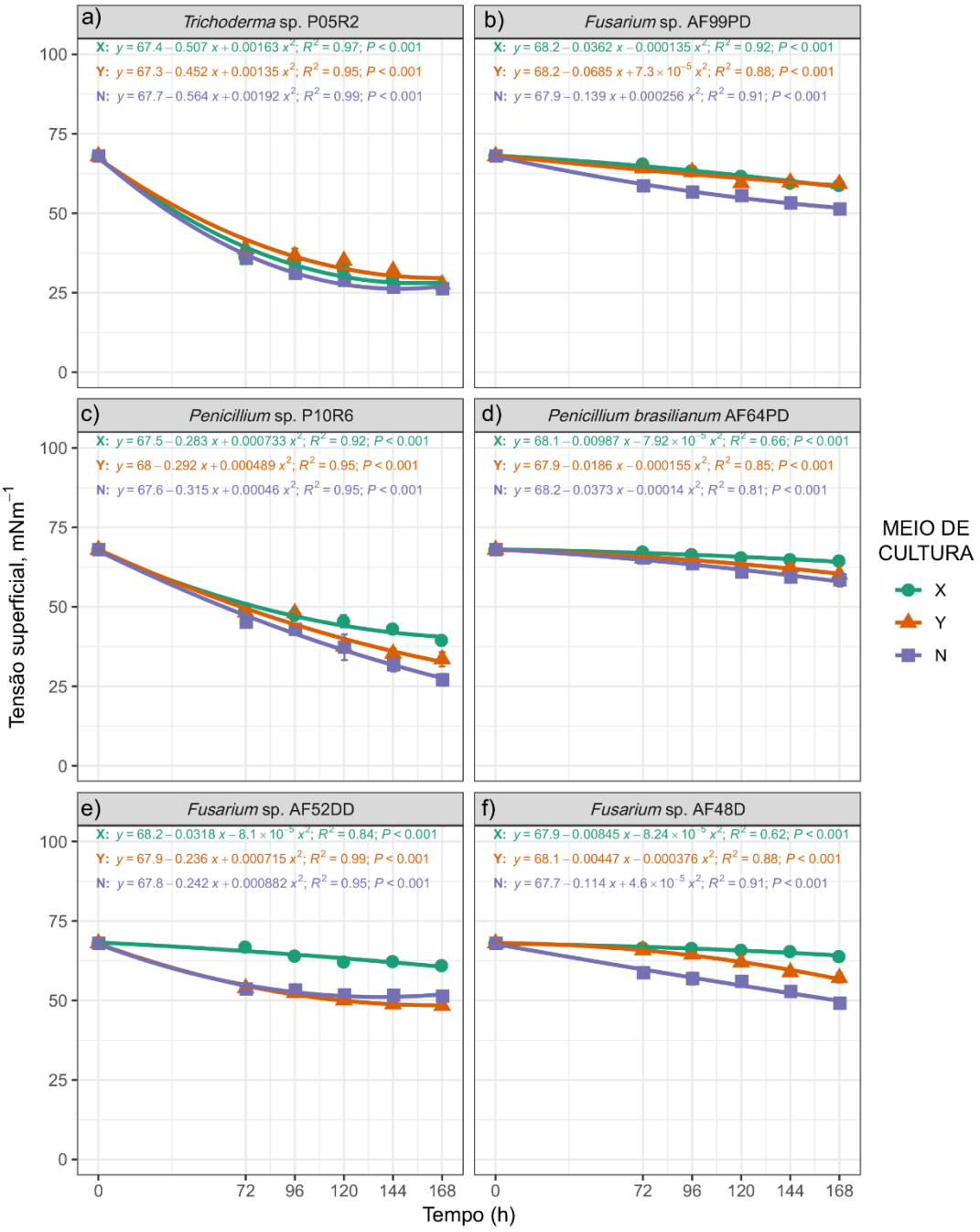
Em 168h, a cultura de *Trichoderma* sp. P05R2, *Pseudogymnoascus* sp. SC23P3, *Penicillium* sp. P10R6 (Fig. 23c) e SCUV02.P1 apresentaram os menores valores de tensão superficial nos meios X (26,9 - 39,9 mN•m^{-1}), Y (27,6 - 33,7 mN•m^{-1}) ou N (26,3 - 33,1 mN•m^{-1}). Não houve diferença significativa da tensão superficial da cultura *Trichoderma* sp. P05R2 em relação aos meios X (26,9 mN•m^{-1}), Y (27,6 mN•m^{-1}) e N (26,3 mN•m^{-1}). Similarmente, não houve diferença significativa entre *Penicillium* sp. P10R6 (39,3 mN•m^{-1}) e SCUV02.P1 (39,9 mN•m^{-1}) usando meio X, enquanto entre *Pseudogymnoascus* sp. SC23P3 (32,4 mN•m^{-1}), *Penicillium* sp. P10R6 (33,5 mN•m^{-1}) e SCUV02.P1 (33,7 mN•m^{-1}) usando meio Y; e *Pseudogymnoascus* sp. SC23P3 (27,1 mN•m^{-1}), *Penicillium* sp. P10R6 (27,0 mN•m^{-1}) e *Trichoderma* sp. P05R2 (26,3 mN•m^{-1}) usando meio N. A partir de 96h, a cultura de *Pseudogymnoascus* sp. SC23P3 não apresentou redução significativa da tensão superficial usando meio X (39,0 mN•m^{-1}).

Figura 22 – Tensão superficial do sobrenadante produzidos nos cultivos de *Trichoderma* sp. RASC1B10122 (a), *Penicillium* sp. SCUV02.P1 (b), *Buergenerula spartinae* (c), *Pseudogymnoascus* sp. SC23P3 (d), *Curvularia* sp. ILRR1A20047 (e) e *Pseudogymnoascus* sp. NU09 (f) a cada 24h entre o tempo 72h e 168h, usando os meios X, Y e N (130 rpm, 25 ºC)



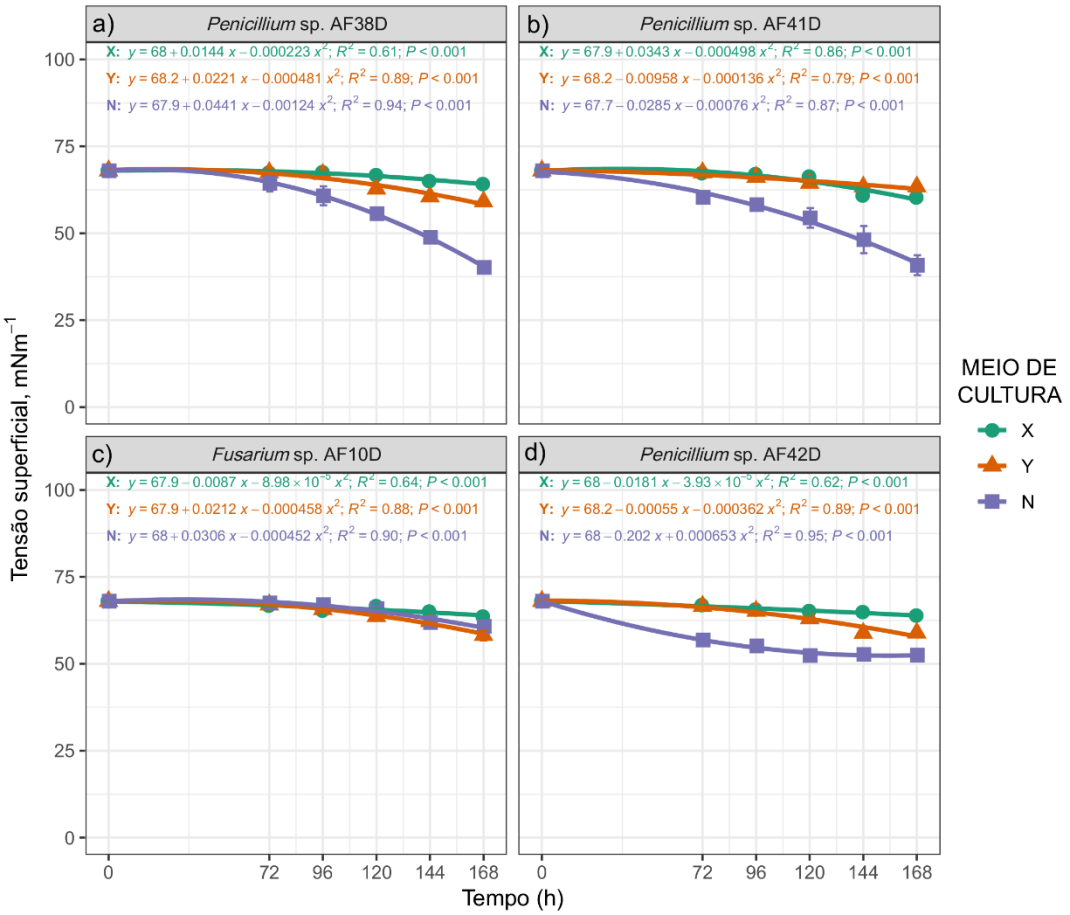
Fonte: Elaborado pelo autor (2023).

Figura 23 – Tensão superficial do sobrenadante produzidos nos cultivos cultivo de *Trichoderma* sp. P05R2 (a), *Fusarium* sp. AF99PD (b), *Penicillium* sp. P10R6 (c), *Penicillium brasilianum* AF64PD (d), *Fusarium* sp. AF52DD (e) e *Fusarium* sp. AF48D (f) a cada 24h entre o tempo 72h e 168h, usando os meios X, Y e N (130 rpm, 25 °C)



Fonte: Elaborado pelo autor (2023).

Figura 24 – Tensão superficial do sobrenadante produzidos nos cultivos cultivo de *Penicillium* sp. AF38D (a), *Penicillium* sp. AF41D (b), *Fusarium* sp. AF10D (c), e *Penicillium* sp. AF42D (d) a cada 24h entre o tempo 72h e 168h, usando os meios X, Y e N (130 rpm, 25 °C)



Fonte: Elaborado pelo autor (2023).

As culturas de *Buergenerula spartinae* (Fig. 22c) e *Trichoderma* sp. RASC1B10122 (Fig. 22a) também apresentaram respectivamente baixos valores de tensão superficial e altos de RTS usando meio X (40,6 mN•m^{-1}; 40%) e Y (36,4 mN•m^{-1}; 46%) em 168h; embora *B. spartinae* apresentou diferença significativa quando cultivada em meio N (48,5 mN•m^{-1}), e *Trichoderma* sp. RASC1B10122 quando cultivada em meio X (42,3 mN•m^{-1}). A partir de 120h, a cultura de *B. spartinae* não apresentou redução significativa da tensão superficial usando meio N (47.7 mN•m^{-1}), enquanto a partir de 144h usando o meio X (41,9 mN•m^{-1}) e Y (44,0 mN•m^{-1}).

As culturas de *Curvularia* sp. ILRR1A20047 (Fig. 22e) também apresentaram baixos valores de tensão superficial e altos de RTS usando o meio Y (40,2 mN•m^{-1}; 41%) e N (37,7 mN•m^{-1}; 45%) diferindo significativamente do meio X (51,3 mN•m^{-1}, 24%) em 168 h, embora a partir de 144 h não houve redução significativa no meio X (49,6 mN•m^{-1}). Além disso, as

culturas de *Penicillium* sp. AF38D (Fig. 24a) e AF41D (Fig. 24b) apresentaram baixos valores de tensão superficial e altos de RTS usando o meio N (40,1 - 40,8 mN•m^{-1}; média 41%) diferindo significativamente do meio X (64,1 – 60,2 mN•m^{-1}; média 8%) e Y (59,0 - 63,3 mN•m^{-1}; média 10%) em 168 h.

Por outro lado, as culturas de *Penicillium brasilianum* AF64PD (Fig. 23d) e *Penicillium* sp. AF42D (Fig. 24d) apresentaram os altos valores de tensão superficial (em mN•m^{-1}) usando os meios X (64,3 - 63,8), Y (60,2 - 58,8) e N (58,5 - 52,5) em 168h, o que representa uma média de RTS em cada meio de 6%, 13% e 18%, respectivamente; embora o meio N promoveu uma houve uma diferença significativa entre estas linhagens. A cultura de *Penicillium* sp. AF38D também apresentou altos valores de tensão superficial e baixos de RTS usando os meios X (64,1 mN•m^{-1}; 6%) e Y (59,0 mN•m^{-1}; 13%) em 168h, enquanto aquela de *Fusarium* sp. AF10D (Fig. 24c) usando os meios X (63,5 mN•m^{-1}, 7%) e N (60,7 mN•m^{-1}, 11%). Similarmente, a linhagem *Fusarium* sp. AF48D (Fig. 23f) apresentou altos valores de tensão superficial usando meio X (63,82 mN•m^{-1}, 6%) em 168h, enquanto *Penicillium* sp. AF41D e *Fusarium* sp. AF99PD (Fig. 23b) usando meio Y (63,36 - 59,23 mN•m^{-1}); e *Pseudogymnoascus* sp. NU09 (Fig. 22f) e *Fusarium* sp. AF52DD (Fig. 23e) usando meio N (52,66 - 51,36 mN•m^{-1}). A tensão superficial da cultura de *Penicillium* sp. AF38D e AF42D, *Penicillium brasilianum* AF64PD, *Fusarium* sp. AF10D e AF48D em meio X não apresentou diferença significativa ao longo do experimento.

É válido reportar que a tensão superficial da cultura de *Trichoderma* sp. RASC1B10122 em meio X, Y e N ainda tende diminuir após 168h provavelmente devido a diferença significativa em relação àquela mensurada em 144h. Este mesmo fato ocorre para *Pseudogymnoascus* sp. NU09 em meio X; *Penicillium* sp. SCUV02.P1 e *Fusarium* sp. AF10D em meio Y; *Fusarium* sp. AF48D, *Penicillium* sp. AF38 e AF41D em meio N; *Penicillium* sp. P10R6 em meio X e N; e *Curvularia* sp. ILRR1A20047 e *Pseudogymnoascus* sp. SC23P3 em meio Y e N.

4 DISCUSSÃO

Neste capítulo foi hipotetizado que fungos isolados de solos contaminados com hidrocarbonetos, solos antárticos e plantas de mangue produzem biosurfactantes. Esta hipótese é fundamentada na premissa que os biosurfactantes são resultados da ecologia e fisiologia microbiana para sua colonização no ambiente. Esse fato é atribuído a capacidade destas moléculas em reduzir a tensão superficial/interfacial entre fases líquido-sólido-vapor, o que

aumenta a disponibilidade de substratos para absorção e metabolismo microbiano (DESAI; BANAT, 1997; SILVA et al., 2021a). Por exemplo, o isolado *Aspergillus* sp. RFC-1 excretou biosurfactantes e teve sua hidrofobicidade celular aumentada com o fornecimento de hidrocarboneto no meio de cultura (AL-HAWASH; ZHANG; MA, 2019). O endófito *Trichoderma camerunense* (de planta de mangue), além de excretar celulases, xilanases e lacases, também excretou biosurfactantes que foram especulados como uma resposta do metabolismo fúngico para aumentar a digestibilidade de biomassa vegetal (MARTINHO et al., 2019). Além disso, a viabilidade celular de *Phoma herbarum* CCFEE 5080 isolado de solo antártico foi aumentada quando a linhagem foi submetida a repetidos ciclos de congelamento-degelo na presença de seu biosurfactante/bioemulsificante produzido, sugerindo que a excreção destas moléculas está relacionada a mecanismo de proteção em ambientes frios (SELBMANN et al., 2002).

O uso combinado de substratos hidrofílicos (por exemplo, glicose, sacarose ou glicerol) e hidrofóbicos (por exemplo, óleos vegetais ou hidrocarbonetos) tem sido uma estratégia para induzir a produção de biosurfactantes em meio de cultura, devido estas fontes de carbono fornecerem as porções hidrofílicas e hidrofóbicas que irão compor a molécula (BHARDWAJ; CAMEOTRA; CHOPRA, 2015; CHOTARD et al., 2023). Além disso, o uso de carboidratos pode suprir o crescimento de biomassa, enquanto os lipídios podem induzir a produção de lipases que estão envolvidas na conversão e/ou síntese de biosurfactantes (BECK; ZIBEK, 2020; ELDIN; KAMEL; HOSSAM, 2019). No entanto, o uso de apenas um tipo de substrato tem também permitido a produção de biosurfactantes fúngicos (PIEGZA et al., 2021; RAMDASS; RAMPERSAD, 2021).

O fornecimento de uma fonte de nitrogênio é essencial para síntese de proteínas e enzimas necessárias no crescimento microbiano, que por sua vez pode também influenciar o rendimento e propriedades físico-químicas dos biosurfactantes fúngicos (ISHAQ et al., 2015; BECK; ZIBEK, 2020). Neste contexto, as principais fontes orgânicas usadas são peptona, extrato de levedura, extrato de malte, extrato de carne e ureia (PARASZKIEWICZ; KANWAL; DLUGOŃSKI, 2002; CHIEWPATTANAKUL et al., 2010; SENA et al., 2018), enquanto as fontes inorgânicas são NH_4NO_3, $(NH_4)_2SO_4$, $NaNO_3$ e KNO_3 (RAUDABAUGH; MILLER, 2013; REIS et al., 2018; OTHMAN et al., 2022; AL-OTIBI; AL-ZAHRANI; MARRAIKI, 2023). Por um lado, o extrato de levedura é composto de células autolisadas de *Saccharomyces cerevisiae*, as quais são ricas em aminoácidos, vitaminas e oligoelementos. A adição de extrato de levedura torna a composição do meio de cultura complexa, e dificulta o balanceamento estequiométrico da razão C/N (QAZI et al., 2014; BECK; ZIBEK, 2020). Por outro lado, meios

quimicamente definidos com sais de amônio tendem a diminuir o pH e inibir a produção de biosurfactantes; enquanto sais de nitrato são menos disponíveis e necessitam ser reduzidos a nitrito antes de sua conversão a amônia, o que torna sua assimilação mais lenta simulando uma condição de limitação de nitrogênio (QAZI et al., 2014; NURFARAHIN; MOHAMED; PHANG, 2018). Uma alta razão de C/N foi reportada como estratégia para induzir a produção de biosurfactantes fúngicos (DESAI; BANAT, 1997; KIRAN et al., 2009), e lipídios em leveduras oleaginosas (TREICHEL et al., 2010). Em outras palavras, a limitação de nitrogênio pode induzir a produção de lipídios que irão constituir a estrutura do biosurfactante produzido (NURFARAHIN; MOHAMED; PHANG, 2018).

A triagem primária foi realizada a partir do cultivo de 115 fungos filamentosos e 3 leveduras em meio de cultura contendo alta concentração de glicose (40 $g \bullet L^{-1}$), baixa concentração de extrato de levedura (0,5 $g \bullet L^{-1}$) e suplementado com óleo de soja (0,5%), usando uma combinação de metodologias como uma estratégia mais efetiva para evitar o descarte de linhagens produtoras de biomoléculas (por exemplo, glicolipídios, lipopeptídeos, polissacarídeos ou proteínas) que apresentaram propriedades surfactantes ou emulsificantes.

De acordo com a literatura, a redução da tensão superficial para valores menores de 40 $mN \bullet m^{-1}$ sugere que a linhagem fúngica produz biosurfactantes, mas as melhores são aquelas que reduzem para valores menores de 30 $mN \bullet m^{-1}$ (REIS et al., 2018; PIEGZA et al., 2021), embora comumente isolados fúngicos que alteram a tensão superficial para valores próximos a 50 $mN \bullet m^{-1}$ são relevantes para estudos mais aprofundados (MURIEL et al., 1996; AL-HAWASH et al., 2018a).

As culturas de *Ilyonectria* sp. AF25D (isolado de solo contaminado) e *Cladosporium* sp. NU07 (isolado de solo antártico) apresentaram uma deformação da gota de 2 mm, e índice de emulsificação de 5% e 2,5%, respectivamente; enquanto *Paecilomyces* sp. AF45D (isolado de solo contaminado) uma deformação de 4 mm e índice de emulsificação de 2,5%; embora todas estas linhagens apresentaram RTS inferior a 4%. Não foi encontrado nenhum relato na literatura sobre a produção de biosurfactantes a partir de espécies do gênero *Ilyonectria.* Um único estudo foi encontrado sobre a produção de biosurfactantes a partir do gênero *Cladosporium,* o qual reportou que *C. resinae* reduziu a tensão superficial de meio líquido para 50 $mN \bullet m^{-1}$ (MURIEL et al., 1996). O gênero *Paecilomyces* foi pouco explorado para produção de biosurfactantes (VIGUERAS et al., 2014; KUMAR et al., 2023). Por exemplo, a linhagem *Purpureocillium lilacinum* (anteriormente denominado *Paecilomyces lilacinum*) foi reportada como produtora de hidrofobinas (proteínas surfactantes) capazes de reduzir a tensão superficial da água (72 $mN \bullet m^{-1}$) para 34,9 $mN \bullet m^{-1}$ (VIGUERAS et al., 2014).

As linhagens *Scolecobasidium* sp. AF33A (isolada de solo contaminado) e *Scopulariopsis* sp. AF34B não apresentaram resultado positivo para nenhum teste realizado neste estudo. A linhagem *Scopulariopsis brevicaulis* foi também reportada como produtoras de ciclodepsipeptídeos (biosurfactante peptídico), embora a atividade surfactante ou emulsificante dessa molécula não foi avaliada, somente a elucidação da estrutura química foi realizada (YU et al., 2008).

As culturas de *Thelebolus* sp. SC29P3 e *Buergenerula spartinaea* apresentaram uma deformação de 4 mm e índice de emulsificação de 5% e 2,5%, respectivamente; enquanto a de *Curvularia* sp. ILRR1A20047 uma deformação da gota de 5 mm e índice de emulsificação de 2,5 %. Estas linhagens apresentaram valores promissores de RTS, tais como 23% (52,1 $mN•m^{-1}$), 29% (47,4 $mN•m^{-1}$) e 30% (47,4 $mN•m^{-1}$), respectivamente; o que sugere seu potencial para produção de biosurfactantes. Não há literatura disponível sobre produção de biosurfactantes a partir do gênero *Buergenerula*, enquanto somente um estudo foi realizado a partir de linhagem do gênero *Curvularia* (PARASZKIEWICZ; KANWAL; DLUGOŃSKI, 2002) e *Thelebolus* (MUKHOPADHYAY et al., 2014). Foi reportado que a linhagem *Curvularia lunata* IM 2901 produziu uma glicoproteína com capacidade para emulsificar hidrocarbonetos alifáticos, alicíclicos e aromáticos (PARASZKIEWICZ; KANWAL; DLUGOŃSKI, 2002); bem como *Thelebolus microsporus* (isolado da Antártica) produziu um polissacarídeo com atividade citotóxica para células cancerígenas (MUKHOPADHYAY et al., 2014). Infelizmente, nenhum destes estudos supracitados analisaram a ação surfactante das biomoléculas produzidas. Portanto, sugere-se que este é o primeiro relato sobre o potencial de *B. spartinaea* e linhagens do gênero *Curvularia* (isolado ILRR1A20047) e *Thelebolus* (isolado SC29P3) para produzir biosurfactantes, o que foi confirmado por tensiometria e colapso da gota.

Linhagens ainda não identificadas apresentaram resultados positivos para produção biosurfactantes; por exemplo, os isolados ACF49 e ACF51 (de solos antárticos) pelo teste colapso da gota (5 mm) e índice de emulsificação (5%), e o isolado RSAF3B20271 (de planta de mangue) reduziu a tensão superficial para 34,17 $mN•m^{-1}$, um dos menores valores alcançados e inferior ao de SDS (36,1 $mN•m^{-1}$). Portanto, é sugerido a identificação polifásica destes isolados para garantir a sua biossegurança em bioprocessos se faz necessária (BUENO et al., 2019; SILVA et al., 2021a).

As culturas de *Aspergillus* sp. SC21P3; *Trichoderma* sp. P05R2 e RASC1B10122; *Pseudogymnoascus* sp. SC23P3 e SC26P3; *Penicillium* sp. P10R5, P10R6, P10R7, SCUV02.P1; *Penicillium brasilianum* AF64PD e *Fusarium* sp. AF99PD apresentaram os maiores resultados no teste colapso da gota (5 mm) e índice de emulsificação (5%); exceto

SCUV02.P1 que apresentou deformação da gota de 4mm, e SC21P3 que apresentou índice de emulsificação de 2,5%. Os menores valores de tensão superficial foram alcançados nas culturas de *Trichoderma* sp. P05R2 (45,8 mN•m^{-1}, isolado de solo contaminado) e RASC1B10122 (33,1 mN•m^{-1}, isolado de planta de mangue); *Aspergillus* sp. SC21P3 (49,3 mN•m^{-1}, isolado de solo antártico); *Penicillium* sp. SCUV02 (46,4 mN•m^{-1}, isolado de solo antártico) e *Pseudogymnoascus* sp. SC23P3 (44,7 mN•m^{-1}, isolado de solo antártico), sugerindo que estas linhagens são potenciais produtoras de biosurfactantes.

Sugere-se que esse é o primeiro relato sobre a produção de biosurfactantes por espécies de *Pseudogymnoascus,* pois segundo Raudabaugh e Miller (2013) as linhagens *P. destructans* MYA-4855 e GdINMSC7 não reduziram a tensão superficial do meio de cultura após 15 dias de cultivo, bem como Souza (2021) reportou que *P. verrucosus* apresentou resultado negativo para o teste colapso da gota.

Por outro lado, as espécies dos gêneros *Trichoderma*, *Penicillium, Aspergillus* e *Fusarium* tem sido fortemente exploradas para produção de biosurfactantes. Por exemplo, as culturas de *Trichoderma citrinoviride* B3 (PIEGZA et al., 2021), *T. camerunense* (MARTINHO et al., 2019) e *T. harzianum* MUT 290 (PITOCCHI et al., 2020) alcançou valores de tensão superficial de 38,2 mN•m^{-1}, 38,0 mN•m^{-1} e 36,4 mN•m^{-1}, respectivamente. Os biosurfactantes produzidos por *Aspergillus awamori*, *A. niger* (MARTINHO et al., 2019), *A. ustus* (KIRAN et al., 2009) e *A. oryzae* (EL-HANAFY et al., 2017) apresentou capacidade para emulsificar óleo de motor (62%), óleo de soja (47%), querosene (42%) e hexadecano (75%), respectivamente. Similarmente, os biosurfactantes produzidos por *Fusarium proliferatum* (BHARDWAJ; CAMEOTRA; CHOPRA, 2015), *F. chlamydosporum* (MARTINHO et al., 2019) e *F. oxysporum* (AL-OTIBI; AL-ZAHRANI; MARRAIKI, 2023) emulsificaram querosene (90%), n-hexano (52%) e diesel (57%), respectivamente; enquanto aqueles produzidos por *Fusarium* sp. BS-8 (QAZI et al., 2014) e *F. fujikuroi* (REIS et al., 2018) alcançaram uma tensão superficial de 34 mN•m^{-1} e 20 mN•m^{-1} , respectivamente. Adicionalmente, as culturas de *Penicillium* sp. RMA1 (AL-HAWASH et al., 2018a), *P. commune* KR137639 (EL-HANAFY et al., 2017) e *P. islandicum* S7-5 (KULKARNI; NENE; JOSHI, 2020b) reduziram a tensão superficial para 50 mN•m^{-1}, 52 mN•m^{-1} e 31 mN•m^{-1}; e emulsificaram óleo bruto (36%), hexadecano (23%) e azeite de oliva (81%), respectivamente.

O maior coeficiente de correlação de Pearson entre os testes de triagem foi de 0,64 para o colapso da gota e índice de emulsificação, enquanto o menor foi de 0,39 para tensiometria e índice de emulsificação. Em geral, a tensiometria permitiu selecionar as linhagens com mais acurácia, as quais poderiam ter sido desprezadas usando somente o teste colapso da gota e/ou

índice de emulsificação. Este fato corrobora com outros trabalhos que verificaram resultados similares (GUSMÃO et al., 2008; OTHMAN et al., 2022). Por exemplo, a cultura de *Aspergillus fumigatus* Shu2 (isolada de borra de petróleo) que apresentou baixo valor de deformação da gota (1 mm) e índice de emulsificação de 3 % para querosene, embora isolado foi considerado um bom produtor de biosurfactantes devido sua capacidade em reduzir a tensão superficial para 30 $mN•m^{-1}$ em 120 h (OTHMAN et al., 2022). Similarmente, a cultura de *Penicillium corilophylum* UFPE-DA-886 apresentou índice de emulsificação de 1,6 % para querosene, porém reduziu a tensão superficial para 37,6 $mN•m^{-1}$ em 13 dias (GUSMÃO et al., 2008).

Neste contexto, é válido reportar que embora a conotação da palavra biosurfactante comumente inclua biomoléculas com potencial emulsificante, a ação surfactante não está diretamente relacionada a capacidade de emulsificação ou vice-versa, apesar que a ação surfactante pode favorecer a emulsificação devido aumentar a interação entre as fases de diferentes polaridades (UZOIGWE et al., 2015). Em suma, o índice de emulsificação para óleo diesel foi baixo para as linhagens triadas em comparação a literatura reportada para algumas espécies pertencentes aos mesmos gêneros deste estudo, enquanto os valores de deformação da gota tenderam ser semelhantes ou superiores (ISHAQ et al., 2015; KUMAR et al., 2023). Por exemplo, os biosurfactantes produzidos por *Penicillium citrinum* e *Paecilomyces variotii* isolados de solos contaminados apresentaram índice de emulsificação de 32% e 40% para óleo diesel (KUMAR et al., 2023). Além disso, a deformação da gota da cultura de *Aspergillus flavus* AF612 foi de 1.7 mm (ISHAQ et al., 2015), enquanto a de *Aspergillus* sp. SC21P3 (deste estudo) foi de 5 mm.

Uma triagem secundária avaliou a influência de $NaNO_3$ (Meio N), ou extrato de levedura (Meio X e Meio Y) em diferentes concentrações no perfil cinético da produção de biosurfactantes, uma vez que a fonte de nitrogênio e tempo de cultivo são parâmetros relevantes nesta produção devido aspectos de rendimento e custos (BANAT et al., 2014). Por exemplo, o preço de 1 kg de extrato de levedura ($ 116,4) é aproximadamente 2,6 vezes maior do que $NaNO_3$ ($ 44,3), valor cotado em 11 de março de 2023 (https://molekula.com/catalog/). Além disso, a produção de lipídios de manosileritritol por *Pseudozyma hubeiensis* em meio líquido contendo glicose, óleo de colza e $NaNO_3$ foi 5,7 vezes maior do que aquele usando extrato de levedura (BECK; ZIBEK, 2020).

Embora seja tácito que o fornecimento de nutrientes e cultivo prolongado pode aumentar o rendimento de biosurfactantes fúngicos (CHIEWPATTANAKUL et al., 2010; PIEGZA et al., 2021), estes devem ser avaliados para especular o período em que a máxima

produção é alcançada, e para garantir a rentabilidade do bioprocesso. Por exemplo, a cultura de *Aspergillus niger* Ph III reduziu a tensão superficial de 68 $mN•m^{-1}$ do meio contendo extrato de levedura e óleo de soja para 44 $mN•m^{-1}$ em 192 h, porém ela aumentou a partir deste período provavelmente devido ao consumo do biosurfactante pela linhagem como uma fonte de carbono alternativa (SILVA et al., 2021a). Além disso, o cultivo prolongado pode favorecer a degradação enzimática do biossurfactante produzido, bem como sua adsorção na superfície da célula, o que pode diminuir o rendimento ou dificultar a extração (LIN; SHARMA; GEORGIOU, 1993).

Quatro linhagens de *Penicillium* (AF38D, AF41D, AF42D, AF64PD) e *Fusarium* (AF10D, AF48D, AF52DD, AF99PD) isoladas de solos contaminados foram selecionadas para triagem secundária, embora estas tenham apresentado baixos valores de RTS na triagem primária em comparação a outras linhagens de solos antárticos e plantas de mangue que apresentaram valores superiores. Este fato foi atribuído a seleção destas linhagens para triagem secundária ter sido realizada antes da triagem primária dos demais isolados promissores deste estudo.

Infelizmente, as linhagens *Aspergillus* sp. SC21P3 e *Thelebolus* sp. SC29P3 não foram selecionadas entre os 16 isolados para triagem secundária; embora elas estejam ranqueadas na posição 8° e 13° dos maiores valores de RTS, respectivamente, e não pertençam a gêneros previamente selecionados. Este fato foi atribuído a identificação destas linhagens ter sido realizada somente após a execução da triagem secundária deste estudo; ou seja, a identificação não foi realizada logo após a triagem primária (CAVALCANTE, 2022).

As linhagens *Trichoderma* sp. P05R2, *Pseudogymnoascus* sp. SC23P3 e *Penicillium* sp. SCUV02.P1 produziram biosurfactantes nas primeiras 72h de cultivo independentemente do meio de cultivo usado. Estas linhagens alcançaram valores de tensão superficial inferiores a 47,3 $mN•m^{-1}$, o que presume para uma maior produtividade na produção de biosurfactantes. Foi reportado que a linhagem *Trichoderma citrinoviride* C1 quando cultivada em meio contendo em ($g•L^{-1}$) $(NH_4)_2SO_4$ (3), peptona (5) e glicose (10) reduziu a tensão superficial para 36 $mN•m^{-1}$ em 48h (PIEGZA et al., 2021), um valor próximo ao obtido do isolado *Trichoderma* sp. P05R2 (deste estudo) nos meios X (36,2 $mN•m^{-1}$), Y (38,7 $mN•m^{-1}$) e N (35,8 $mN•m^{-1}$). O isolado *Penicillium* sp. SCUV02.P1 (deste estudo) reduziu a tensão superficial do meio X (46,4 $mN•m^{-1}$), Y (47,3 $mN•m^{-1}$) e N (45,1 $mN•m^{-1}$) em 72h para valores inferiores ao reportado para *Penicillium* sp. RMA2 (51 $mN•m^{-1}$) quando cultivado em meio contendo (em $g•L^{-1}$) óleo bruto (10), $(NH_4)_2SO_4$ (0.1) e $NaNO_3$ (0.2) em 13 dias (AL-HAWASH et al., 2018a).

Ao final do cultivo (em 168 h), o isolado *Penicillium* sp. P10R6 alcançou baixos e significativamente diferentes ($p<0,05$) valores de tensão superficial em meio X (39,3 $mN•m^{-1}$),

Y (33,5 mN•m^{-1}) e N (27,0 mN•m^{-1}); bem como *Pseudogymnoascus* sp. SC23P3 em meio X (36,2 mN•m^{-1}), Y (32,4 mN•m^{-1}) e N (27,1 mN•m^{-1}). Por outro lado, a linhagem *Trichoderma* sp. P05R2 e *Penicillium* sp. SCUV02.P1 também apresentaram baixos valores de tensão superficial, porém estes não diferiram significativamente nos meios X (26,9 mN•m^{-1}), Y (27,6 mN•m^{-1}) e N (26,3 mN•m^{-1}) para a primeira, enquanto a segunda somente não diferiu nos meios Y (33,7 mN•m^{-1}) e N (33,1 mN•m^{-1}) ao contrário do meio X (39,9 mN•m^{-1}). Em outras palavras, a linhagem *Trichoderma* sp. P05R2 alcançou os menores valores de tensão superficial nos três meios em 168 h, os quais diferiram significativamente de *Pseudogymnoascus* sp. SC23P3, *Penicillium* sp. SCUV02.P1 e P10R6 no meio X e Y. Em relação ao meio N, os menores valores de tensão superficial foram alcançados usando este meio para as linhagens supracitadas em 168h, exceto para *Penicillium* sp. SCUV02.P1 que apresentou o maior valor de 33,1 mN•m^{-1}, o qual diferiu significativamente das linhagens supracitadas. Similarmente, as linhagens *Trichoderma* sp. RASC1B10122, *Curvularia* sp. ILRR1A20047, *Penicillium* sp. AF38D e AF41D alcançaram baixos valores de tensão superficial (37,7- 40,8 mN•m^{-1}) que não diferiram significativamente entre si em 168 h usando o meio N. É válido reportar que *Penicillium* sp. AF38D e AF41D produziram biosurfactantes somente em meio N, este resultado diverge do isolado *Penicillium* sp. 8CC2 que não produziu biosurfactantes quando cultivado em meio contendo $NaNO_3$, porém produziu com extrato de levedura (SENA et al., 2018). Os menores valores de tensão alcançados por *B. spartinae, Trichoderma* sp. RASC1B10122 e *Curvularia* sp. ILRR1A20047 foram em meio X (40,6 mN•m^{-1}), Y (36,4 mN•m^{-1}) e N (37,7 mN•m^{-1}) em 168h, respectivamente; embora a redução alcançada por *B. spartinae* não diferiu significativamente com *Trichoderma* sp. RASC1B10122 (42,3 mN•m^{-1}) em meio X, e com *Curvularia* sp. ILRR1A20047 (40,2 mN•m^{-1}) em meio Y.

Os menores valores alcançados pelas linhagens do gênero *Fusarium* usando o meio X (58,7 mN•m^{-1}), Y (48,3 mN•m^{-1}) e N (49,1 mN•m^{-1}) foram para o isolado AF99PD, AF52DD e AF48D, respectivamente. Estes valores são superiores aos anteriormente descritos para outras linhagens deste estudo. Estes valores também são superiores ao reportado para *Fusarium neocosmosporiellum* RH-10 (isolado também de solo contaminado) que reduziu a tensão superficial para 26.5 mN•m^{-1}em meio contendo (em g•L^{-1}) $NaNO_3$ (2) e óleo bruto (10) em 168 h (AZIN; MOGHIMI; HEIDARYTABAR, 2018). Além disso, o isolado *Fusarium proliferatum* WC416 (isolado de refinaria de petróleo) reduziu a tensão superficial para 27.4 mN•m^{-1} em meio contendo (em g•L^{-1}) $(NH_4)_2SO_4$ (0.67) e naftaleno (1) em 15 dias (GUPTA; PATHAK; RAVI, 2023).

Em suma, embora os valores de tensão superficial alcançados variaram entre as linhagens e meio de cultura usado, eles estão de acordo com valores reportados para sugerir a produção de biosurfactante pelas linhagens supracitadas (exceto, *Fusarium*), bem como estão próximos ou inferiores ao do surfactante químico SDS (36,1 $mN•m^{-1}$). Em relação a linhagem *Trichoderma* sp. P05R2 que não apresentou diferença significativa para nenhum meio de cultivo em 168 h, sugere-se o uso do meio X devido à baixa concentração de extrato de levedura necessária embora seu preço seja maior do que ao nitrato de sódio usado em maior concentração (10x) no meio N. Similarmente, recomenda-se o uso do meio X para as linhagens *Fusarium* sp. AF99PD, *Pseudogymnoascus* sp. NU09 e *B. spartinae* que não apresentaram diferenças significativas entre Meio X e Y em 168 h, devido a menor concentração de extrato de levedura fornecida ser uma alternativa para minimizar a formação de espuma. A formação de espuma está relacionada a presença de biosurfactantes e/ou ácidos graxos no meio, mas as proteínas e peptídeos constituintes do extrato de levedura pode favorecer esta formação, que por sua vez, dificulta a transferência de oxigênio e recuperação do biosurfactante (REIS et al., 2018; BECK; ZIBEK, 2020; ONWOSI et al., 2021). Adicionalmente, o meio N é recomendado para as linhagens *Fusarium* sp. AF52DD, *Curvularia* sp. ILRR1A20047, *Trichoderma* sp. RASC1B10122 e P05R2, *Penicillium* sp. P10R6 e SCUV02.P1 que não apresentaram diferenças significativas entre meio Y e N, devido o $NaNO_3$ ser uma fonte mais barata, além de possibilitar o balanceamento estequiométrico em meio não contendo substratos hidrofóbicos (BECK; ZIBEK, 2020).

É válido reportar que a redução da tensão superficial alcançados na triagem secundária são maiores do que aos obtidos na triagem primária, exceto para *Trichoderma* sp. RASC1B10122. Em outras palavras, a formulação dos meios X, Y e N com uma menor concentração de glicose (20 $g•L^{-1}$) em comparação a usada na triagem primária (40 $g•L^{-1}$) foi relevante para aumentar a RTS do cultivo em 168 h, além reduzir o custo pela metade devido a quantidade de substrato fornecido. Estes menores valores de tensão superficial obtidos na triagem secundária provavelmente estão relacionados ao melhor desempenho promovido pelo tamanho (10%) do inóculo fornecido, o qual foi previamente preparado sem óleo de soja e com glicose em menor concentração (10 $g•L^{-1}$), e inoculado com células em alta atividade metabólica. Infelizmente, a cultura de pré-inóculo não foi submetida a tensiometria para avaliar a influência de substrato hidrofóbico nesta produção.

É presumível que linhagens que promoveram uma redução da tensão superficial em 168h significativamente diferente daquela obtida em 144 h, podem alcançar valores ainda menores após o período avaliado neste estudo. Por exemplo, *Trichoderma* sp. RASC1B10122

em meio X, Y e N; ou *Pseudogymnoascus* sp. NU09 e SC23P3 e *Curvularia* sp. ILRR1A20047 em meio X, Y e N, respectivamente. Foi reportado que a cultura de *Trichoderma citrinoviride* B11 alcançou menor valor de tensão superficial (38 $mN•m^{-1}$) em meio contendo (em $g•L^{-1}$) NH_4NO_3 (0.6), $NaNO_3$ (3.8), peptona (5) e glicose (10) por mais de 240 h (PIEGZA et al., 2021), porém a linhagem *Trichoderma* sp. RASC1B10122 (deste estudo) alcançou resultados semelhantes em meio X (36,4 $mN•m^{-1}$) e Y (37,9 $mN•m^{-1}$) em 168 h. Adicionalmente, ressalta-se que a tensiometria usada para estimar a produção e ação de biosurfactantes não deve ser considerada como um parâmetro para mensurar a produtividade ou rendimento de processo (DESAI; BANAT, 1997; JAHAN et al., 2020). Este fato é justificado devido a tensão superficial tornar-se constante quando o biosurfactante produzido atinge sua Concentração Micelar Crítica (CMC), que corresponde a concentração mínima do biosurfactantes necessária para formar micelas (estrutura de automontagem) (DESAI; BANAT, 1997; SILVA et al., 2021a). Em outras palavras, os valores de tensão superficial subestimam a produtividade e rendimento do biosurfactante produzido pela linhagem triada, pois embora a cultura tenha alcançado uma redução máxima de tensão superficial (na CMC do biosurfactante), isto não significa que a linhagem não esteja ainda produzindo biosurfactantes (JAHAN et al., 2020; SILVA et al., 2021a).

5 CONCLUSÃO

Alguns isolados fúngicos de solos contaminados com hidrocarbonetos, solos antárticos e endofíticos de mangue foram triados para produção de biosurfactantes. Os melhores resultados alcançados na triagem primária realizada pelo teste colapso da gota, índice de emulsificação e tensiometria sugerem que os isolados de solos antárticos e endofíticos de plantas de mangue são mais promissores do que aqueles de solo contaminado.

A tensiometria foi o melhor método para seleção de linhagens produtoras de biosurfactantes. A capacidade surfactante e emulsificante divergiram entre estas linhagens devido aos baixos índices de emulsificação alcançados para óleo diesel.

Esse é o primeiro relato sobre a produção de biosurfactantes para espécie *B. spartinaea,* bem como para linhagens dos gêneros *Thelebolus* (isolado SC29P3) e *Curvularia* (isolado ILRR1A20047) usando tensiomeria, e *Ilyonectria* (isolado AF25D) usando colapso da gota. O isolado *Paecilomyces* sp. AF45D apresentou resultado positivo somente para o teste colapso da gota e índice de emulsificação.

Em geral, os valores de tensão superficial alcançados na triagem secundária são maiores do que aqueles da triagem primária. Este fato corrobora para afirmar que um processo sequencial de triagem pode melhor garantir a seleção de linhagens produtoras de biosurfactantes.

Os meios de cultura formulados contendo extrato de levedura (Meio X e Y) ou nitrato de sódio (Meio N) influenciaram na ação surfactante da biomolécula produzida. O extrato de levedura não apresentou efeito positivo para maioria das linhagens, exceto para *B. spartinae* no meio X. Neste contexto, sugere-se que o uso meio N é indicado para as linhagens *Trichoderma* sp. P05R2 e RASC1B10122 *Pseudogymnoascus* sp. SC23P3, *Penicillium* sp. SCUV02.P1, P10R6, AF38D e AF41D e *Curvularia* sp. ILRR1A20047 produzirem biosurfactantes com maior ação de superfície, além do $NaNO_3$ ser uma fonte mais barata.

Em suma, os métodos de triagem e estudo inicial de cultivo permitiram selecionar linhagens promissoras ao desenvolvimento de bioprocessos voltados a produção de biosurfactantes, os quais futuramente poderão ser submetidos a etapas de recuperação, caracterização, melhoramento genético e/ou serem avaliados como substitutos aos químicos em diversos campos, tais como: agricultura, saúde, alimentos e/ou remediação ambiental.

CONSIDERAÇÕES FINAIS

A partir da premissa que um objeto de estudo é fundamentado na sintropia de teorias e metodologias científicas para sua investigação (MOXLEY, 1974; THOMAS; JAMES, 2006), o principal objeto de pesquisa desta tese foi explorar a *práxis* (teoria e prática) sobre o uso de fungos como recursos biológicos para micorremediação e produção de surfactantes. Este trabalho resultou em duas sistemáticas revisões de literatura sob uma perspectiva integral e abrangente em biotecnologia industrial, usando o método de investigação empírico-indutivo; e duas pesquisas experimentais usando o método hipotético-dedutivo.

A primeira revisão de literatura (Capítulo I) forneceu uma visão geral sobre estudos de micologia objetivados no desenvolvimento de bioprocessos e tecnologias usando fungos e seus metabólitos para remediação de hidrocarbonetos e metais tóxicos. A partir deste estudo, foi possível concluir que fungos cultiváveis de solos contaminados (ou outros ambientes) são promissores inoculantes em processos de bioaugmentação (Tabela 1 e 3; Capítulo I); bem como o isolamento destes fungos cultiváveis é uma etapa preliminar para obtenção de inóculos produtores de biosurfactantes que são úteis em processos de bioestimulação, bioaugmentação e lavagem de solos (Fig. 5; Capítulo I).

O capítulo seguinte (Cap II) segue como uma extensão/continuação do capítulo I, no que tange em abordar os principais pilares do desenvolvimento de um bioprocesso (SCHMIDELL et al. 2001). Na segunda revisão de literatura se apresentou sistematicamente o processo envolvido na produção de biosurfactantes fúngicos desde o isolamento de linhagens potencialmente produtoras até a formulação de produtos comerciais contendo estas biomoléculas. Neste contexto, foi possível concluir que estudos de bioprospecção tem selecionado linhagens fúngicas para produção de biosurfactantes dentro de um ambiente industrial; bem como a formulação de meios de cultura pode influenciar rendimento e propriedades físico-químicas dos biosurfactantes.

Em suma, os levantamentos bibliográficos obtidos auxiliaram a fundamentar o delineamento das hipóteses testadas nas pesquisas experimentais. A primeira pesquisa experimental (Capítulo III) investigou o isolamento fungos cultiváveis isolados dos solos contaminados com diferentes hidrocarbonetos e com diferentes processos de remediação ou não (do REMA/UFSC). Se obteve uma grande quantidade de isolados (n= 58) com diferença entre o tipo de solo contaminado. A partir da identificação morfológica ou molecular, sugere-se a colonização de linhagens de diferentes gêneros de ascomicetos como *Fusarium, Penicillium*, *Paecilomyces, Scolecobasidium, Scopulariopsis, Ilyonectria* nestes solos.

Entretanto, é interessante o uso de outros marcadores moleculares, além do ITS, para a especiação destas linhagens; para garantir a biossegurança do uso destas linhagens em bioprocessos.

Na segunda pesquisa experimental (Capítulo IV), as linhagens fúngicas de solos contaminados (isoladas no Capítulo III) e outras de solos antárticos e plantas de mangue foram triadas para produção de biosurfactantes; bem como foi avaliada a influência de extrato de levedura e nitrato de sódio no perfil cinético desta produção. Dentre os isolados de cada gênero triado, as culturas de *Trichoderma* sp. RASC1B10122, *Pseudogymnoascus* sp. SC23P3, *Penicillium* sp. SCUV02.P1, *Curvularia* sp. ILRR1A20047, *Thelebolus* sp. SC29P3, *Buergenerula spartinae, Aspergillus* sp. SC21P3 e *Fusarium* sp. AF99PD foram as mais promissoras para produção de biosurfactantes, uma vez que estes alcançaram os menores valores de tensão superficial. Este é o primeiro relato da produção de biosurfactantes para linhagens do gênero *Pseudogymnoascus* e *Buergenerula spartinae,* bem como para *Curvularia* sp. ILRR1A20047 e *Thelebolus* sp. SC29P3 usando a tensiometria como metodologia de triagem. Em relação a influência da fonte de nitrogênio, sugere-se o uso de $NaNO_3$ (5 g•L^{-1}) para as linhagens *Trichoderma* sp. P05R2 e RASC1B10122, *Pseudogymnoascus* sp. SC23P3, *Penicillium* sp. SCUV02.P1, P10R6, AF38D e AF41D e *Curvularia* sp. ILRR1A20047, uma vez que estas alcançaram uma alta redução da tensão superficial, além de ser uma fonte com custo menor. Por outro lado, o extrato de levedura em baixa concentração (0.5 g•L^{-1}) foi sugerido para *B. spartinae,* embora esta fonte apresente um custo maior, a concentração fornecida é dez vezes menor que $NaNO_3$.

Por fim, estudos de micologia objetivados a remediação ambiental e/ou produção de produtos biotecnológicos, usando ferramentas de engenharia de bioprocessos, tem sido uma alternativa promissora em substituição aos tratamentos físico-químicos e aos surfactantes químicos, respectivamente. Assim, os trabalhos apresentados nesta tese demonstraram a relevância e descoberta de fungos cultiváveis se o objetivo é produzir biosurfactantes, bem como os resultados alcançados lançam luzes para o desenvolvimento de bioprocessos objetivados na recuperação e aplicação destes surfactantes para remediação ambiental.

REFERÊNCIAS

ABDEL-MAWGOUD, A. M.; STEPHANOPOULOS, G. Simple glycolipids of microbes: Chemistry, biological activity and metabolic engineering. **Synthetic and Systems Biotechnology**, v. 3, n. 1, p. 3-19, 2018.

ADAMCZAK, M.; BEDNARSKI, W. O. Influence of medium composition and aeration on the synthesis of biosurfactants produced by *Candida antarctica*. **Biotechnology Letters**, v. 22, n. 4, p. 313-316, 2000.

ADNAN, M. et al. Physiological and molecular characterization of biosurfactant producing endophytic fungi *Xylaria regalis* from the cones of *Thuja plicata* as a potent plant growth promoter with its potential application. **BioMed Research International**, v. 2018, p. 1-11, 2018.

AHMAD, I. Microalgae–bacteria consortia: A review on the degradation of polycyclic aromatic hydrocarbons (PAHs). **Arabian Journal for Science and Engineering**, v. 47, n. 1, p. 19-43, 2022.

AHMED, E.; HOLMSTRÖM, S. J. M. Siderophores in environmental research: roles and applications: Siderophores in environmental research. **Microbial Biotechnology**, v. 7, n. 3, p. 196-208, 2014.

AIMANIANDA, V. et al. Surface hydrophobin prevents immune recognition of airborne fungal spores. **Nature**, v. 460, n. 7259, p. 1117-1121, 2009.

AJELLO, L.; MCGINNIS, M.R.; CAMPER, J. An outbreak of phaeohyphomycosis in rainbow trout caused by Scolecobasidium humicola. **Mycopathologia**, v. 62, n. 1, p. 15-22, 1977.

AKHTAR, N.; MANNAN, M. A.-U. Mycoremediation: Expunging environmental pollutants. **Biotechnology Reports**, v. 26, n. 26, p. 1-10, 2020.

ALCALDE, M. Engineering the ligninolytic enzyme consortium. **Trends in Biotechnology**, v. 33, n. 3, p. 155-162, 2015.

AL-FAWWAZ, A. T.; JACOB, J. H.; AL-WAHISHE, T. E. Bioremoval capacity of phenol by green micro-algal and fungal species isolated from dry environment. **International Journal of Scientific & Technology Research**, v. 5, n. 2, p. 155-160, 2016.

AL-HAWASH, A. B. et al. Biodegradation of n-hexadecane by *Aspergillus* sp. RFC^{-1} and its mechanism. **Ecotoxicology and Environmental Safety**, v. 164, p. 398-408, 2018c.

AL-HAWASH, A. B. et al. Isolation and characterization of two crude oil-degrading fungi strains from Rumaila oil field, Iraq. **Biotechnology Reports**, v. 17, p. 104-109, 2018a.

AL-HAWASH, A. B. et al. Principles of microbial degradation of petroleum hydrocarbons in the environment. **Egyptian Journal of Aquatic Research**, v. 44, n. 2, p. 71-76, 2018b.

AL-HAWASH, A. B.; ZHANG, X.; MA, F. Removal and biodegradation of different petroleum hydrocarbons using the filamentous fungus *Aspergillus* sp. RFC-1. **MicrobiologyOpen**, v. 8, n. 1, p. 1-14, 2019.

ALIMADADI, N.; SOUDI, M. R.; TALEBPOUR, Z. Efficient production of tri-acetylated mono-acylated mannosylerythritol lipids by *Sporisorium* sp. aff. *sorghi* SAM20. **Journal of Applied Microbiology**, v. 124, n. 2, p. 457-468, 2018.

ALMEIDA, D. G. et al. Biosurfactants: Promising molecules for petroleum biotechnology advances. **Frontiers in Microbiology**, v. 7, p. 1-14, 2016.

ALMEIDA, D. G. et al. Response surface methodology for optimizing the production of biosurfactant by *Candida tropicalis* on industrial waste substrates. **Frontiers in Microbiology**, v. 8, p. 1-13, 2017.

AL-OTIBI, F.; AL-ZAHRANI, R. M.; MARRAIKI, N. Biodegradation of selected hydrocarbons by *Fusarium* species isolated from contaminated soil samples in Riyadh, Saudi Arabia. **Journal of Fungi**, v. 9, n. 2, p. 1-20, 2023.

ALPAT, Ş. et al. Effects of biosorption parameter: Kinetics, isotherm and thermodynamics for Ni(II) biosorption from aqueous solution by *Circinella* sp. **Electronic Journal of Biotechnology**, v. 13, n. 5, p. 1-19, 2010.

ALSHARARI, S. F.; TAYEL, A. A.; MOUSSA, S. H. Soil emendation with nano-fungal chitosan for heavy metals biosorption. **International Journal of Biological Macromolecules**, v. 118, p. 2265-2268, 2018.

AL-ZABAN, M. I.; ALHARBI, M. A.; MAHMOUD, M. A. Hydrocarbon biodegradation and transcriptome responses of cellulase, peroxidase, and laccase encoding genes inhabiting rhizospheric fungal isolates. **Saudi Journal of Biological Sciences**, v. 28, n. 4, p. 2083–2090, 2021.

AMARAL, P. F. F. et al. Production and characterization of a bioemulsifier from *Yarrowia lipolytica*. **Process Biochemistry**, v. 41, n. 8, p. 1894-1898, 2006.

AMEEN, F. et al. Biodegradation of diesel fuel hydrocarbons by mangrove fungi from Red Sea Coast of Saudi Arabia. **Saudi Journal of Biological Sciences**, v. 23, n. 2, p. 211-218, 2016.

ANDRADE, C. J. et al. A novel approach for the production and purification of mannosylerythritol lipids (MEL) by *Pseudozyma tsukubaensis* using cassava wastewater as substrate. **Separation and Purification Technology**, v. 180, p. 157-167, 2017.

ANDRADE, R. F. S. et al. Promising biosurfactant produced by *Cunninghamella echinulate* UCP 1299 using renewable resources and its application in cotton fabric cleaning process. **Advances in Materials Science and Engineering**, v. 2018, p. 1-12, 2018.

ANTONY, J. Screening Designs. *In*: **Design of Experiments for Engineers and Scientists**. [s.l.] Elsevier, p. 51-62, 2014.

APHALO, P. J. **ggpmisc**: Miscellaneous Extensions to 'ggplot2'. R Package (v. 0.5.2), 2017. Disponível em: https://cran.r-project.org/web/packages/ggpmisc/index.html. Acesso em: 17/02/2023

ARAB, F.; MULLIGAN, C. N. An eco-friendly method for heavy metal removal from mine tailings. **Environmental Science and Pollution Research International**, v. 25, n. 16, p. 16202-16216, 2018.

ARANDA, E. et al. Isolation of *Ascomycota* fungi with capability to transform PAHs: Insights into the biodegradation mechanisms of *Penicillium oxalicum*. **International Biodeterioration & Biodegradation**, v. 122, p. 141–150, 2017.

ARAÚJO, S. C. S. et al. MBSP1: a biosurfactant protein derived from a metagenomic library with activity in oil degradation. **Scientific Reports**, v. 10, n. 1, p. 1-13, 2020.

ARCHANA, K. et al. Isolation and characterization of sophorolipid producing yeast from fruit waste for application as antibacterial agent. **Environmental Sustainability**, v. 2, n. 2, p. 107-115, 2019.

ARIMA, K.; KAKINUMA, A.; TAMURA, G. Surfactin, a crystalline peptidelipid surfactant produced by *Bacillus subtilis*: isolation, characterization and its inhibition of fibrin clot formation. **Biochemical and Biophysical Research Communications**, v. 31, n. 3, p. 488–494, 1968.

ARISTE, A. F. et al. Mycoremediation of phenols and polycyclic aromatic hydrocarbons from a biorefinery wastewater and concomitant production of lignin modifying enzymes. **Journal of Cleaner Production**, v. 253, n. 119810, p. 1-12, 2020.

ARNOLD, J. B. **ggthemes**: Extra Themes, Scales and Geoms for 'ggplot2'. R Package (v. 4.2.4), 2021. Disponível em: https://rdrr.io/cran/ggthemes/. Acesso em:17/02/2023

ARUTCHELVI, J. I. et al. Mannosylerythritol lipids: a review. **Journal of Industrial Microbiology & Biotechnology**, v. 35, n. 12, p. 1559-1570, 2008.

ASGHER, M. et al. Improved biosurfactant production from *Aspergillus niger* through chemical mutagenesis: characterization and RSM optimization. **SN Applied Sciences**, v. 2, n. 5, p. 1-11, 2020.

ATAKPA, E. O. et al. Improved degradation of petroleum hydrocarbons by co-culture of fungi and biosurfactant-producing bacteria. **Chemosphere**, v. 290, n. 133337, p. 133337, 2022.

AZIN, E.; MOGHIMI, H.; HEIDARYTABAR, R. Petroleum degradation, biosurfactant and laccase production by *Fusarium neocosmosporiellum* RH-10: A microcosm study. **Soil & Sediment Contamination**, v. 27, n. 4, p. 329-342, 2018.

BA, S.; KUMAR, V. V. Recent developments in the use of tyrosinase and laccase in environmental applications. **Critical Reviews in Biotechnology**, v. 37, n. 7, p. 819-832, 2017.

BACCILE, N. et al. Development of a cradle-to-grave approach for acetylated acidic sophorolipid biosurfactants. **ACS Sustainable Chemistry & Engineering**, v. 5, n. 1, p. 1186-1198, 2017.

BACCILE, N. et al. Sophorolipids-functionalized iron oxide nanoparticles. **Physical Chemistry Chemical Physics**, v. 15, n. 5, p. 1606-1620, 2013.

BAE, I.-H. et al. Mannosylerythritol lipids inhibit melanogenesis via suppressing ERK-CREB-MiTF-tyrosinase signalling in normal human melanocytes and a three-dimensional human skin equivalent. **Experimental Dermatology**, v. 28, n. 6, p. 738-741, 2019.

BAHIA, F. M. et al. Rhamnolipids production from sucrose by engineered *Saccharomyces cerevisiae*. **Scientific Reports**, v. 8, n. 1, p. 1-10, 2018.

BAJAJ, V. K.; ANNAPURE, U. S. Castor oil as secondary carbon source for production of sophorolipids using *Starmerella bombicola* NRRL Y-17069. **Journal of Oleo Science**, v. 64, n. 3, p. 315-323, 2015.

BAJPAI, P. Bioprocesses in industrial biotechnology. *In*: **Biotechnology in the Chemical Industry**. [s.l.] Elsevier, p. 37-56, 2020.

BAKUR, A. et al. Synthesis of gold nanoparticles derived from mannosylerythritol lipid and evaluation of their bioactivities. **AMB Express**, v. 9, n. 1, p. 1-9, 2019.

BALAN, S. S.; GANESH KUMAR, C.; JAYALAKSHMI, S. Physicochemical, structural and biological evaluation of Cybersan (trigalactomargarate), a new glycolipid biosurfactant produced by a marine yeast, *Cyberlindnera saturnus* strain SBPN-27. **Process Biochemistry**, v. 80, p. 171-180, 2019.

BALCÁZAR-LÓPEZ, E. et al. Xenobiotic compounds degradation by heterologous expression of a *Trametes sanguineus* Laccase in *Trichoderma atroviride*. **PloS One**, v. 11, n. 2, p. 1-13, 2016.

BALDEWEG, F. et al. Fungal biosurfactants from *Mortierella alpina*. **Organic Letters**, v. 21, n. 5, p. 1444-1448, 2019.

BAMI, M. S et al. Biosurfactants aided bioremediation mechanisms: A mini-review. **Soil & Sediment Contamination**, v. 31, n. 7, p. 801-817, 2022.

BANAT, I. M. et al. Biosurfactants: The green generation of speciality chemicals and potential production using Solid-State fermentation (SSF) technology. **Bioresource Technology**, v. 320, p. 1-52, 2021.

BANAT, I. M. et al. Cost effective technologies and renewable substrates for biosurfactants' production. **Frontiers in Microbiology**, v. 5, p. 1-18, 2014.

BANAT, I. M. et al. Microbial biosurfactants production, applications and future potential. **Applied Microbiology and Biotechnology**, v. 87, n. 2, p. 427-444, 2010.

BAO, Q. et al. Study on the treatment of oily sludge in oil fields with lipopeptide/sophorolipid complex bio-surfactant. **Ecotoxicology and Environmental Safety**, v. 212, n. 111964, p. 1-12, 2021.

BARANGER, C. et al. A compartmentalized microsystem helps understanding the uptake of benzo[a]pyrene by fungi during soil bioremediation processes. **The Science of The Total Environment**, v. 784, n. 147151, p. 1-12, 2021.

BARCELOS, G. S. et al. Spray drying as a strategy for biosurfactant recovery, concentration and storage. **SpringerPlus**, v. 3, n. 1, p. 1-19, 2014.

BAREA-SEPÚLVEDA, M. et al. Toxic elements and trace elements in *Macrolepiota procera* mushrooms from southern Spain and northern Morocco. **Journal of Food Composition and Analysis**, v. 108, n. 104419, p. 1-12, 2022.

BARNES, N. M. et al. Bioremediation potential of hydrocarbon-utilizing fungi from select marine niches of India. **3 Biotech**, v. 8, n. 1, p. 1-10, 2018.

BARON, N. C. et al. Black fungi and hydrocarbons: An environmental survey for alkylbenzene assimilation. **Microorganisms**, v. 9, n. 5, p. 1008, 2021.

BASTOS, I. M. A. S. **Isolamento e identificação de fungos endofíticos de manguezais da ilha de Santa Catarina**. 117 f. Dissertação (Mestrado em Biotecnologia e Biociências) – Universidade Federal de Santa Catarina, Florianópolis, 2022.

BATISTA, R. M. et al. Effect of medium components on the production of a biosurfactant from *Candida tropicalis* applied to the removal of hydrophobic contaminants in soil. **Water Environment Research,** v. 82, n. 5, p. 418-425, 2010.

BATISTA-GARCÍA, R. A. et al. Simple screening protocol for identification of potential mycoremediation tools for the elimination of polycyclic aromatic hydrocarbons and phenols from hyperalkalophile industrial effluents. **Journal of Environmental Management**, v. 198, n. Pt 2, p. 1-11, 2017.

BAZIOLI, J. M. et al. Insights into *Penicillium brasilianum* secondary metabolism and its biotechnological potential. **Molecules**, v. 22, n. 6, p. 1-23, 2017.

BECARELLI, S. et al. A new *Ciboria* sp. For soil mycoremediation and the bacterial contribution to the depletion of total petroleum hydrocarbons. **Frontiers in Microbiology**, v. 12, p. 1-20, 2021.

BECK, A. et al. Influence of microorganism and plant oils on the structure of mannosylerythritol lipid (MEL) biosurfactants revealed by a novel thin layer chromatography mass spectrometry method. **Journal of Industrial Microbiology & Biotechnology**, v. 46, n. 8, p. 1191-1204, 2019.

BECK, A.; ZIBEK, S. Growth behavior of selected Ustilaginaceae fungi used for mannosylerythritol lipid (MEL) biosurfactant production - evaluation of a defined culture medium. **Frontiers in Bioengineering and Biotechnology**, v. 8, p. 1-17, 2020.

BENGUENAB, A.; CHIBANI, A. Biodegradation of petroleum hydrocarbons by filamentous fungi (*Aspergillus ustus* and *Purpureocillium lilacinum*) isolated from used engine oil contaminated soil. **Acta Ecologica Sinica**, v. 41, n. 5, p. 416-423, 2021.

BERGER, B. W.; SALLADA, N. D. Hydrophobins: Multifunctional biosurfactants for interface engineering. **Journal of Biological Engineering. Journal of Biological Engineering**, v. 13, n. 1, p. 1-8, 2019.

BETTIN, F. et al. Phenol removal by laccases and other phenol oxidases of *Pleurotus sajor-caju* PS-2001 in submerged cultivations and aqueous mixtures. **Journal of Environmental Management**, v. 236, p. 581-590, 2019.

BEVITÓRIO, L. Z. et al. Impacts of tailings of Fundão dam (Brazil) rupture on marine fish: Metals bioaccumulation and physiological responses. **Marine Pollution Bulletin**, v. 177, n. 113511, p. 113511, 2022.

BHARDWAJ, G.; CAMEOTRA, S. S.; CHOPRA, H. K. Isolation and purification of a new enamide biosurfactant from *Fusarium proliferatum* using rice-bran. **RSC Advances**, v. 5, n. 67, p. 54783-54792, 2015.

BIK, H. M. et al. Dramatic shifts in benthic microbial eukaryote communities following the Deepwater Horizon oil spill. **PloS One**, v. 7, n. 6, p. 1-6, 2012.

BILLS, G. F. et al. *Aspergillus mulundensis* sp. nov., a new species for the fungus producing the antifungal echinocandin lipopeptides, mulundocandins. **The Journal of Antibiotics**, v. 69, n. 3, p. 141-148, 2016.

BIROLLI, W. G. et al. Biodegradation of anthracene and several PAHs by the marine-derived fungus *Cladosporium* sp. CBMAI 1237. **Marine Pollution Bulletin**, v. 129, n. 2, p. 525-533, 2018.

BISCHOFF, K. M. et al. Susceptibility of Streptococcus suis to liamocins from *Aureobasidium pullulans*. **Biocatalysis and Agricultural Biotechnology**, v. 15, p. 291-294, 2018.

BOCZONÁDI, I. et al. Increased Cd^{2+} biosorption capability of *Aspergillus nidulans* elicited by crpA deletion. **Journal of Basic Microbiology**, v. 60, n. 7, p. 574-584, 2020.

BODIN, H.; ASP, H.; HULTBERG, M. Effects of biopellets composed of microalgae and fungi on cadmium present at environmentally relevant levels in water. **International Journal of Phytoremediation**, v. 19, n. 5, p. 500-504, 2017.

BODOUR, A. A.; MILLER-MAIER, R. M. Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. **Journal of Microbiological Methods**, v. 32, n. 3, p. 273-280, 1998.

BÖLKER, M.; BASSE, C. W.; SCHIRAWSKI, J. *Ustilago maydis* secondary metabolism-from genomics to biochemistry. **Fungal Genetics and Biology**, v. 45, p. 88-93, 2008.

BORIOVÁ, K. et al. Bioaccumulation and biovolatilization of various elements using filamentous fungus *Scopulariopsis brevicaulis*. **Letters in Applied Microbiology**, v. 59, n. 2, p. 217-223, 2014.

BOVIO, E. et al. The culturable mycobiota of a Mediterranean marine site after an oil spill: isolation, identification and potential application in bioremediation. **The Science of the Total Environment**, v. 576, p. 310-318, 2017.

BRUMANO, L. P. et al. Biosurfactant production by *Aureobasidium pullulans* in stirred tank bioreactor: New approach to understand the influence of important variables in the process. **Bioresource Technology**, v. 243, p. 264-272, 2017.

BRUNEEL, J. et al. Dynamic performance of a fungal biofilter packed with perlite for the abatement of hexane polluted gas streams using SIFT-MS and packing characterization with advanced X-ray spectroscopy. **Chemosphere**, v. 253, n. 126684, p. 1-10, 2020.

BUENO, J. L. et al. Biosurfactant production by yeasts from different types of soil of the South Shetland Islands (Maritime Antarctica). **Journal of Applied Microbiology**, v. 126, n. 5, p. 1402-1413, 2019.

CAJKA, T. et al. Multiplatform mass spectrometry-based approach identifies extracellular glycolipids of the yeast *Rhodotorula babjevae* UCDFST 04-877. **Journal of Natural Products**, v. 79, n. 10, p. 2580-2589, 2016.

CAMARGO, F. P. et al. Bioleaching of toxic metals from sewage sludge by co-inoculation of *Acidithiobacillus* and the biosurfactant-producing yeast *Meyerozyma guilliermondii*. **Journal of Environmental Management**, v. 211, p. 28-35, 2018.

CAMARGO-DE-MORAIS, M. M. Production of an extracellular polysaccharide with emulsifier properties by *Penicillium citrinum*. **World Journal of Microbiology and Biotechnology**, v. 19, n. 2, p. 191-194, 2003.

CAMEOTRA, S. S. et al. Synthesis of biosurfactants and their advantages to microorganisms and mankind. **Advances in Experimental Medicine and Biology**, v. 672, p. 261-280, 2010.

CAMERON, D. R.; COOPER, D. G.; NEUFELD, R. J. The mannoprotein of *Saccharomyces cerevisiae* is an effective bioemulsifie. **Applied and Environmental Microbiology**, v. 54, n. 6, p. 1420-1425, 1988.

CAMPOS, J. M. et al. Formulation of mayonnaise with the addition of a bioemulsifier isolated from *Candida utilis*. **Toxicology Reports**, v. 2, p. 1164-1170, 2015.

CAMPOS, J. M.; STAMFORD, T. L. M.; SARUBBO, L. A. Characterization and application of a biosurfactant isolated from *Candida utilis* in salad dressings. **Biodegradation**, v. 30, n. 4, p. 313-324, 2019.

CAÑAS, A. I.; CAMARERO, S. Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes. **Biotechnology Advances**, v. 28, n. 6, p. 694-705, 2010.

CASTIGLIONI, G. et al. Analytical modeling and numerical optimization of the biosurfactants production in solid-state fermentation by *Aspergillus fumigatus*. **Acta Scientiarum Technology**, v. 36, n. 1, p. 67-67, 2014.

CATHERINE, H.; PENNINCKX, M.; FRÉDÉRIC, D. Product formation from phenolic compounds removal by laccases: A review. **Environmental Technology & Innovation**, v. 5, p. 250-266, 2016.

CAVALCANTE, S. B. **Produção de pigmentos por fungos antárticos e seu potencial antimicrobiano**. 120 f. Dissertação (Mestrado em Biotecnologia e Biociências) – Universidade Federal de Santa Catarina, Florianópolis, 2022.

ÇELIK, P. A. et al. Biosurfactants' potential role in combating COVID-19 and similar future microbial threats. **Applied Sciences**, v. 11, n. 1, p. 1-16, 2020.

CERDA, A. et al. Valorisation of digestate from biowaste through solid-state fermentation to obtain value added bioproducts: A first approach. **Bioresource Technology**, v. 271, p. 409-416, 2019.

CERESA, C. et al. The effect of sophorolipids against microbial biofilms on medical-grade silicone. **Journal of Biotechnology**, v. 309, p. 34-43, 2020.

CHACHINA, S. B.; VORONKOVA, N. A.; BAKLANOVA, O. N. Biological remediation of the engine lubricant oil-contaminated soil with three kinds of earthworms, *Eisenia fetida*, *Eisenia Andrei*, dendrobena Veneta, and a mixture of microorganisms. **Procedia Engineering**, v. 113, p. 113-123, 2015.

CHAILLAN, F. et al. Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. **Research in Microbiology**, v. 155, n. 7, p. 587-595, 2004.

CHAKRABORTY, S.; MUKHERJEE, A.; DAS, T. K. Biochemical characterization of a lead-tolerant strain of *Aspergillus foetidus*: An implication of bioremediation of lead from liquid media. **International Biodeterioration & Biodegradation**, v. 84, p. 134-142, 2013.

CHANDRA, S. et al. Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. **Annals of Microbiology**, v. 63, n. 2, p. 417-431, 2013.

CHANDRAN, P.; DAS, N. Degradation of diesel oil by immobilized *Candida tropicalis* and biofilm formed on gravels. **Biodegradation**, v. 22, n. 6, p. 1181-1189, 2011.

CHANDRAN, P.; DAS, N. Role of sophorolipid biosurfactant in degradation of diesel oil by *Candida tropicalis*. **Bioremediation Journal**, v. 16, n. 1, p. 19-30, 2012.

CHANG, J. et al. Bioremediation of Hg-contaminated soil by combining a novel Hg-volatilizing *Lecythophora* sp. fungus, DC-F1, with biochar: Performance and the response of soil fungal community. **Science of the Total Environment**, v. 671, p. 676-684, 2019.

CHAPRÃO, M. J. et al. Application of bacterial and yeast biosurfactants for enhanced removal and biodegradation of motor oil from contaminated sand. **Electronic Journal of Biotechnology**, v. 18, p. 471-479, 2015.

CHAU, H. W. et al. A novel method for identifying hydrophobicity on fungal surfaces. **Mycological Research**, v. 113, n. Pt 10, p. 1046-1052, 2009.

CHAUDHARY, P. et al. Unloading of hazardous Cr and Tannic Acid from real and synthetic waste water by novel fungal consortia. **Environmental Technology & Innovation**, v. 26, n. 102230, p. 1-11, 2022.

CHAUDHARY, T. et al. Techniques for improving formulations of bioinoculants. **3 Biotech**, v. 10, n. 5, p. 1-9, 2020.

CHEN, C.; HU, J.; WANG, J. Biosorption of uranium by immobilized *Saccharomyces cerevisiae*. **Journal of Environmental Radioactivity**, v. 213, n. 106158, p. 1-10, 2020.

CHEN, C.-Y.; BAKER, S. C.; DARTON, R. C. The application of a high throughput analysis method for the screening of potential biosurfactants from natural sources. **Journal of Microbiological Methods**, v. 70, n. 3, p. 503-510, 2007.

CHEN, G.-Q.; JIANG, X.-R. Next generation industrial biotechnology based on extremophilic bacteria. **Current Opinion in Biotechnology**, v. 50, p. 94-100, 2018.

CHEN, H.-L.; CHEN, Y.-S.; JUANG, R.-S. Flux decline and membrane cleaning in cross-flow ultrafiltration of treated fermentation broths for surfactin recovery. **Separation and Purification Technology**, v. 62, n. 1, p. 47-55, 2008.

CHEN, J. et al. Metal ions can affect the composition and production of sophorolipids by *Wickerhamiella domercqiae* Y 2A CGMCC 3798: Metal ions affect sophorolipid composition and production. **European Journal of Lipid Science and Technology**, v. 116, n. 11, p. 1505-1512, 2014b.

CHEN, S. et al. Glomalin-related soil protein reduces the sorption of polycyclic aromatic hydrocarbons by soils. **Chemosphere**, v. 260, n. 127603, p. 1-10, 2020.

CHEN, W. et al. Fungal cytochrome p450 monooxygenases: their distribution, structure, functions, family expansion, and evolutionary origin. **Genome Biology and Evolution**, v. 6, n. 7, p. 1620-1634, 2014a.

CHEN, X. et al. Enhanced biocatalysis of phenanthrene in aqueous phase by novel CA-Ca-SBE-laccase biocatalyst: Performance and mechanism. **Colloids and Surfaces A**: Physicochemical and Engineering Aspects, v. 611, n. 125884, p. 1-8, 2021.

CHENG, Y. et al. Insertional mutagenesis of a fungal biocontrol agent led to discovery of a rare cellobiose lipid with antifungal activity. **Applied and Environmental Microbiology**, v. 69, n. 5, p. 2595-2602, 2003.

CHIADÒ, A. et al. Rational engineering of the lccβ *T. versicolor* laccase for the mediator-less oxidation of large polycyclic aromatic hydrocarbons. **Computational and Structural Biotechnology Journal**, v. 19, p. 2213-2222, 2021.

CHIEWPATTANAKUL, P. et al. Bioproduction and anticancer activity of biosurfactant produced by the dematiaceous fungus *Exophiala dermatitidis* SK80. **Journal of Microbiology and Biotechnology**, v. 20, n. 12, p. 1664-1671, 2010.

CHORMARE, R.; KUMAR, M. A. Environmental health and risk assessment metrics with special mention to biotransfer, bioaccumulation and biomagnification of environmental pollutants. **Chemosphere**, v. 302, n. 134836, p. 134836, 2022.

CHOTARD, M. et al. Fungal diversity and surfactant-producing fungi in oil contaminated environments. **Journal of Applied Microbiology**, v. 134, n. 2, p. 1-13, 2023.

CHUO, S. C. et al. Utilization of green sophorolipids biosurfactant in reverse micelle extraction of antibiotics: Kinetic and mass transfer studies. **Journal of Molecular Liquids**, v. 276, p. 225-232, 2019.

CIRIGLIANO, M. C.; CARMAN, G. M. Isolation of a bioemulsifier from *Candida lipolytica*. **Applied and Environmental Microbiology**, v. 48, n. 4, p. 747-750, 1984.

CLAUS, S.; VAN BOGAERT, I. N. A. Sophorolipid production by yeasts: a critical review of the literature and suggestions for future research. **Applied Microbiology and Biotechnology**, v. 101, n. 21, p. 7811-7821, 2017.

COBAS, M. et al. Development of permeable reactive biobarrier for the removal of PAHs by *Trichoderma longibrachiatum*. **Chemosphere**, v. 91, n. 5, p. 711-716, 2013.

COLLA, L. M. et al. Simultaneous production of lipases and biosurfactants by submerged and solid-state bioprocesses. **Bioresource Technology**, v. 101, n. 21, p. 8308-8314, 2010.

COLLADO, J. et al. High-throughput culturing of fungi from plant litter by a dilution-to-extinction technique: High-throughput culturing of fungi from plant litter. **FEMS Microbiology Ecology**, v. 60, n. 3, p. 521–533, 2007.

COOPER, D. G.; GOLDENBERG, B. G. Surface-active agents from two bacillus species. **Applied and Environmental Microbiology**, v. 53, n. 2, p. 224-229, 1987.

CORREA, H. T. et al. L-asparaginase and biosurfactants produced by extremophile yeasts from antarctic environments. **Industrial Biotechnology**, v. 16, n. 2, p. 107-116, 2020.

CORSEUIL, H. X. et al. Nitrate addition to groundwater impacted by ethanol-blended fuel accelerates ethanol removal and mitigates the associated metabolic flux dilution and inhibition of BTEX biodegradation. **Journal of Contaminant Hydrology**, v. 174, p. 1–9, 2015.

CORTÉS-SÁNCHEZ, A. DE J.; HERNÁNDEZ-SÁNCHEZ, H.; JARAMILLO-FLORES, M. E. Biological activity of glycolipids produced by microorganisms: new trends and possible therapeutic alternatives. **Microbiological Research**, v. 168, n. 1, p. 22-32, 2013.

COVINO, S. et al. Assessment of degradation potential of aliphatic hydrocarbons by autochthonous filamentous fungi from a historically polluted clay soil. **The Science of the Total Environment**, v. 505, p. 545-554, 2015.

COWAN-ELLSBERRY, C. et al. Environmental safety of the use of major surfactant classes in north America. **Critical Reviews in Environmental Science and Technology**, v. 44, n. 17, p. 1893-1993, 2014.

DACCÒ, C. et al. Key fungal degradation patterns, enzymes and their applications for the removal of aliphatic hydrocarbons in polluted soils: A review. **International Biodeterioration & Biodegradation**, v. 147, n. 104866, p. 1-11, 2020.

DAI, Y.; YIN, L.; NIU, J. Laccase-carrying electrospun fibrous membranes for adsorption and degradation of PAHs in shoal soils. **Environmental Science & Technology**, v. 45, n. 24, p. 10611-10618, 2011.

DALLINGER, A. et al. Biotransformation of cyclohexane and related alicyclic hydrocarbons by *Candida maltosa* and *Trichosporon* species. **International Biodeterioration & Biodegradation**, v. 107, p. 132-139, 2016.

DAMODARAN, D.; VIDYA SHETTY, K.; RAJ MOHAN, B. Effect of chelaters on bioaccumulation of Cd (II), Cu (II), Cr (VI), Pb (II) and Zn (II) in *Galerina vittiformis* from soil. **International Biodeterioration & Biodegradation**, v. 85, p. 182-188, 2013.

DAVEREY, A.; PAKSHIRAJAN, K. Sophorolipids from *Candida bombicola* using mixed hydrophilic substrates: production, purification and characterization. **Colloids and surfaces B**: Biointerfaces, v. 79, n. 1, p. 246-253, 2010.

DAVIES, J. S.; WESTLAKE, D. W. Crude oil utilization by fungi. **Canadian Journal of Microbiology**, v. 25, n. 2, p. 146-156, 1979.

DEHGHAN-NOUDEH, G.; HOUSAINDOKHT, M.; BAZZAZ, B. S. F. Isolation, characterization, and investigation of surface and hemolytic activities of a lipopeptide biosurfactant produced by ATCC 6633. **The Journal of Microbiology**, v. 43, n. 3, p. 272-276, 2005.

DEINZER, H.-T. et al. Elucidation of substrate specificities of decorating enzymes involved in mannosylerythritol lipid production by cross-species complementation. **Fungal Genetics and Biology**, v. 130, p. 91-97, 2019.

DELBEKE, E. I. P. et al. Petroselinic acid purification and its use for the fermentation of new sophorolipids. **AMB Express**, v. 6, n. 1, p. 1-9, 2016.

DELBEKE, E. I. P. et al. Sophorolipid modification: The power of yeasts and enzymes. *In*: **Lipid Modification by Enzymes and Engineered Microbes**. [s.l.] Elsevier, p. 315-341, 2018.

DELSARTE, I. et al. Lipid metabolism and benzo[a]pyrene degradation by *Fusarium solani*: an unexplored potential. **Environmental Science and Pollution Research International**, v. 25, n. 12, p. 12177-12182, 2018.

DEML, G. et al. Schizonellin A and B, new glycolipids from *Schizonella melanogramma*. **Phytochemistry**, v. 19, n. 1, p. 83-87, 1980.

DENG, J. et al. Catalyzed degradation of polycyclic aromatic hydrocarbons by recoverable magnetic chitosan immobilized laccase from *Trametes versicolor*. **Chemosphere**, v. 301, n. 134753, p. 134753, 2022.

DENG, S.; ZENG, D. Removal of phenanthrene in contaminated soil by combination of alfalfa, white-rot fungus, and earthworms. **Environmental Science and Pollution Research International**, v. 24, n. 8, p. 7565-7571, 2017.

DERGUINE-MECHERI, L. et al. Screening and biosurfactant/bioemulsifier production from a high-salt-tolerant halophilic *Cryptococcus* strain YLF isolated from crude oil. **Journal of Petroleum Science & Engineering**, v. 162, p. 712-724, 2018.

DERGUINE-MECHERI, L. et al. Screening and biosurfactant/bioemulsifier production from a high-salt-tolerant halophilic *Cryptococcus strain* YLF isolated from crude oil. **Journal of Petroleum Science & Engineering**, v. 162, p. 712–724, 2018.

DESAI, J. D.; BANAT, I. M. Microbial Production of Surfactants and Their Commercial Potential. **Microbiology and Molecular Biology Reviews**, v. 61, n. 1, p. 47-64, 1997.

DHANJAL, D. S.; SHARMA, D. Microbial metagenomics for industrial and environmental bioprospecting: The unknown envoy. *In*: **Microbial Bioprospecting for Sustainable Development**. Singapore: Springer Singapore, p. 327-352, 2018.

DHAR, P. et al. Toward green flotation: Interaction of a sophorolipid biosurfactant with a copper sulfide. **Journal of Colloid and Interface Science**, v. 585, p. 386-399, 2021.

DOKOUHAKI, M. et al. Hydrophobins and chaplins: Novel bio-surfactants for food dispersions a review. **Trends in Food Science & Technology**, v. 111, p. 378-387, 2021.

DOLMAN, B. M. et al. Integrated sophorolipid production and gravity separation. **Process Biochemistry**, v. 54, p. 162-171, 2017.

DOLMAN, B. M.; WANG, F.; WINTERBURN, J. B. Integrated production and separation of biosurfactants. **Process Biochemistry**, v. 83, p. 1-8, 2019.

DRAKE, H. et al. Fossilized anaerobic and possibly methanogenesis-fueling fungi identified deep within the Siljan impact structure, Sweden. **Communications Earth & Environment**, v. 2, n. 1, p. 1-10, 2021.

DU NOÜY, P. L. An interfacial tensiometer for universal use. **The Journal of General Physiology**, v. 7, n. 5, p. 625-631, 1925.

DUSENGEMUNGU, L. et al. Recent advances in biosorption of copper and cobalt by filamentous fungi. **Frontiers in Microbiology**, v. 11, p. 1-16, 2020.

EBNESAJJAD, S. Surface Tension and Its Measurement. *In*: **Handbook of Adhesives and Surface Preparation**. [s.l.] Elsevier, p. 21-30, 2011.

EIBES, G. et al. Enzymatic technologies for remediation of hydrophobic organic pollutants in soil. **Applied Microbiology and Biotechnology**, v. 99, n. 21, p. 8815-8829, 2015.

ELDIN, A. M.; KAMEL, Z.; HOSSAM, N. Isolation and genetic identification of yeast producing biosurfactants, evaluated by different screening methods. **Microchemical Journal, Devoted to the Application of Microtechniques in all Branches of Science**, v. 146, p. 309-314, 2019.

EL-HANAFY, A. A.-E.-M. et al. Characterization of native fungi responsible for degrading crude oil from the coastal area of Yanbu, Saudi Arabia. **Biotechnology, Biotechnological Equipment**, v. 31, n. 1, p. 105-111, 2017.

EL-MORSY, E.-S. M. *Cunninghamella echinulata* a new biosorbent of metal ions from polluted water in Egypt. **Mycologia**, v. 96, n. 6, p. 1183-1189, 2004.

ELSHAFIE, H. S. et al. Mycoremediation effect of *Trichoderma harzianum* strain T22 combined with ozonation in diesel-contaminated sand. **Chemosphere**, v. 252, n. 126597, p. 1-8, 2020.

ELSHIKH, M. et al. Rhamnolipids and lactonic sophorolipids: natural antimicrobial surfactants for oral hygiene. **Journal of Applied Microbiology**, v. 123, n. 5, p. 1111-1123, 2017.

EVONIK INDUSTRIES. Evonik and Unilever team up for large-scale production of world's first 'green' biosurfactant. **Focus on Surfactants**, v. 2020, n. 2, 2020.

FAN, L. et al. Characterization and inducing melanoma cell apoptosis activity of mannosylerythritol lipids-A produced from *Pseudozyma aphidis*. **PloS One**, v. 11, n. 2, p. 1-17, 2016.

FAN, L.-L. et al. Production and identification of mannosylerythritol lipid-A homologs from the ustilaginomycetous yeast *Pseudozyma aphidis* ZJUDM34. **Carbohydrate Research**, v. 392, p. 1-6, 2014.

FAN, M.-Y.; XIE, R.-J.; QIN, G. Bioremediation of petroleum-contaminated soil by a combined system of biostimulation-bioaugmentation with yeast. **Environmental Technology**, v. 35, n. 1–4, p. 391-399, 2014.

FARDET, A.; LEBREDONCHEL, L.; ROCK, E. Empirico-inductive and/or hypothetico-deductive methods in food science and nutrition research: which one to favor for a better global health? **Critical Reviews in Food Science and Nutrition**, v. 8, p. 1-14, 2021.

FARIA, N. T. et al. Direct xylan conversion into glycolipid biosurfactants, mannosylerythritol lipids, by *Pseudozyma antarctica* PYCC 5048(T). **Enzyme and Microbial Technology**, v. 71, p. 58-65, 2015.

FARIAS, J. M. et al. Mouthwash containing a biosurfactant and chitosan: An eco-sustainable option for the control of cariogenic microorganisms. **International Journal of Biological Macromolecules**, v. 129, p. 853-860, 2019.

FASIM, A.; MORE, V. S.; MORE, S. S. Large-scale production of enzymes for biotechnology uses. **Current Opinion in Biotechnology**, v. 69, p. 68-76, 2021.

FAYEULLE, A. et al. Energy-dependent uptake of benzo[a]pyrene and its cytoskeleton-dependent intracellular transport by the telluric fungus *Fusarium solani*. **Environmental Science and Pollution Research International**, v. 21, n. 5, p. 3515-3523, 2014.

FAYEULLE, A. et al. PAH biodegradation by telluric saprotrophic fungi isolated from aged PAH-contaminated soils in mineral medium and historically contaminated soil microcosms. **Journal of Soils and Sediments**, v. 19, n. 7, p. 3056-3067, 2019.

FAZLI, M. M. et al. Highly cadmium tolerant fungi: their tolerance and removal potential. **Journal of Environmental Health Science & Engineering**, v. 13, n. 1, p. 1-9, 2015.

FENG, L. et al. Petroleum hydrocarbon-contaminated soil bioremediation assisted by isolated bacterial consortium and sophorolipid. **Environmental Pollution**, v. 273, n. 116476, p. 1-8, 2021.

FERNANDES, N. A. T. et al. Eco-friendly biosurfactant from *Wickerhamomyces anomalus* CCMA 0358 as larvicidal and antimicrobial. **Microbiological Research**, v. 241, n. 126571, p. 1-8, 2020.

FERNÁNDEZ, P. M. et al. Bioremediation strategies for chromium removal: Current research, scale-up approach and future perspectives. **Chemosphere**, v. 208, p. 139-148, 2018.

FERNÁNDEZ, P. M. et al. Phenol degradation and heavy metal tolerance of Antarctic yeasts. **Extremophiles**: Life Under Extreme Conditions, v. 21, n. 3, p. 445-457, 2017.

FERRARI, B. C.; ZHANG, C.; VAN DORST, J. Recovering greater fungal diversity from pristine and diesel fuel contaminated sub-antarctic soil through cultivation using both a high and a low nutrient media approach. **Frontiers in Microbiology**, v. 2, p. 1-14, 2011.

FERREIRA, I. N. S. et al. Biosurfactant and bioemulsifier as promising molecules produced by *Mucor hiemalis* isolated from Caatinga soil. **Electronic Journal of Biotechnology**, v. 47, p. 51-58, 2020.

FRANZETTI, A. et al. Production and applications of trehalose lipid biosurfactants. **European Journal of Lipid Science and Technology**, v. 112, n. 6, p. 617-627, 2010.

FREITAS, B. G. et al. Formulation of a commercial biosurfactant for application as a dispersant of petroleum and by-products spilled in oceans. **Frontiers in microbiology**, v. 7, p. 1-9, 2016.

FRISVAD, J.C.; SAMSON, R. A. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium* - A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. **Studies in Mycology**, v. 49, 1-174, 2004.

FRISVAD, J.C.; SAMSON, R. A.; STOLK, A. C. Disposition of recently described species of *Penicillium*. **Persoonia**, v. 14, n. 2, p. 209-232, 1990.

FUKUOKA, T. et al. Application of glycolipid biosurfactants as surface modifiers in bioplastics. **Journal of Oleo Science**, v. 67, n. 12, p. 1609-1616, 2018.

FUKUOKA, T. et al. Enzymatic synthesis of a novel glycolipid biosurfactant, mannosylerythritol lipid-D and its aqueous phase behavior. **Carbohydrate Research**, v. 346, n. 2, p. 266-271, 2011.

GAŁĄZKA, A. et al. Fungal community, metabolic diversity, and glomalin-related soil proteins (GRSP) content in soil contaminated with crude oil after long-term natural bioremediation. **Frontiers in Microbiology**, v. 11, p. 1-17, 2020.

GANESH KUMAR, A. et al. Genome sequence analysis of deep sea *Aspergillus sydowii* BOBA1 and effect of high pressure on biodegradation of spent engine oil. **Scientific Reports**, v. 11, n. 1, p. 1-19, 2021.

GARAY, L. A. et al. Discovery of synthesis and secretion of polyol esters of fatty acids by four basidiomycetous yeast species in the order Sporidiobolales. **Journal of Industrial Microbiology & Biotechnology**, v. 44, n. 6, p. 923-936, 2017a.

GARAY, L. A. et al. Extracellular fungal polyol lipids: A new class of potential high value lipids. **Biotechnology Advances**, v. 36, n. 2, p. 397-414, 2018.

GARAY, L. A. et al. Simultaneous production of intracellular triacylglycerols and extracellular polyol esters of fatty acids by *Rhodotorula babjevae* and *Rhodotorula* aff. *paludigena*. **Journal of Industrial Microbiology & Biotechnology**, v. 44, n. 10, p. 1397-1413, 2017b.

GARG, M.; PRIYANKA; CHATTERJEE, M. Isolation, characterization and antibacterial effect of biosurfactant from *Candida parapsilosis*. **Biotechnology Reports**, v. 18, p. 1-6, 2018.

GARTSHORE, J.; LIM, Y. C.; COOPER, D. G. Quantitative analysis of biosurfactants using Fourier Transform Infrared (FT-IR) spectroscopy. **Biotechnology Letters**, v. 22, n. 2, p. 169-172, 2000.

GARZOLI, L. et al. Sink or swim: Updated knowledge on marine fungi associated with wood substrates in the Mediterranean Sea and hints about their potential to remediate hydrocarbons. **Progress in Oceanography**, v. 137, p. 140-148, 2015.

GAUR, V. K. et al. Biosynthesis and characterization of sophorolipid biosurfactant by *Candida* spp.: Application as food emulsifier and antibacterial agent. **Bioresource Technology**, v. 285, n. 121314, p. 1-4, 2019.

GAUTAM, G. A cost effective strategy for production of bio-surfactant from locally isolated *Penicillium chrysogenum* SNP5 and its applications. **Journal of Bioprocessing & Biotechniques**, v. 04, n. 06, p. 1-7, 2014.

GEETHA, S.J.; BANAT, I. M.; JOSHI, S. J. Biosurfactants: Production and potential applications in microbial enhanced oil recovery (MEOR). **Biocatalysis and Agricultural Biotechnology**, v. 14, p. 23-32, 2018.

GEYS, R.; SOETAERT, W.; VAN BOGAERT, I. Biotechnological opportunities in biosurfactant production. **Current Opinion in Biotechnology**, v. 30, p. 66-72, 2014.

GHORBANNEZHAD, H.; MOGHIMI, H.; DASTGHEIB, S. M. M. Evaluation of pyrene and tetracosane degradation by mixed-cultures of fungi and bacteria. **Journal of Hazardous Materials**, v. 416, n. 126202, p. 1-9, 2021.

GHORBANNEZHAD, H.; MOGHIMI, H.; DASTGHEIB, S. M. M. Evaluation of heavy petroleum degradation using bacterial-fungal mixed cultures. **Ecotoxicology and Environmental Safety**, v. 164, p. 434-439, 2018.

GHOSAL, D. et al. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. **Frontiers in Microbiology**, v. 7, p. 1-27, 2016.

GOMES, C. et al. Isolation and taxonomy of filamentous fungi in endodontic infections. **Journal of Endodontics**, v. 36, n. 4, p. 626–629, 2010.

GONÇALVES, M. F. M. et al. Biodiversity of *Penicillium* species from marine environments in Portugal and description of *Penicillium lusitanum* sp. nov., a novel species isolated from sea water. **International Journal of Systematic and Evolutionary Microbiology**, v. 69, n. 10, p. 3014–3021, 2019.

GORFER, M. et al. Identification of heavy metal regulated genes from the root associated ascomycete *Cadophora finlandica* using a genomic microarray. **Mycological Research**, v. 113, n. Pt 12, p. 1377-1388, 2009.

GOSWAMI, T. et al. Remediation of aviation kerosene-contaminated soil by sophorolipids from *Candida bombicola* CB 2107. **Applied Sciences**, v. 10, n. 6, p. 1-11, 2020.

GREEN, M. R.; SAMBROOK, J. Precipitation of DNA with isopropanol. **Cold Spring Harbor Protocols**, v. 2017, n. 8, p. 673-674, 2017.

GU, D. et al. Synergy between fungi and bacteria promotes polycyclic aromatic hydrocarbon cometabolism in lignin-amended soil. **Journal of Hazardous Materials**, v. 425, n. 127958, p. 127958, 2022.

GUERFALI, M. et al. Triacylglycerols accumulation and glycolipids secretion by the oleaginous yeast *Rhodotorula babjevae* Y-SL7: Structural identification and biotechnological applications. **Bioresource Technology**, v. 273, p. 326-334, 2019.

GUPTA, S.; PATHAK, B.; RAVI, R. K. Biodegradation of naphthalene using biosurfactant producing *Fusarium proliferatum* WC416 isolated from refinery effluent. Applied Biochemistry and Biotechnology, p. 1-17, 2023.

GUSMÃO, C. A. B.; RUFINO, R. D.; SARUBBO, L. A. Laboratory production and characterization of a new biosurfactant from *Candida glabrata* UCP1002 cultivated in vegetable fat waste applied to the removal of hydrophobic contaminant. **World Journal of Microbiology and Biotechnology**, v. 26, n. 9, p. 1683-1692, 2010.

GUSMÃO, N. B. et al. Biosurfactant Production by Fungi. *In*: Latin American Biodeterioration Society Meeting Abstracts. **International Biodeterioration & Biodegradation**, v. 62, n. 1, p. 8, 2008.

HABA, E. et al. Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. **Journal of Applied Microbiology**, v. 88, n. 3, p. 379-387, 2000.

HADIBARATA, T.; TACHIBANA, S.; ITOH, K. Biodegradation of chrysene, an aromatic hydrocarbon by *Polyporus* sp. S133 in liquid medium. **Journal of Hazardous Materials**, v. 164, n. 2–3, p. 911-917, 2009.

HALL, S. M. Bioprocessing. *In*: **Rules of Thumb for Chemical Engineers**. [s.l.] Elsevier, p. 331-344, 2018.

HAMAD, A. A. et al. Petroleum hydrocarbon bioremediation using native fungal isolates and consortia. **The Scientific World Journal**, v. 2021, p. 1-13 2021.

HAN, J. et al. Preparation and uranium (VI) biosorption for tri-amidoxime modified marine fungus material. **Environmental Science and Pollution Research International**, v. 27, n. 30, p. 37313-37323, 2020.

HAQUE, S. et al. Functional microbiome strategies for the bioremediation of petroleum-hydrocarbon and heavy metal contaminated soils: A review. **The Science of the Total Environment**, v. 833, n. 155222, p. 155222, 2022.

HARMS, H.; SCHLOSSER, D.; WICK, L. Y. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. **Nature Reviews**: Microbiology, v. 9, n. 3, p. 177-192, 2011.

HASHEM, M. et al. Biodegradation and detoxification of aliphatic and aromatic hydrocarbons by new yeast strains. **Ecotoxicology and Environmental Safety**, v. 151, p. 28-34, 2018.

HASSAN, A. et al. Effective bioremediation of heavy metal–contaminated landfill soil through bioaugmentation using consortia of fungi. **Journal of Soils and Sediments**, v. 20, n. 1, p. 66-80, 2020.

HASSAN, S. E.; HIJRI, M.; ST-ARNAUD, M. Effect of arbuscular mycorrhizal fungi on trace metal uptake by sunflower plants grown on cadmium contaminated soil. **New Biotechnology**, v. 30, n. 6, p. 780-787, 2013.

HELLE, S. S.; DUFF, S. J.; COOPER, D. G. Effect of surfactants on cellulose hydrolysis. **Biotechnology and Bioengineering**, v. 42, n. 5, p. 611-617, 1993.

HESHAM, A. E.-L. et al. Biodegradation of high molecular weight PAHs using isolated yeast mixtures: application of meta-genomic methods for community structure analyses. **Environmental Science and Pollution Research International**, v. 19, n. 8, p. 3568-3578, 2012.

HEWALD, S. et al. Identification of a gene cluster for biosynthesis of mannosylerythritol lipids in the basidiomycetous fungus *Ustilago maydis*. **Applied and Environmental Microbiology**, v. 72, n. 8, p. 5469-5477, 2006.

HOREL, A.; SCHIEWER, S. Microbial degradation of different hydrocarbon fuels with mycoremediation of volatiles. **Microorganisms**, v. 8, n. 2, p. 1-15, 2020.

HUBERT, J. et al. New perspectives for microbial glycolipid fractionation and purification processes. **Comptes Rendus Chimie**, v. 15, n. 1, p. 18-28, 2012.

HUGHES, K. A.; BRIDGE, P.; CLARK, M. S. Tolerance of Antarctic soil fungi to hydrocarbons. **The Science of the Total Environment**, v. 372, n. 2-3, p. 539-548, 2007.

KULSHRESTHA, G.; KUMARI, A. Fungal degradation of chlorpyrifos by Acremonium sp. strain (GFRC-1) isolated from a laboratory-enriched red agricultural soil. **Biology and Fertility of Soils**, v. 47, n. 2, p. 219-225, 2011.

IMURA, T. et al. Aqueous gel formation from sodium salts of cellobiose lipids. **Journal of Oleo Science**, v. 63, n. 10, p. 1005-1010, 2014.

IMURA, T. et al. Low molecular weight gelators based on biosurfactants, cellobiose lipids by *Cryptococcus humicola*. **Journal of Oleo Science**, v. 61, n. 11, p. 659-664, 2012.

INOH, Y. et al. Biosurfactant MEL-A dramatically increases gene transfection via membrane fusion. **Journal of Controlled Release**, v. 94, n. 2-3, p. 423-431, 2004.

ISHAQ, U. et al. Production and characterization of novel self-assembling biosurfactants from *Aspergillus flavus*. **Journal of Applied Microbiology**, v. 119, n. 4, p. 1035-1045, 2015.

JACKSON, S. A. et al. Metagenomics for the discovery of novel biosurfactants of environmental interest from marine ecosystems. **Current Opinion in Biotechnology**, v. 33, p. 176-182, 2015.

JADHAV, J. V.; PRATAP, A. P.; KALE, S. B. Evaluation of sunflower oil refinery waste as feedstock for production of sophorolipid. **Process Biochemistry**, v. 78, p. 15-24, 2019.

JAHAN, R. et al. Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications. **Advances in Colloid and Interface Science**, v. 275, n. 102061, p. 102061, 2020.

JAIN, D. K. et al. A drop-collapsing test for screening surfactant-producing microorganisms. **Journal of Microbiological Methods**, v. 13, n. 4, p. 271-279, 1991.

JAUREGI, P.; KOURMENTZA, K. Membrane Filtration of Biosurfactants. *In*: **Separation of Functional Molecules in Food by Membrane Technology**. [s.l.] Elsevier, p. 79-112, 2019.

JIANG, J. et al. Recent progress towards industrial rhamnolipids fermentation: Process optimization and foam control. **Bioresource Technology**, v. 298, n. 122394, p. 122394, 2020.

JIMÉNEZ-PEÑALVER, P. et al. Production and characterization of sophorolipids from stearic acid by solid-state fermentation, a cleaner alternative to chemical surfactants. **Journal of Cleaner Production**, v. 172, p. 2735-2747, 2018.

JIMÉNEZ-PEÑALVER, P. et al. Production of sophorolipids from winterization oil cake by solid-state fermentation: Optimization, monitoring and effect of mixing. **Biochemical Engineering Journal**, v. 115, p. 93-100, 2016.

JIMOH, A. A. et al. Development and genetic engineering of hyper-producing microbial strains for improved synthesis of biosurfactants. **Molecular Biotechnology**, v. 63, n. 4, p. 267-288, 2021.

JIMOH, A. A.; LIN, J. Biosurfactant: A new frontier for greener technology and environmental sustainability. **Ecotoxicology and Environmental Safety**, v. 184, n. 109607, p. 1-19, 2019.

JOHNSON, P. et al. Effect of synthetic surfactants on the environment and the potential for substitution by biosurfactants. **Advances in Colloid and Interface Science**, v. 288, n. 102340, p. 102340, 2021.

JOSHI-NAVARE, K.; SINGH, P. K.; PRABHUNE, A. A. New yeast isolate *Pichia caribbica* synthesizes xylolipid biosurfactant with enhanced functionality: Synthesis of xylolipid using new yeast with novel characters. **European Journal of Lipid Science and Technology**, v. 116, n. 8, p. 1070-1079, 2014.

KABBOUT, R.; TAHA, S. Biodecolorization of textile dye effluent by biosorption on fungal biomass materials. **Physics Procedia**, v. 55, p. 437-444, 2014.

KADRI, T. et al. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review. **Journal of Environmental Sciences**, v. 51, p. 52-74, 2017.

KAMYABI, A.; NOURI, H.; MOGHIMI, H. Synergistic effect of *Sarocladium* sp. and *Cryptococcus* sp. co-culture on crude oil biodegradation and biosurfactant production. **Applied Biochemistry and Biotechnology**, v. 182, n. 1, p. 324-334, 2017.

KÄSTNER, M. et al. Classification and modelling of nonextractable residue (NER) formation of xenobiotics in soil – A synthesis. **Critical Reviews in Environmental Science and Technology**, v. 44, n. 19, p. 2107-2171, 2014.

KASTURE, M. et al. Multiutility sophorolipids as nanoparticle capping agents: synthesis of stable and water dispersible Co nanoparticles. **Langmuir**: The ACS Journal of Surfaces and Colloids, v. 23, n. 23, p. 11409-11412, 2007.

KAUR, G. et al. Efficient sophorolipids production using food waste. **Journal of Cleaner Production**, v. 232, p. 1-11, 2019.

KAUR, K.; SANGWAN, S.; KAUR, H. Biosurfactant production by yeasts isolated from hydrocarbon polluted environments. **Environmental Monitoring and Assessment**, v. 189, n. 12, p. 1-13, 2017.

KERBER, S. S. **Prospecção de bactérias com potencial na biorremediação de áreas contaminadas com BTX (benzeno, tolueno e xileno)**. 76 f. Dissertação (Mestrado em Biotecnologia e Biociências) – Universidade Federal de Santa Catarina, Florianópolis, 2020.

KERN, M. E.; BLEVINS, K. S. **Micologia Médica**. 2 ed. São Paulo: Editora Premier, 1999.

KHAN, I. et al. Mycoremediation: a treatment for heavy metal-polluted soil using indigenous metallotolerant fungi. **Environmental Monitoring and Assessment**, v. 191, n. 10, p. 1-15, 2019.

KHATOON, H.; RAI, J. P. N.; JILLANI, A. Role of fungi in bioremediation of contaminated soil. *In*: **Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nanotechnology**. [s.l.] Elsevier, p. 121-156, 2021.

KHUNA, S. et al. Morphology, molecular identification, and pathogenicity of two novel *Fusarium* species associated with postharvest fruit rot of cucurbits in northern Thailand. **Journal of Fungi**, v. 8, n. 11, p. 1-18, 2022.

KIM, J. S.; LEE, I. K.; YUN, B. S. A novel biosurfactant produced by *Aureobasidium pullulans* L3-GPY from a tiger lily wild flower, *Lilium lancifolium* Thunb. **PloS One**, v. 10, n. 4, p. 1-12, 2015.

KIM, J.-H. et al. Value-added conversion of biodiesel into the versatile biosurfactant sophorolipid using *Starmerella bombicola*. **Cleaner Engineering and Technology**, v. 1, n. 100027, p. 1-6, 2020.

KIM, Y.-B.; YUN, H. S.; KIM, E.-K. Enhanced sophorolipid production by feeding-rate-controlled fed-batch culture. **Bioresource Technology**, v. 100, n. 23, p. 6028-6032, 2009.

KIRAN, G. S. et al. Optimization and production of a biosurfactant from the sponge-associated marine fungus *Aspergillus ustus* MSF3. **Colloids and Surfaces B**: Biointerfaces, v. 73, n. 2, p. 250-256, 2009.

KIRAN, G. S.; THOMAS, T. A.; SELVIN, J. Production of a new glycolipid biosurfactant from marine *Nocardiopsis lucentensis* MSA04 in solid-state cultivation. **Colloids and Surfaces B**: Biointerfaces, v. 78, p. 8-16, 2010.

KITAGAWA, M. et al. **Skin care cosmetic and skin and agent for preventing skin roughness containing biosurfactants**. European Patent Specification - EP 1964546 B1.

Global Patent Index, 2008. Disponível em: https://data.epo.org/gpi/EP1964546A1. Acesso em:10/02/2021

KITAMOTO, D. et al. Remarkable antiagglomeration effect of a yeast biosurfactant, diacylmannosylerythritol, on ice-water slurry for cold thermal storage: Remarkable antiagglomeration effect of a yeast biosurfactant, diacylmannosylerythritol, on ice-water slurry for cold thermal storage. **Biotechnology Progress**, v. 17, n. 2, p. 362-365, 2001.

KITAMOTO, D. et al. Self-assembling properties of glycolipid biosurfactants and their potential applications. **Current Opinion in Colloid & Interface Science**, v. 14, n. 5, p. 315-328, 2009.

KITAMOTO, D.; ISODA, H.; NAKAHARA, T. Functions and potential applications of glycolipid biosurfactants — from energy-saving materials to gene delivery carriers. **Journal of Bioscience and Bioengineering**, v. 94, n. 3, p. 187-201, 2002.

KODZIUS, R.; GOJOBORI, T. Marine metagenomics as a source for bioprospecting. **Marine Genomics**, v. 24, Pt 1, p. 21-30, 2015.

KOLHE, N. et al. Transcriptome response of the tropical marine yeast *Yarrowia lipolytica* on exposure to uranium. **Current Microbiology**, v. 78, n. 5, p. 2033-2043, 2021.

KONISHI, M.; MAKINO, M. Selective production of deacetylated mannosylerythritol lipid, MEL-D, by acetyltransferase disruption mutant of *Pseudozyma hubeiensis*. **Journal of Bioscience and Bioengineering**, v. 125, n. 1, p. 105-110, 2018.

KRELING, N. E. et al. Extracellular biosurfactants from yeast and soil–biodiesel interactions during bioremediation. **International Journal of Environmental Science and Technology**, v. 17, n. 1, p. 395-408, 2020a.

KRELING, N. E. et al. Simultaneous production of lipases and biosurfactants in solid-state fermentation and use in bioremediation. **Journal of Environmental Engineering**, v. 146, n. 9, p. 1-10, 2020b.

KRIEGER, N.; CAMILIOS NETO, D.; MITCHELL, D. A. Production of microbial biosurfactants by solid-state cultivation. **Advances in Experimental Medicine and Biology**, v. 672, p. 203-210, 2010.

KÜES, U. Fungal enzymes for environmental management. **Current Opinion in Biotechnology**, v. 33, p. 268-278, 2015.

KUIPER, I. et al. Characterization of two *Pseudomonas putida* lipopeptide biosurfactants, putisolvin I and II, which inhibit biofilm formation and break down existing biofilms: Putisolvins inhibit biofilm formation and break down existing biofilms. **Molecular Microbiology**, v. 51, n. 1, p. 97-113, 2004.

KULAKOVSKAYA, E.; BASKUNOV, B.; ZVONAREV, A. The antibiotic and membrane-damaging activities of cellobiose lipids and sophorose lipids. **Journal of Oleo Science**, v. 63, n. 7, p. 701-707, 2014.

KULAKOVSKAYA, T. V. et al. Characterization of an antifungal glycolipid secreted by the yeast *Sympodiomycopsis paphiopedili*. **FEMS Yeast Research**, v. 5, n. 3, p. 247-252, 2004.
KULAKOVSKAYA, T. V. et al. Production of antifungal cellobiose lipids by *Trichosporon porosum*. **Mycopathologia**, v. 169, n. 2, p. 117-123, 2010.

KULAKOVSKAYA, T. V. et al. Ustilagic acid secretion by *Pseudozyma fusiformata* strains. **FEMS Yeast Research**, v. 5, n. 10, p. 919-923, 2005.

KULKARNI, S. S.; NENE, S. N.; JOSHI, K. S. A comparative study of production of hydrophobin like proteins (HYD-LPs) in submerged liquid and solid state fermentation from white rot fungus *Pleurotus ostreatus*. **Biocatalysis and Agricultural Biotechnology**, v. 23, n. 101440, p. 1-7, 2020a.

KULKARNI, S. S.; NENE, S. N.; JOSHI, K. S. Exploring malted barley waste for fungi producing surface active proteins like hydrophobins. **SN Applied Sciences**, v. 2, n. 11, p. 1-14, 2020b.

KULKARNI, S.; NENE, S.; JOSHI, K. Production of hydrophobins from fungi. **Process Biochemistry**, v. 61, p. 1-11, 2017.

KUMAR, V. et al. Studies on the morphology, phylogeny, and bioremediation potential of *Penicillium citrinum* and *Paecilomyces variotii* (Eurotiales) from oil-contaminated areas. **Archives of Microbiology**, v. 205, n. 1, p. 50, 2023.

KUMAR, V.; DWIVEDI, S. K. Bioremediation mechanism and potential of copper by actively growing fungus *Trichoderma lixii* CR700 isolated from electroplating wastewater. **Journal of Environmental Management**, v. 277, n. 111370, p. 1-9, 2021.

LAWRENCE, D. P.; NOURI, M. T.; TROUILLAS, F. P. Taxonomy and multi-locus phylogeny of cylindrocarpon-like species associated with diseased roots of grapevine and other fruit and nut crops in California. **Fungal Systematics and Evolution**, v. 4, p. 59–75, 2019.

LEAHY, J. G.; COLWELL, R. R. Microbial degradation of hydrocarbons in the environment. **Microbiological Reviews**, v. 54, n. 3, p. 305-315, 1990.

LEATHERS, T. D. et al. Inhibition of *Streptococcus mutans* and *S. sobrinus* biofilms by liamocins from *Aureobasidium pullulans*. **Biotechnology Reports**, v. 21, p. 1-4, 2019.

LEATHERS, T. D. et al. Medium optimization for production of anti-streptococcal liamocins by *Aureobasidium pullulans*. **Biocatalysis and Agricultural Biotechnology**, v. 13, p. 53-57, 2018.

LEE, A. H. et al. A proposed stepwise screening framework for the selection of polycyclic aromatic hydrocarbon (PAH)-degrading white rot fungi. **Bioprocess and Biosystems Engineering**, v. 43, n. 5, p. 767-783, 2020.

LEE, H. et al. Biotechnological procedures to select white rot fungi for the degradation of PAHs. **Journal of Microbiological Methods**, v. 97, p. 56-62, 2014.

LENG, L. et al. Co-culture of fungi-microalgae consortium for wastewater treatment: A review. **Bioresource Technology**, v. 330, n. 125008, p. 1-14, 2021.

LI, J. et al. Transmembrane transport mechanism of n-hexadecane by *Candida tropicalis*: Kinetic study and proteomic analysis. **Ecotoxicology and Environmental Safety**, v. 209, n. 111789, p. 1-10, 2021c.

LI, Q. et al. Diversity and structure of phenanthrene degrading bacterial communities associated with fungal bioremediation in petroleum contaminated soil. **Journal of Hazardous Materials**, v. 403, n. 123895, p. 1-9, 2021a.

LI, Q.; LIU, J.; GADD, G. M. Fungal bioremediation of soil co-contaminated with petroleum hydrocarbons and toxic metals. **Applied Microbiology and Biotechnology**, v. 104, n. 21, p. 8999-9008, 2020.

LI, X. et al. Applying β-cyclodextrin to amaranth inoculated with white-rot fungus for more efficient remediation of soil co-contaminated with Cd and BDE-209. **The Science of the Total Environment**, v. 634, p. 417-426, 2018.

LI, X. et al. Biosorption capacity of *Mucor circinelloides* bioaugmented with *Solanum nigrum* L. for the cleanup of lead, cadmium and arsenic. **Ecotoxicology and Environmental Safety**, v. 212, n. 112014, p. 1-9, 2021b.

LI, X. et al. Degradation of polycyclic aromatic hydrocarbons by crude extracts from spent mushroom substrate and its possible mechanisms. **Current Microbiology**, v. 60, n. 5, p. 336-342, 2010b.

LI, X. et al. Isolation of *Penicillium marneffei* from soil and wild rodents in Guangdong, SE China. **Mycopathologia**, v. 172, n. 6, p. 447–451, 2011.

LI, X. et al. Optimization of laccase-mediated Benzo[a]pyrene oxidation and the bioremedial application in aged polycyclic aromatic hydrocarbons-contaminated soil. **Journal of Health Science**, v. 56, n. 5, p. 534-540, 2010a.

LIN, S. C.; SHARMA, M. M.; GEORGIOU, G. Production and deactivation of biosurfactant by *Bacillus licheniformis* JF-2. **Biotechnology Progress**, v. 9, n. 2, p. 138-145, 1993.

LINDER, M. B. Hydrophobins: Proteins that self assemble at interfaces. **Current Opinion in Colloid & Interface Science**, v. 14, n. 5, p. 356-363, 2009.

LIU, H. et al. Enhancement of arbuscular mycorrhizal fungus (*Glomus versiforme*) on the growth and Cd uptake by Cd-hyperaccumulator *Solanum nigrum*. **Applied Soil Ecology**: a Section of Agriculture, Ecosystems & Environment, v. 89, p. 44-49, 2015.

LIU, J. et al. Styrene removal with an acidic biofilter with four packing materials: Performance and fungal bioaerosol emissions. **Environmental Research**, v. 191, n. 110154, p. 1-12, 2020.

LIU, P.-W. G. et al. Bioremediation of petroleum hydrocarbon contaminated soil: Effects of strategies and microbial community shift. International **Biodeterioration & Biodegradation**, v. 65, n. 8, p. 1119-1127, 2011.

LIU, Z. et al. Efficient sophorolipids production via a novel in situ separation technology by *Starmerella bombicola*. **Process Biochemistry**, v. 81, p. 1-10, 2018.

LU, H. et al. Non-equilibrium micelles formed by sugar-based surfactants under their Krafft temperature. **Colloids and Surfaces A**: Physicochemical and Engineering Aspects, v. 540, p. 167-176, 2018.

LU, N. et al. Fungal cell with artificial metal container for heavy metals biosorption: Equilibrium, kinetics study and mechanisms analysis. **Environmental Research**, v. 182, n. 109061, p. 1-10, 2020.

LUKONDEH, T.; ASHBOLT, N. J.; ROGERS, P. L. Evaluation of *Kluyveromyces marxianus* FII 510700 grown on a lactose-based medium as a source of a natural bioemulsifier. **Journal of Industrial Microbiology & Biotechnology**, v. 30, n. 12, p. 715-720, 2003.

LUNA, J. M. et al. Environmental applications of the biosurfactant produced by *Candida sphaerica* cultivated in low-cost substrates. **Colloids and Surfaces A**: Physicochemical and Engineering Aspects, v. 480, p. 413-418, 2015.

LUNA, J. M.; RUFINO, R. D.; SARUBBO, L. A. Biosurfactant from *Candida sphaerica* UCP0995 exhibiting heavy metal remediation properties. **Process Safety and Environmental Protection**, v. 102, p. 558-566, 2016.

LYDON, H. L. et al. Adjuvant antibiotic activity of acidic sophorolipids with potential for facilitating wound healing. **Antimicrobial Agents and Chemotherapy**, v. 61, n. 5, p. 1-9, 2017.

LYMAN, M. et al. *Rhodotorula taiwanensis* MD1149 produces hypoacetylated PEFA compounds with increased surface activity compared to *Rhodotorula babjevae* MD1169. **PloS One**, v. 13, n. 1, p. 1-19, 2018.

MA, X. et al. Efficient biosorption of lead(II) and cadmium(II) ions from aqueous solutions by functionalized cell with intracellular $CaCO_3$ mineral scaffolds. **Bioresource Technology**, v. 185, p. 70-78, 2015.

MA, X. et al. Soil microbial community succession and interactions during combined plant/white-rot fungus remediation of polycyclic aromatic hydrocarbons. **The Science of the Total Environment**, v. 752, n. 142224, p. 1-12, 2021.

MA, X.-J. et al. Effects of nitrogen sources on production and composition of sophorolipids by *Wickerhamiella domercqiae* var. *sophorolipid* CGMCC 1576. **Applied Microbiology and Biotechnology**, v. 91, n. 6, p. 1623-1632, 2011.

MADDIKERI, G. L.; GOGATE, P. R.; PANDIT, A. B. Improved synthesis of sophorolipids from waste cooking oil using fed batch approach in the presence of ultrasound. **Chemical Engineering Journal**, v. 263, p. 479-487, 2015.

MAENG, Y. et al. A novel microbial technique for producing high-quality sophorolipids from horse oil suitable for cosmetic applications. **Microbial Biotechnology**, v. 11, n. 5, p. 917-929, 2018.

MAGALHÃES, K. M. et al. Polycyclic aromatic hydrocarbons (PAHs) in fishery resources affected by the 2019 oil spill in Brazil: Short-term environmental health and seafood safety. **Marine Pollution Bulletin**, v. 175, n. 113334, p. 1-13, 2022.

MÄKELÄ, M. R. et al. Fungal laccases and their potential in bioremediation Applications. *In*: **Microbiology Monographs**. Cham: Springer International Publishing, p. 1-25, 2020.

MANIGLIA, B. C. et al. Production of active cassava starch films; effect of adding a biosurfactant or synthetic surfactant. **Reactive & Functional Polymers**, v. 144, n. 104368, p. 104368, 2019.

MAO, J.; GUAN, W. Fungal degradation of polycyclic aromatic hydrocarbons (PAHs) by *Scopulariopsis brevicaulis* and its application in bioremediation of PAH-contaminated soil. **Acta Agriculturae Scandinavica**: Section B, Soil and Plant Science, v. 66, n. 5, p. 399–405, 2016.

MARCELINO, P. R. F. et al. Biosurfactants produced by *Scheffersomyces stipitis* cultured in sugarcane bagasse hydrolysate as new green larvicides for the control of *Aedes aegypti*, a vector of neglected tropical diseases. **PloS One**, v. 12, n. 11, p. 1-16, 2017.

MARCELINO, P. R. F. et al. Biosurfactants production by yeasts using sugarcane bagasse hemicellulosic hydrolysate as new sustainable alternative for lignocellulosic biorefineries. **Industrial Crops and Products**, v. 129, p. 212-223, 2019.

MARCHAND, C. et al. Petroleum biodegradation capacity of bacteria and fungi isolated from petroleum-contaminated soil. **International Biodeterioration & Biodegradation**, v. 116, p. 48-57, 2017.

MARCHANT, R.; BANAT, I. M. Biosurfactants: a sustainable replacement for chemical surfactants? **Biotechnology Letters**, v. 34, n. 9, p. 1597-1605, 2012a.

MARCHANT, R.; BANAT, I. M. Microbial biosurfactants: challenges and opportunities for future exploitation. **Trends in Biotechnology**, v. 30, n. 11, p. 558-565, 2012b.

MARQUES, N. S. A. A. et al. Lipopeptide biosurfactant produced by *Mucor circinelloides* UCP/WFCC 0001 applied in the removal of crude oil and engine oil from soil. **Acta Scientiarum. Technology**, v. 41, p. 1-10, 2019.

MARTI, M. E. et al. Production and characterization of microbial biosurfactants for potential use in oil-spill remediation. **Enzyme and Microbial Technology**, v. 55, p. 31-39, 2014.

MARTINHO, V. et al. Enzymatic potential and biosurfactant production by endophytic fungi from mangrove forest in Southeastern Brazil. **AMB Express**, v. 9, n. 1, p. 1-8, 2019.

MARTINS, M. P. **Avaliação do comportamento da barreira física como técnica de remediação para o derramamento de biodiesel**. 67 f. Trabalho de Conclusão de Curso (Graduação em Engenharia Sanitária e Ambiental) – Universidade Federal de Santa Catarina, Florianópolis, 2022.

MATE, D. M.; ALCALDE, M. Laccase engineering: From rational design to directed evolution. **Biotechnology Advances**, v. 33, n. 1, p. 25-40, 2015.

MEDAURA, M. C. et al. Bioaugmentation of native fungi, an efficient strategy for the bioremediation of an aged industrially polluted soil with heavy hydrocarbons. **Frontiers in Microbiology**, v. 12, p. 1-18, 2021.

MENESES, D. P. et al. The yeast-like fungus *Aureobasidium thailandense* LB01 produces a new biosurfactant using olive oil mill wastewater as an inducer. **Microbiological Research**, v. 204, p. 40-47, 2017.

MENON, V. et al. Biocatalytic approach for the utilization of hemicellulose for ethanol production from agricultural residue using thermostable xylanase and thermotolerant yeast. **Bioresource Technology**, v. 101, n. 14, p. 5366-5373, 2010.

MERCADÉ, M. E. et al. Screening and selection of surfactant-producing bacteria from waste lubricating oil. **The Journal of Applied Bacteriology**, v. 81, n. 2, p. 161-166, 1996.

MIMEE, B.; LABBÉ, C.; BÉLANGER, R. R. Catabolism of flocculosin, an antimicrobial metabolite produced by *Pseudozyma flocculosa*. **Glycobiology**, v. 19, n. 9, p. 995-1001, 2009.

MISHRA, A.; MALIK, A. Novel fungal consortium for bioremediation of metals and dyes from mixed waste stream. **Bioresource Technology**, v. 171, p. 217-226, 2014.

MISHRA, S. et al. Biosurfactant is a powerful tool for the bioremediation of heavy metals from contaminated soils. **Journal of Hazardous Materials**, v. 418, n. 126253, p. 1-23, 2021.

MNIF, I.; GHRIBI, D. Microbial derived surface active compounds: properties and screening concept. **World Journal of Microbiology and Biotechnology**, v. 31, n. 7, p. 1001-1020, 2015.

MOGHIMI, H.; TABAR, R.H.; HAMEDI, J. Assessing the biodegradation of polycyclic aromatic hydrocarbons and laccase production by new fungus *Trematophoma* sp. UTMC 5003. **World Journal of Microbiology and Biotechnology**, v. 33, n. 7, p. 1-10, 2017.

MOHAMMADI, M. et al. Immobilization of laccase on epoxy-functionalized silica and its application in biodegradation of phenolic compounds. **International Journal of Biological Macromolecules**, v. 109, p. 443-447, 2018.

MOHANRAM, R.; JAGTAP, C.; KUMAR, P. Isolation, screening, and characterization of surface-active agent-producing, oil-degrading marine bacteria of Mumbai Harbor. **Marine Pollution Bulletin**, v. 105, n. 1, p. 131-138, 2016.

MORALES-BARRERA, L.; CRISTIANI-URBINA, E. Removal of hexavalent chromium by *Trichoderma viride* in an airlift bioreactor. **Enzyme and Microbial Technology**, v. 40, n. 1, p. 107-113, 2006.

MORITA, T. et al. Accumulation of cellobiose lipids under nitrogen-limiting conditions by two ustilaginomycetous yeasts, *Pseudozyma aphidis* and *Pseudozyma hubeiensis*. **FEMS Yeast Research**, v. 13, n. 1, p. 44–49, 2013.

MORITA, T. et al. Glycolipid Biosurfactants. *In*: **Reference Module in Chemistry, Molecular Sciences and Chemical Engineering**. [s.l.] Elsevier, p. 1-7, 2016.

MORITA, T. et al. Isolation of *Pseudozyma churashimaensis* sp. *nov.*, a novel ustilaginomycetous yeast species as a producer of glycolipid biosurfactants, mannosylerythritol lipids. **Journal of Bioscience and Bioengineering**, v. 112, n. 2, p. 137-144, 2011.

MORITA, T. et al. Physiological differences in the formation of the glycolipid biosurfactants, mannosylerythritol lipids, between *Pseudozyma antarctica* and *Pseudozyma aphidis*. **Applied Microbiology and Biotechnology**, v. 74, n. 2, p. 307-315, 2007.

MOSA, K. A. et al. Potential biotechnological strategies for the cleanup of heavy metals and metalloids. **Frontiers in Plant Science**, v. 7, p. 1-14, 2016.

MOTA, E. A. et al. Manganese (II) removal from aqueous solutions by *Cladosporium halotolerans* and *Hypocrea jecorina*. **Biotechnology Reports**, v. 25, p. 1-8, 2020.

MOUSAVI, F.; BEHESHTI-MAAL, K.; MASSAH, A. Production of sophorolipid from an identified current yeast, *Lachancea thermotolerans* BBMCZ7FA20, isolated from honey bee. **Current Microbiology**, v. 71, n. 2, p. 303-310, 2015.

MOXLEY, R. A. Jr. Formative and non-formative evaluation. **Instructional Science**, v. 3, n. 3, p. 243-283, 1974.

MUKHERJEE, T. et al. Bioremediation potential of arsenic by non-enzymatically biofabricated silver nanoparticles adhered to the mesoporous carbonized fungal cell surface of *Aspergillus foetidus* MTCC8876. **Journal of Environmental Management**, v. 201, p. 435-446, 2017.

MUKHOPADHYAY, S. K. et al. Isolation and characterization of extracellular polysaccharide Thelebolan produced by a newly isolated psychrophilic Antarctic fungus *Thelebolus*. **Carbohydrate Polymers**, v. 104, p. 204-212, 2014.

MÜLLER, J. B. et al. Combined iron and sulfate reduction biostimulation as a novel approach to enhance BTEX and PAH source-zone biodegradation in biodiesel blend-contaminated groundwater. **Journal of Hazardous Materials**, v. 326, p. 229–236, 2017.

MULLIGAN, C. N. Environmental applications for biosurfactants. **Environmental Pollution**, v. 133, n. 2, p. 183-198, 2005.

MULLIGAN, C. N.; COOPER, D. G. Selection of microbes producing biosurfactants in media without hydrocarbons. **Journal of Fermentation Technology**, v. 62, n. 4, p. 311–314, 1984.

MULLIGAN, C. N.; YONG, R. N.; GIBBS, B. F. An evaluation of technologies for the heavy metal remediation of dredged sediments. **Journal of Hazardous Materials**, v. 85, n. 1-2, p. 145-163, 2001.

MUÑOZ, A. J. et al. Heavy metal tolerance of microorganisms isolated from wastewaters: Identification and evaluation of its potential for biosorption. **Chemical Engineering Journal**, v. 210, p. 325-332, 2012.

MURIEL, J. et al. Production of biosurfactants by *Cladosporium resinae*. **Biotechnology Letters**, v. 18, n. 3, p. 235-240, 1996.

NAJAFPOUR, G. D. Industrial Microbiology. *In*: **Biochemical Engineering and Biotechnology**. [s.l.] Elsevier, p. 1-18, 2015.

NAJMI, Z. et al. In situ downstream strategies for cost-effective bio/surfactant recovery. **Biotechnology and Applied Biochemistry**, v. 65, n. 4, p. 523-532, 2018.

NASHIDA, J. et al. Systematic and stereoselective total synthesis of mannosylerythritol lipids and evaluation of their antibacterial activity. **The Journal of Organic Chemistry**, v. 83, n. 13, p. 7281-7289, 2018.

NAUGHTON, P. J. et al. Microbial biosurfactants: current trends and applications in agricultural and biomedical industries. **Journal of Applied Microbiology**, v. 127, n. 1, p. 12-28, 2019.

NEUWIRTH, E. **RColorBrewer**: ColorBrewer Palettes. R Package (v. 1.1.3), 2022.

NIKOLAIVITS, E. et al. Versatile fungal polyphenol oxidase with chlorophenol bioremediation potential: Characterization and protein engineering. **Applied and Environmental Microbiology**, v. 84, n. 23, p. 1-16, 2018.

NIU, Y. et al. Characterization, enhancement and modelling of mannosylerythritol lipid production by fungal endophyte *Ceriporia lacerate* CHZJU. **Food Chemistry**, v. 228, p. 610-617, 2017.

NIU, Y. et al. Production and characterization of a new glycolipid, mannosylerythritol lipid, from waste cooking oil biotransformation by *Pseudozyma aphidis* ZJUDM34. **Food Science & Nutrition**, v. 7, n. 3, p. 937-948, 2019.

NJOKU, K. L.; AKINYEDE, O. R.; OBIDI, O. F. Microbial Remediation of Heavy Metals Contaminated Media by *Bacillus megaterium* and *Rhizopus stolonifer*. **Scientific African**, v. 10, n. e00545, p. 1-8, 2020.

NOWAK, K. et al. Fungal (1 → 3)-α-d-glucans as a new kind of biosorbent for heavy metals. **International Journal of Biological Macromolecules**, v. 137, p. 960-965, 2019.

NURFARAHIN, A. H.; MOHAMED, M. S.; PHANG, L. Y. Culture medium development for microbial-derived surfactants production-an overview. **Molecules**, v. 23, n. 5, p. 1-26, 2018.
O'BRIEN, M. et al. Morphological and molecular characterisation of *Penicillium roqueforti* and *P. paneum* isolated from baled grass silage. **Mycological Research**, v. 112, p. 921–932, 2008.

O'CALLAHAN, D. et al. *Penicillium rotoruae*, a new Species from an In-Ground Timber Durability Test Site in New Zealand. **Current Microbiology**, v. 77, n. 12, p. 4129–4139, 2020.

OCHOA, S. Fed-batch fermentation – design strategies. *In*: **Comprehensive Biotechnology**. [s.l.] Elsevier, p. 586-600, 2019.

OGAKI, M. B. et al. Diversity and bioprospecting of cultivable fungal assemblages in sediments of lakes in the *Antarctic Peninsula*. **Fungal Biology**, v. 124, n. 6, p. 601–611, 2020.

OH, J.-J. et al. Utilization of extracellular fungal melanin as an eco-friendly biosorbent for treatment of metal-contaminated effluents. **Chemosphere**, v. 272, n. 129884, p. 1-9, 2021.

OJHA, N.; MANDAL, S. K.; DAS, N. Enhanced degradation of indeno(1,2,3-cd)pyrene using *Candida tropicalis* NN4 in presence of iron nanoparticles and produced biosurfactant: a statistical approach. **3 Biotech**, v. 9, n. 3, p. 1-13, 2019.

OLANIRAN, A. O.; BALGOBIND, A.; PILLAY, B. Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. **International Journal of Molecular Sciences**, v. 14, n. 5, p. 10197-10228, 2013.

OLIVEIRA, J. S. et al. BioSurfDB: knowledge and algorithms to support biosurfactants and biodegradation studies. **Database:** The Journal of Biological Databases and Curation, v. 2015, p. 1-8, 2015.

OLIVEIRA, V. H. et al. Bioremediation potential of Cd by transgenic yeast expressing a metallothionein gene from *Populus trichocarpa*. **Ecotoxicology and Environmental Safety**, v. 202, n. 110917, p. 1-9, 2020.

ONG, S. A.; WU, J. C. A simple method for rapid screening of biosurfactant-producing strains using bromothymol blue alone. **Biocatalysis and Agricultural Biotechnology**, v. 16, p. 121-125, 2018.

ONWOSI, C. O. et al. Microbial-derived glycolipids in the sustainable formulation of biomedical and personal care products: A consideration of the process economics towards commercialization. **Process Biochemistry**, v. 100, p. 124-139, 2021.

ORTEGA-GONZÁLEZ, D. K. et al. Evaluation of the removal of pyrene and fluoranthene by *Ochrobactrum anthropi*, *Fusarium* sp. and their coculture. **Applied Biochemistry and Biotechnology,** v. 175, n. 2, p. 1123-1138, 2015.

OSTREM LOSS, E. M.; YU, J.-H. Bioremediation and microbial metabolism of benzo(a)pyrene. **Molecular Microbiology**, v. 109, n. 4, p. 433-444, 2018.

OTHMAN, A. R. et al. Potential of indigenous biosurfactant-producing fungi from real crude oil sludge in total petroleum hydrocarbon degradation and its future research prospects. **Journal of Environmental Chemical Engineering**, v. 10, n. 3, p. 107621, 2022.

OTZEN, D. E. Biosurfactants and surfactants interacting with membranes and proteins: Same but different? **Biochimica et Biophysica Acta**, v. 1859, n. 4, p. 639-649, 2017.

OZDENER, M. H. et al. Sophorolipid biosurfactants activate taste receptor type 1 member 3-mediated taste responses and block responses to bitter taste *in vitro* and *in vivo*. **Journal of Surfactants and Detergents**, v. 22, n. 3, p. 441-449, 2019.

OZYUREK, S. B.; AVCIOGLU, N. H.; BILKAY, I. S. Mycoremediation potential of *Aspergillus ochraceus* NRRL 3174. **Archives of Microbiology**, v. 203, n. 10, p. 5937-5950, 2021.

PANDEY, A. K. et al. Biocatalytic remediation of industrial pollutants for environmental sustainability: Research needs and opportunities. **Chemosphere**, v. 272, n. 129936, p. 1-12, 2021.

PARASZKIEWICZ, K.; KANWAL, A.; DŁUGONSKI, J. Emulsifier production by steroid transforming filamentous fungus *Curvularia lunata*. Growth and product characterization. **Journal of Biotechnology**, v. 92, n. 3, p. 287–294, 2002.

PAREKH, V. J.; PANDIT, A. B. Solid state fermentation (SSF) for the production of sophorolipids from *Starmerella bombicola* NRRL Y-17069 using glucose, wheat bran and oleic acid. **Current Trends in Biotechnology and Pharmacy**, v. 6, n. 4, p. 418-424, 2012.

PARK, H. et al. Comprehensive genomic and transcriptomic analysis of polycyclic aromatic hydrocarbon degradation by a mycoremediation fungus, *Dentipellis* sp. KUC8613. **Applied Microbiology and Biotechnology**, v. 103, n. 19, p. 8145-8155, 2019.

PAULINO, B. N. et al. Current status in biotechnological production and applications of glycolipid biosurfactants. **Applied Microbiology and Biotechnology**, v. 100, n. 24, p. 10265-10293, 2016.

PEIDRO-GUZMÁN, H. et al. Transcriptomic analysis of polyaromatic hydrocarbon degradation by the halophilic fungus *Aspergillus sydowii* at hypersaline conditions. **Environmental Microbiology**, v. 23, n. 7, p. 3435-3459, 2021.

PELE, M. A. et al. Conversion of renewable substrates for biosurfactant production by *Rhizopus arrhizus* UCP 1607 and enhancing the removal of diesel oil from marine soil. **Electronic Journal of Biotechnology**, v. 38, p. 40-48, 2019.

PERFUMO, A. et al. Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. *In*: **Handbook of Hydrocarbon and Lipid Microbiology**. Berlin, Heidelberg: Springer Berlin Heidelberg, p. 1501-1512, 2010.

PERFUMO, A.; BANAT, I. M.; MARCHANT, R. Going green and cold: Biosurfactants from low-temperature environments to biotechnology applications. **Trends in Biotechnology**, v. 36, n. 3, p. 277-289, 2018.

PESSÔA, M. G. et al. Newly isolated microorganisms with potential application in biotechnology. **Biotechnology Advances**, v. 37, n. 2, p. 319-339, 2019.

PIEGZA, M. et al. Biosurfactants from *Trichoderma* filamentous fungi-A preliminary study. **Biomolecules**, v. 11, n. 4, p. 1-13, 2021.

PINO-HERRERA, D. O. et al. Removal mechanisms in aerobic slurry bioreactors for remediation of soils and sediments polluted with hydrophobic organic compounds: An overview. **Journal of Hazardous Materials**, v. 339, p. 427-449, 2017.

PIOTROWSKI, M.; LEWANDOWSKA, J.; WOJCIECHOWSKI, K. Biosurfactant-protein mixtures: Quillaja Bark Saponin at water/air and water/oil interfaces in presence of β-lactoglobulin. **The Journal of Physical Chemistry B**, v. 116, n. 16, p. 4843-4850, 2012.

PITOCCHI, R. et al. Cerato-platanins from marine fungi as effective protein biosurfactants and bioemulsifiers. **International Journal of Molecular Sciences**, v. 21, n. 8, p. 1-12, 2020.

POSCH, A. E.; HERWIG, C.; SPADIUT, O. Science-based bioprocess design for filamentous fungi. **Trends in Biotechnology**, v. 31, n. 1, p. 37-44, 2013.

POTIN, O.; RAFIN, C.; VEIGNIE, E. Bioremediation of an aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil by filamentous fungi isolated from the soil. **International Biodeterioration & Biodegradation**, v. 54, n. 1, p. 45-52, 2004.

POYNTNER, C. et al. Selective screening: isolation of fungal strains from contaminated soils in Austria. **Bodenkultur**, v. 68, n. 3, p. 157-169, 2018.

PRENAFETA-BOLDÚ, F. X. et al. Isolation and characterisation of fungi growing on volatile aromatic hydrocarbons as their sole carbon and energy source. **Mycological Research**, v. 105, n. 4, p. 477-484, 2001.

PRENAFETA-BOLDÚ, F. X.; HOOG, G. S.; SUMMERBELL, R. C. Fungal communities in hydrocarbon degradation. *In*: **Microbial Communities Utilizing Hydrocarbons and Lipids**: Members, Metagenomics and Ecophysiology. Cham: Springer International Publishing, p. 1-36, 2019.

PRICE, N. P. et al. Polyols, not sugars, determine the structural diversity of anti-streptococcal liamocins produced by *Aureobasidium pullulans* strain NRRL 50380. **The Journal of Antibiotics**, v. 70, n. 2, p. 136-141, 2017.

PRICE, N. P. J. et al. Structural characterization of novel extracellular liamocins (mannitol oils) produced by *Aureobasidium pullulans* strain NRRL 50380. **Carbohydrate Research**, v. 370, p. 24-32, 2013.

PUCHKOV, E. O. et al. The mycocidal, membrane-active complex of *Cryptococcus humicola* is a new type of cellobiose lipid with detergent features. **Biochimica et Biophysica Acta. Biomembranes**, v. 1558, n. 2, p. 161-170, 2002.

PUGLISI, I. et al. Identification of differentially expressed genes in response to mercury I and II stress in *Trichoderma harzianum*. **Gene**, v. 506, n. 2, p. 325-330, 2012.

PURCHASE, D. et al. Effects of temperature on metal tolerance and the accumulation of Zn and Pb by metal-tolerant fungi isolated from urban runoff treatment wetlands. **Journal of Applied Microbiology**, v. 106, n. 4, p. 1163-1174, 2009.

QAZI, M. A. et al. Isolation and characterization of a biosurfactant-producing *Fusarium* sp. BS-8 from oil contaminated soil. **Biotechnology Progress**, v. 30, n. 5, p. 1065-1075, 2014.

QI, X. et al. Removal of cadmium and lead from contaminated soils using sophorolipids from fermentation culture of *Starmerella bombicola* CGMCC 1576 fermentation. **International Journal of Environmental Research and Public Health**, v. 15, p. 1-12, 2018.

QIAO, N.; SHAO, Z. Isolation and characterization of a novel biosurfactant produced by hydrocarbon-degrading bacterium *Alcanivorax dieselolei* B-5. **Journal of Applied Microbiology**, v. 108, n. 4, p. 1207-1216, 2010.

QUINTELLA, C. M.; MATA, A. M. T.; LIMA, L. C. P. Overview of bioremediation with technology assessment and emphasis on fungal bioremediation of oil contaminated soils. **Journal of Environmental Management**, v. 241, p. 156-166, 2019.

RAAIJMAKERS, J. M. et al. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. **FEMS Microbiology Reviews**, v. 34, n. 6, p. 1037-1062, 2010.

RADHA, P. et al. Chicken tallow, a renewable source for the production of biosurfactant by *Yarrowia lipolytica* MTCC9520, and its application in silver nanoparticle synthesis. **Journal of Surfactants and Detergents**, v. 23, n. 1, p. 119-135, 2020.

RAMDASS, A. C.; RAMPERSAD, S. N. Biodiversity and biocatalyst activity of culturable hydrocarbonoclastic fungi isolated from Marac-Moruga mud volcano in South Trinidad. **Scientific Reports**, v. 11, n. 1, p. 1-18, 2021.

RAMOS, D. T. et al. Assessment of microbial communities associated with fermentative-methanogenic biodegradation of aromatic hydrocarbons in groundwater contaminated with a biodiesel blend (B20). **Biodegradation**, v. 25, n. 5, p. 681–691, 2014.

RAMOS, D. T. et al. Biodiesel presence in the source zone hinders aromatic hydrocarbons attenuation in a B20-contaminated groundwater. **Journal of Contaminant Hydrology**, v. 193, p. 48–53, 2016.

RANJANA, M. et al. Sophorolipid induced hydrothermal synthesis of Cu nanowires and its modulating effect on Cu nanostructures. **Nano-structures & Nano-objects**, v. 18, n. 100285, p. 1-7, 2019.

RATHANKUMAR, A. K. et al. Surfactant-aided mycoremediation of soil contaminated with polycyclic aromatic hydrocarbon (PAHs): progress, limitation, and countermeasures. **Journal of Chemical Technology and Biotechnology**, v. 97, n. 2, p. 391-408, 2021.

RAU, U. et al. Fed-batch bioreactor production of mannosylerythritol lipids secreted by *Pseudozyma aphidis*. **Applied Microbiology and Biotechnology**, v. 68, n. 5, p. 607-613, 2005.

RAUDABAUGH, D. B.; MILLER, A. N. Nutritional capability of and substrate suitability for *Pseudogymnoascus destructans*, the causal agent of bat white-nose syndrome. **PloS One**, v. 8, n. 10, p. 1-9, 2013.

REHMAN, R. et al. Crude oil biodegradation potential of biosurfactant-producing *Pseudomonas aeruginosa* and *Meyerozyma* sp. **Journal of Hazardous Materials**, v. 418, n. 126276, p. 1-12, 2021.

REIS, C. B. L. D. et al. First report of the production of a potent biosurfactant with α, β-trehalose by *Fusarium fujikuroi* under optimized conditions of submerged fermentation. **Brazilian Journal of Microbiology**, v. 49, Suppl 1, p. 185-192, 2018.

RESENDE, A. H. M. et al. Application of biosurfactants and chitosan in toothpaste formulation. **Colloids and Surfaces B**: Biointerfaces, v. 181, p. 77-84, 2019.

REYES-CÉSAR, A. et al. Biodegradation of a mixture of PAHs by non-ligninolytic fungal strains isolated from crude oil-contaminated soil. **World Journal of Microbiology and Biotechnology**, v. 30, n. 3, p. 999-1009, 2014.

RIENZO, M. A. D. D. et al. Antimicrobial properties of sophorolipids produced by *Candida bombicola* ATCC 22214 against gram positive and Gram-negative bacteria. **New Biotechnology**, v. 31, p. 66-67, 2014.

RIZZO, C. et al. Efficiency in hydrocarbon degradation and biosurfactant production by *Joostella* sp. A8 when grown in pure culture and consortia. **Journal of Environmental Sciences**, v. 67, p. 115-126, 2018.

ROBICHAUD, K. et al. Local fungi, willow and municipal compost effectively remediate petroleum-contaminated soil in the Canadian North. **Chemosphere**, v. 220, p. 47-55, 2019.

ROCHA JUNIOR, R. B. et al. Application of a low-cost biosurfactant in heavy metal remediation processes. **Biodegradation**, v. 30, n. 4, p. 215-233, 2019.

RODRIGUES, L. R. Microbial surfactants: fundamentals and applicability in the formulation of nano-sized drug delivery vectors. **Journal of Colloid and Interface Science**, v. 449, p. 304-316, 2015.

ROLLEMBERG, N. C. et al. Identification of fungi in flaxseed (*L. usitatissimum* L.) using the ITS1 and ITS2 intergenic regions. **Microbiology Research**, v. 13, n. 2, p. 315-322, 2022.

ROSENBERG, M.; GUTNICK, D.; ROSENBERG, E. Adherence of bacteria to hydrocarbons: A simple method for measuring cell-surface hydrophobicity. **FEMS Microbiology Letters**, v. 9, n. 1, p. 29-33, 1980.

ROSHANDEL, F. et al. Mycoremediation of oil contaminant by *Pleurotus florida* (P.Kumm) in liquid culture. **Fungal Biology**, v. 125, n. 9, p. 667-678, 2021.

ROSS, I. S. Some effects of heavy metals on fungal cells. **Transactions of the British Mycological Society**, v. 64, n. 2, p. 175-193, 1975.

ROTTA, L. H. S. et al. The 2019 Brumadinho tailings dam collapse: Possible cause and impacts of the worst human and environmental disaster in Brazil. **International Journal of Applied Earth Observation and Geoinformation**, v. 90, n. 102119, p. 1-12, 2020.

RUFINO, R. D. et al. Characterization and properties of the biosurfactant produced by *Candida lipolytica* UCP 0988. **Electronic Journal of Biotechnology**, v. 17, n. 1, p. 34-38, 2014.

RUFINO, R. D. et al. Removal of petroleum derivative adsorbed to soil by biosurfactant Rufisan produced by *Candida lipolytica*. **Journal of Petroleum Science & Engineering**, v. 109, p. 117-122, 2013.

RUSINOVA-VIDEVA, S. et al. Antarctic yeast *Cryptococcus laurentii* (AL 65): biomass and exopolysaccharide production and biosorption of metals. **Journal of Chemical Technology and Biotechnology**, v. 95, n. 5, p. 1372-1379, 2020.

SAIKA, A. et al. Biosynthesis of mono-acylated mannosylerythritol lipid in an acyltransferase gene-disrupted mutant of *Pseudozyma tsukubaensis*. **Applied Microbiology and Biotechnology**, v. 102, n. 4, p. 1759-1767, 2018b.

SAIKA, A. et al. Enhanced production of a diastereomer type of mannosylerythritol lipid-B by the basidiomycetous yeast *Pseudozyma tsukubaensis* expressing lipase genes from *Pseudozyma antarctica*. **Applied Microbiology and Biotechnology,** v. 101, n. 23–24, p. 8345-8352, 2017.

SAIKA, A. et al. Screening and isolation of the liamocin-producing yeast *Aureobasidium melanogenum* using xylose as the sole carbon source. **Journal of Bioscience and Bioengineering**, v. 129, n. 4, p. 428-434, 2020.

SAIKA, A. et al. Tailor-made mannosylerythritol lipids: current state and perspectives. **Applied Microbiology and Biotechnology**, v. 102, n. 16, p. 6877-6884, 2018a.

SAŁEK, K.; EUSTON, S. R. Sustainable microbial biosurfactants and bioemulsifiers for commercial exploitation. **Process Biochemistry**, v. 85, p. 143-155, 2019.

SALLADA, N. D.; HARKINS, L. E.; BERGER, B. W. Effect of gene copy number and chaperone coexpression on recombinant hydrophobin HFBI biosurfactant production in *Pichia pastoris*. **Biotechnology and Bioengineering**, v. 116, n. 8, p. 2029-2040, 2019.

SANCHES, M. et al. Biosurfactant Production by *Fusarium oxysporum* LM 5634 using peels from the fruit of *Bactris gasipaes* (Kunth) as substrate. **Biotechnology Journal International**, v. 21, n. 1, p. 1–9, 2018.

SÁNCHEZ-VÁZQUEZ, V. et al. Polycyclic aromatic hydrocarbon-emulsifier protein produced by *Aspergillus brasiliensis* (niger) in an airlift bioreactor following an electrochemical pretreatment. **Bioresource Technology**, v. 256, p. 408-413, 2018.

SANDERS, D. A. How to write (and how not to write) a scientific review article. **Clinical Biochemistry**, v. 81, p. 65-68, 2020.

SANTOS, D. K. F. et al. Biosurfactant production from *Candida lipolytica* in bioreactor and evaluation of its toxicity for application as a bioremediation agent. **Process Biochemistry**, v. 54, p. 20-27, 2017b.

SANTOS, D. K. F. et al. *Candida lipolytica* UCP0988 biosurfactant: Potential as a bioremediation agent and in formulating a commercial related product. **Frontiers in Microbiology**, v. 8, p. 1-11, 2017a.

SANTOS, D. K. F. et al. Optimization of cultural conditions for biosurfactant production from *Candida lipolytica*. **Biocatalysis and Agricultural Biotechnology**, v. 3, n. 3, p. 48-57, 2014.

SANTOS, J. E. Á. et al. First report of *Fusarium kalimantanense* causing *Fusarium wilt* on banana tree in Brazil. **Plant Disease**, v. 107, n. 1, p. 227, 2022.

SANTOS, M. V. et al. Production of mannosylerythritol lipids from lignocellulose hydrolysates: tolerance thresholds of *Moesziomyces antarcticus* to inhibitors: *Moesziomyces antarcticus* tolerance to inhibitors in biosurfactants production. **Journal of Chemical Technology and Biotechnology**, v. 94, n. 4, p. 1064-1072, 2019.

SARUBBO, L. A. et al. Some aspects of heavy metals contamination remediation and role of biosurfactants. **Chemistry in Ecology**, v. 31, n. 8, p. 707-723, 2015.

SATOW, M. M. et al. Selective factors involved in oil flotation isolation of black yeasts from the environment. **Studies in Mycology**, v. 61, p. 157-163, 2008.

SATPUTE, S. K. et al. Methods for investigating biosurfactants and bioemulsifiers: a review. **Critical Reviews in Biotechnology**, v. 30, n. 2, p. 127-144, 2010.

SAUR, K. M. et al. A pH shift induces high-titer liamocin production in *Aureobasidium pullulans.* **Applied Microbiology and Biotechnology**, v. 103, n. 12, p. 4741-4752, 2019.

SCHAFFER, S.; WESSEL, M.; THIESSENHUSEN, A. **Cells, Nucleic Acids, Enzymes and Use Thereof, and Methods for the Production of Sophorolipids**- US 8.911.982 B2. Lens, 2014. Disponível em: https://www.lens.org/lens/patent/127-281-411-967-478. Acesso em: 28/07/2020

SCHMIDT, P.-A. et al. Illumina metabarcoding of a soil fungal community. **Soil Biology & Biochemistry**, v. 65, p. 128–132, 2013.

SCHMIDELL, W. et al. **Biotecnologia Industrial** - Engenharia Bioquímica. São Paulo: Edgard Blucher Ltda, 2001.

SCHMIDT, S. N.; CHRISTENSEN, J. H.; JOHNSEN, A. R. Fungal PAH-metabolites resist mineralization by soil microorganisms. **Environmental Science & Technology**, v. 44, n. 5, p. 1677–1682, 2010.

SCHULTZ, J.; ROSADO, A. S. Extreme environments: a source of biosurfactants for biotechnological applications. **Extremophiles**: Life Under Extreme Conditions, v. 24, n. 2, p. 189-206, 2020.

SCOTT, M. J.; JONES, M. N. The biodegradation of surfactants in the environment. **Biochimica et Biophysica Acta. Biomembranes**, v. 1508, n. 1-2, p. 235-251, 2000.

SELBMANN, L. et al. Production and structural characterization of the exopolysaccharide of the Antarctic fungus *Phoma herbarum* CCFEE 5080. **Research in Microbiology**, v. 153, n. 9, p. 585-592, 2002.

SEN, S. et al. Production, characterization, and antifungal activity of a biosurfactant produced by *Rhodotorula babjevae* YS3. **Microbial Cell Factories**, v. 16, n. 1, p. 1-14, 2017.

SENA, H. H. et al. Production of biosurfactants by soil fungi isolated from the Amazon forest. **International Journal of Microbiology**, v. 2018, p. 1-8, 2018.

SHAH, M. U. H. et al. Production of sophorolipids by *Starmerella bombicola* yeast using new hydrophobic substrates. **Biochemical Engineering Journal**, v. 127, p. 60-67, 2017.

SHAH, V. et al. Sophorolipids, microbial glycolipids with anti-human immunodeficiency virus and sperm-immobilizing activities. **Antimicrobial Agents and Chemotherapy**, v. 49, n. 10, p. 4093-4100, 2005.

SHAH, V.; DAVEREY, A. Effects of sophorolipids augmentation on the plant growth and phytoremediation of heavy metal contaminated soil. **Journal of Cleaner Production**, v. 280, n. 124406, p. 124406, 2021.

SHARMA, R. et al. Bioremediation potential of novel fungal species isolated from wastewater for the removal of lead from liquid medium. **Environmental Technology & Innovation**, v. 18, n. 100757, p. 1-12, 2020.

SHEN, L. et al. Isolation and purification of biosurfactant mannosylerythritol lipids from fermentation broth with methanol/water/n-hexane. **Separation and Purification Technology**, v. 219, p. 1-8, 2019.

SHETAIA, Y. M. H. et al. Potential biodegradation of crude petroleum oil by newly isolated halotolerant microbial strains from polluted Red Sea area. **Marine Pollution Bulletin**, v. 111, n. 1–2, p. 435-442, 2016.

SHI, Z. et al. Vermiremediation of organically contaminated soils: Concepts, current status, and future perspectives. **Applied Soil Ecology**: a section of Agriculture, Ecosystems & Environment, v. 147, n. 103377, p. 1-11, 2020.

SHIMIZU, G. D.; MARUBAYASHI, R. Y. P.; GONCALVES, L. S. A. **AgroR**: Experimental Statistics and Graphics for Agricultural Sciences. R Package (v. 1.3.1), 2022. Disponível em: https://agronomiar.github.io/AgroR_package/index.html. Acesso em:17/02/2023

SIEGMUND, I.; WAGNER, F. New method for detecting rhamnolipids excreted by *Pseudomonas* species during growth on mineral agar. **Biotechnology Techniques**, v. 5, n. 4, p. 265-268, 1991.

SIEVERDING, E. **Increasing Yields By The Use Of Sophorolipids** - US 2017/0094968 A1. Google Patents, 2017. Disponível em: https://patents.google.com/patent/EP3142490A1/en. Acesso em:28/07/2020

SILVA, A. F. et al. Fungal bioproducts for petroleum hydrocarbons and toxic metals remediation: recent advances and emerging technologies. **Bioprocess and Biosystems Engineering**, v. 46, n. 3, p. 393–428, 2023.

SILVA, A. F. et al. Fungal biosurfactants, from nature to biotechnological product: bioprospection, production and potential applications. Bioprocess and **Biosystems Engineering**, v. 44, n. 10, p. 2003-2034, 2021c.

SILVA, I. G. S. et al. Application of green surfactants in the remediation of soils contaminated by hydrocarbons. **Processes**, v. 9, n. 9, p. 1-21, 2021b.

SILVA, J. F. et al. Bioemulsifier produced by *Yarrowia lipolytica* using residual glycerol as a carbon source. **Journal of Environmental Analysis and Progress**, v. 5, n. 1, p. 031-037, 2020.

SILVA, M. E. T. et al. Biosurfactant production of *Piper hispidum* endophytic fungi. **Journal of Applied Microbiology**, v. 130, n. 2, p. 561-569, 2021a.

SILVA, N. R. A. et al. Biosurfactant-and-bioemulsifier produced by a promising *Cunninghamella echinulata* isolated from Caatinga soil in the northeast of Brazil. **International Journal of Molecular Sciences**, v. 15, n. 9, p. 15377-15395, 2014.

SILVA, S. N. R. L. et al. Glycerol as substrate for the production of biosurfactant by *Pseudomonas aeruginosa* UCP0992. **Colloids and Surface B**: Biointerfaces, v. 79, n. 1, p. 174-183, 2010.

SILVEIRA, V. A. I. et al. Characterization and antimicrobial properties of bioactive packaging films based on polylactic acid-sophorolipid for the control of foodborne pathogens. **Food Packaging and Shelf Life**, v. 26, n. 100591, p. 1-7, 2020.

SILVEIRA, V. A. I. et al. Production and antimicrobial activity of sophorolipid against *Clostridium perfringens* and *Campylobacter jejuni* and their additive interaction with lactic acid. **Biocatalysis and Agricultural Biotechnology**, v. 21, n. 101287, p. 1-7, 2019.

SIMISTER, R. L. et al. Degradation of oil by fungi isolated from Gulf of Mexico beaches. **Marine pollution bulletin**, v. 100, n. 1, p. 327–333, 2015.

SINGH, P. C. et al. Mycoremediation mechanisms for heavy metal resistance/tolerance in plants. *In*: **Fungal Biology**. Cham: Springer International Publishing, p. 351-381, 2018.

SINGH, P.; PATIL, Y.; RALE, V. Biosurfactant production: emerging trends and promising strategies. **Journal of Applied Microbiology**, v. 126, n. 1, p. 2-13, 2019.

SINGH, R. K. et al. Fungi as potential candidates for bioremediation. *In*: **Abatement of Environmental Pollutants**. [s.l.] Elsevier, p. 177-191, 2020.

SINGH, R. K. et al. Fungi as potential candidates for bioremediation. In: **Abatement of Environmental Pollutants**. Elsevier, p. 177–191, 2020.

SINGH, S. K. et al. Biosurfactant producing microbes for clean-up of soil contaminants. *In*: **Microbe Mediated Remediation of Environmental Contaminants**. [s.l.] Elsevier, p. 89-93, 2021.

SMITH, D. Culture Collections. *In*: **Advances in Applied Microbiology**. San Diego, CA: Elsevier, 2012.

SMITS, T. H. M. et al. Characterization of the surface hydrophobicity of filamentous fungi. **Environmental Microbiology**, v. 5, n. 2, p. 85-91, 2003.

SMYTH, T. J. P. et al. Isolation and analysis of low molecular weight microbial glycolipids. *In*: **Handbook of Hydrocarbon and Lipid Microbiology**. Springer, Berlin, Heidelberg, p. 3705-3724, 2010.

SOBRINHO, H. B. S. et al. Utilization of two agroindustrial by-products for the production of a surfactant by *Candida sphaerica* UCP0995. **Process Biochemistry**, v. 43, n. 9, p. 912-917, 2008.

SOETAERT, W.; VAN BOGAERT, I.; ROELANTS, S. **Methods to produce bolaamphiphilic glycolipids** - WO 2015/028278. Google Patents, 2013. Disponível em: https://patents.google.com/patent/US20160168612A1/en. Acesso em:08/03/2020

SOLAIMAN, D. K. Y. et al. Cloning, characterization, and heterologous expression of a novel glucosyltransferase gene from sophorolipid-producing *Candida bombicola*. **Gene**, v. 540, n. 1, p. 46-53, 2014.

SOLAIMAN, D. K. Y. et al. Production of sophorolipids by *Candida bombicola* grown on soy molasses as substrate. **Biotechnology Letters**, v. 26, n. 15, p. 1241-1245, 2004.

SOUZA, E. C.; VESSONI-PENNA, T. C.; OLIVEIRA, R. P. S. Biosurfactant-enhanced hydrocarbon bioremediation: An overview. **International Biodeterioration & Biodegradation**, v. 89, p. 88-94, 2014.

SOUZA, H. M. L. et al. Filamentous fungi isolates of contaminated sediment in the Amazon region with the potential for Benzo(a)pyrene degradation. **Water, Air, and Soil Pollution**, v. 227, n. 12, p. 1-13, 2016.

SOUZA, K. S. T. et al. Improvement of biosurfactant production by *Wickerhamomyces anomalus* CCMA 0358 and its potential application in bioremediation. **Journal of Hazardous Materials**, v. 346, p. 152-158, 2018a.

SOUZA, K. S. T. et al. New glycolipid biosurfactants produced by the yeast strain *Wickerhamomyces anomalus* CCMA 0358. **Colloids and Surfaces B**: Biointerfaces, v. 154, p. 373-382, 2017.

SOUZA, L. M. D. **Diversidade de fungos presentes em lagos da antártica e detecção de enzimas e biossurfactantes de interesse industrial**. 112 f. Dissertação (Mestrado em Microbiologia) – Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, 2021.

SOUZA, P. et al. Production of a biosurfactant by *Cunninghamella echinulata* using renewable substrates and its applications in enhanced oil spill recovery. **Colloids and Interfaces**, v. 2, n. 4, p. 1-12, 2018b.

SPINA, F. et al. Screening of Anionic Biosurfactants Production among Fungi and Bacteria. **Chemical Engineering Transactions**, v. 64, p. 493-498, 2018.

SPINI, G. et al. Molecular and microbiological insights on the enrichment procedures for the isolation of petroleum degrading bacteria and fungi. **Frontiers in microbiology**, v. 9, p. 1-19, 2018.

SPOECKNER, S. et al. Glycolipids of the smut fungus *Ustilago maydis* from cultivation on renewable resources. **Applied Microbiology and Biotechnology**, v. 51, n. 1, p. 33-39, 1999.

SRIVASTAVA, A. K.; SINGH, R. K.; SINGH, D. Microbe-based bioreactor system for bioremediation of organic contaminants: present and future perspective. *In*: **Microbe Mediated Remediation of Environmental Contaminants**. Woodhead Publishing Series in Food Science, Technology and Nutrition, p. 241-253, 2021.

STANBURY, P. F.; WHITAKER, A.; HALL, S. J. Culture preservation and inoculum development. In: **Principles of Fermentation Technology**. [s.l.] Elsevier, p. 335-399, 2017a.

STANBURY, P. F.; WHITAKER, A.; HALL, S. J. Media for industrial fermentations. In: **Principles of Fermentation Technology**. [s.l.] Elsevier, p. 213-272, 2017b.

STEINKELLNER, S.; LANGER, I. Impact of tillage on the incidence of *Fusarium* spp. in soil. **Plant and soil**, v. 267, n. 1–2, p. 13–22, 2004.

SUN, Y. et al. Enhanced cometabolism of benzo(a)anthracene by the lignin monomer vanillate is related to structural and functional responses of the soil microbiome. **Soil Biology & Biochemistry**, v. 149, n. 107908, p. 1-10, 2020.

SUNDE, M.; PHAM, C. L. L.; KWAN, A. H. Molecular characteristics and biological functions of surface-active and surfactant proteins. **Annual Review of Biochemistry**, v. 86, n. 1, p. 585-608, 2017.

SY-CORDERO, A. A.; PEARCE, C. J.; OBERLIES, N. H. Revisiting the enniatins: a review of their isolation, biosynthesis, structure determination and biological activities. **The Journal of Antibiotics**, v. 65, n. 11, p. 541-549, 2012.

SYED, K. et al. CYP63A2, a catalytically versatile fungal P450 monooxygenase capable of oxidizing higher-molecular-weight polycyclic aromatic hydrocarbons, alkylphenols, and alkanes. **Applied and Environmental Microbiology**, v. 79, n. 8, p. 2692-2702, 2013.

SYED, K. et al. Genome-to-function characterization of novel fungal P450 monooxygenases oxidizing polycyclic aromatic hydrocarbons (PAHs). **Biochemical and Biophysical Research Communications**, v. 399, n. 4, p. 492-497, 2010.

TAKAHASHI, M. et al. Glycolipid biosurfactants, mannosylerythritol lipids, show antioxidant and protective effects against $H(_2)O(_2)$-induced oxidative stress in cultured human skin fibroblasts. **Journal of Oleo Science**, v. 61, n. 8, p. 457-464, 2012.

TAMURA, K.; NEI, M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. **Molecular biology and evolution**, v. 10, n. 3, p. 512-526, 1993.

TANG, J. et al. Biosurfactants enhanced heavy metals removal from sludge in the electrokinetic treatment. **Chemical Engineering Journal**, v. 334, p. 2579-2592, 2018.

R CORE TEAM. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2022. Disponível em: https://www.eea.europa.eu/data-and-maps/indicators/oxygen-consuming-substances-in-rivers/r-development-core-team-2006. Acesso em:

TEKE, G. et al. Assessment of foliar uptake and accumulation of airborne polyaromatic hydrocarbons under laboratory conditions. **Bulletin of Environmental Contamination and Toxicology**, v. 104, n. 4, p. 444-448, 2020.

TÉLLEZ-JURADO, A. et al. Expression of a heterologous laccase by *Aspergillus niger* cultured by solid-state and submerged fermentations. **Enzyme and Microbial Technology**, v. 38, n. 5, p. 665-669, 2006.

THIES, S. et al. Metagenomic discovery of novel enzymes and biosurfactants in a slaughterhouse biofilm microbial community. **Scientific Reports**, v. 6, p. 1-12, 2016.

THOMAS, G.; JAMES, D. Reinventing grounded theory: some questions about theory, ground and discovery. **British Educational Research Journal**, v. 32, n. 6, p. 767-795, 2006.

THOMSEN, C. N.; HART, M. M. Using invasion theory to predict the fate of arbuscular mycorrhizal fungal inoculants. **Biological Invasions**, v. 20, n. 10, p. 2695-2706, 2018.

TOLEDO, A. G. R. et al. Innovative method for encapsulating highly pigmented biomass from *Aspergillus nidulans* mutant for copper ions removal and recovery. **PloS One**, v. 16, n. 11, p. 1-12, 2021.

TREICHEL, H. et al. A review on microbial lipases production. **Food and Bioprocess Technology**, v. 3, n. 2, p. 182-196, 2010.

TRIPPE, K. M. et al. RNAi silencing of a cytochrome P450 monoxygenase disrupts the ability of a filamentous fungus, *Graphium* sp., to grow on short-chain gaseous alkanes and ethers. **Biodegradation**, v. 25, n. 1, p. 137-151, 2014.

TRUDGEON, B. et al. Low-temperature biosurfactants from polar microbes. **Microorganisms**, v. 8, n. 8, p. 1-14, 2020.

TWIGG, M. S. et al. Microbial biosurfactant research: time to improve the rigour in the reporting of synthesis, functional characterization and process development. **Microbial Biotechnology**, v. 14, n. 1, p. 147-170, 2021.

UZOIGWE, C. et al. Bioemulsifiers are not biosurfactants and require different screening approaches. **Frontiers in Microbiology**, v. 6, p. 1-6, 2015.

VALDEZ, J. G. Identification, Pathogenicity and Distribution of *Penicillium* Spp. Isolated from Garlic in Two Regions in Argentina. **Plant Pathology**, v. 58, n. 2, p. 352–361, 2009.

VALENTÍN, L. et al. Biodegradation of dibenzothiophene, fluoranthene, pyrene and chrysene in a soil slurry reactor by the white-rot fungus *Bjerkandera* sp. BOS55. **Process Biochemistry,** v. 42, n. 4, p. 641-648, 2007.

VAN BOGAERT, I. N. A. et al. Microbial production and application of sophorolipids. **Applied Microbiology and Biotechnology**, v. 76, n. 1, p. 23-34, 2007.

VAN DIEN, S. From the first drop to the first truckload: commercialization of microbial processes for renewable chemicals. **Current Opinion in Biotechnology**, v. 24, n. 6, p. 1061-1068, 2013.

VAN HAMME, J. D.; SINGH, A.; WARD, O. P. Physiological aspects. Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. **Biotechnology Advances**, v. 24, n. 6, p. 604-620, 2006.

VAN RENTERGHEM, L. et al. From lab to market: An integrated bioprocess design approach for new-to-nature biosurfactants produced by *Starmerella bombicola*. **Biotechnology and Bioengineering**, v. 115, n. 5, p. 1195-1206, 2018.

VARJANI, S. J. Microbial degradation of petroleum hydrocarbons. **Bioresource Technology**, v. 223, p. 277-286, 2017.

VARJANI, S. J.; UPASANI, V. N. Critical review on biosurfactant analysis, purification and characterization using rhamnolipid as a model biosurfactant. **Bioresource Technology**, v. 232, p. 389-397, 2017.

VELIOGLU, Z.; UREK, R.O. Concurrent biosurfactant and ligninolytic enzyme production by *Pleurotus* spp. in solid-state fermentation. **Applied Biochemistry and Biotechnology**, v. 174, n. 4, p. 1354-1364, 2014.

VELIOGLU, Z.; UREK, R.O. Optimization of cultural conditions for biosurfactant production by *Pleurotus djamor* in solid state fermentation. **Journal of Bioscience and Bioengineering**, v. 120, n. 5, p. 526-531, 2015.

VELIOGLU, Z.; UREK, R.O. Physicochemical and structural characterization of biosurfactant produced by *Pleurotus djamor* in solid-state fermentation. **Biotechnology and Bioprocess Engineering**, v. 21, n. 3, p. 430-438, 2016.

VERGARA-FERNÁNDEZ, A. et al. Biofiltration of benzo[α]pyrene, toluene and formaldehyde in air by a consortium of *Rhodococcus erythropolis* and *Fusarium solani*: Effect of inlet loads, gas flow and temperature. **Chemical Engineering Journal**, v. 332, p. 702-710, 2018a.

VERGARA-FERNÁNDEZ, A. et al. Biofiltration of volatile organic compounds using fungi and its conceptual and mathematical modeling. **Biotechnology Advances**, v. 36, n. 4, p. 1079-1093, 2018b.

VIGUERAS, G. et al. Growth of the fungus *Paecilomyces lilacinus* with n-hexadecane in submerged and solid-state cultures and recovery of hydrophobin proteins. **Process Biochemistry**, v. 49, n. 10, p. 1606-1611, 2014.

VIPOTNIK, Z.; MICHELIN, M.; TAVARES, T. Biodegradation of chrysene and benzo[a]pyrene and removal of metals from naturally contaminated soil by isolated Trametes versicolor strain and laccase produced thereof. **Environmental Technology & Innovation**, v. 28, n. 102737, p. 1-12, 2022a.

VIPOTNIK, Z.; MICHELIN, M.; TAVARES, T. Rehabilitation of a historically contaminated soil by different laccases and laccase-mediator system. **Journal of Soils and Sediments**, v. 22, n. 5, p. 1546-1554, 2022b.

VISAGIE, C. M. et al. Identification and nomenclature of the genus *Penicillium*. **Studies in mycology**, v. 78, n. 1, p. 343–371, 2014.

WADEKAR, S. D. et al. Jatropha oil and karanja oil as carbon sources for production of sophorolipids. **European Journal of Lipid Science and Technology**, v. 114, n. 7, p. 823-832, 2012.

WALTER, V.; SYLDATK, C.; HAUSMANN, R. Screening concepts for the isolation of biosurfactant producing microorganisms. **Advances in Experimental Medicine and Biology**, v. 672, p. 1-13, 2010.

WANG, C. W. et al. *Fusarium acuminatum*: A new pathogen causing postharvest rot on stored kiwifruit in China. **Plant disease**, v. 99, n. 11, p. 1644–1644, 2015.

WANG, G.; WANG, L.; MA, F. Effects of earthworms and arbuscular mycorrhizal fungi on improvement of fertility and microbial communities of soils heavily polluted by cadmium. **Chemosphere**, v. 286, n. Pt 2, p. 131567, 2022.

WANG, H. et al. Efficient in-situ separation design for long-term sophorolipids fermentation with high productivity. **Journal of Cleaner** Production, v. 246, n. 118995, p. 118995, 2020a.

WANG, H. et al. *Starmerella bombicola*: recent advances on sophorolipid production and prospects of waste stream utilization: *Starmerella bombicola*: recent advances on sophorolipid

production. **Journal of Chemical Technology and Biotechnology**, v. 94, n. 4, p. 999-1007, 2019b.

WANG, H. et al. Techno-economic evaluation of a biorefinery applying food waste for sophorolipid production - A case study for Hong Kong. **Bioresource Technology**, v. 303, n. 122852, p. 1-11, 2020b.

WANG, M. et al. Efficient simultaneous production of extracellular polyol esters of fatty acids and intracellular lipids from inulin by a deep-sea yeast *Rhodotorula paludigena* P4R5. **Microbial Cell Factories**, v. 18, n. 1, p. 1-13, 2019a.

WEBER, A. et al. Downstream processing of biosurfactants. **Chemical Engineering Transactions**, v. 27, p. 115-120, 2012.

WHITE, T. J. et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: **PCR Protocols**. San Diego, CA: Elsevier, 1990.

WICKHAM, H. **ggplot2**: Elegant Graphics for Data Analysis. New York: Springer, Verlag, 2016. Disponível em: https://link.springer.com/book/10.1007/978-0-387-98141-3. Acesso em:17/02/2023

WILLIAMS, W.; TRINDADE, M. Metagenomics for the discovery of novel biosurfactants. In: **Functional Metagenomics**: Tools and Applications. Cham: Springer International Publishing, p. 95-117, 2017.

WILSON, S. C.; JONES, K. C. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. **Environmental Pollution**, v. 81, n. 3, p. 229-249, 1993.

WINTERBURN, J. B.; RUSSELL, A. B.; MARTIN, P. J. Integrated recirculating foam fractionation for the continuous recovery of biosurfactant from fermenters. **Biochemical Engineering Journal**, v. 54, n. 2, p. 132-139, 2011.

WOLLENBERG, A. et al. Uranium (VI) bioassociation by different fungi - a comparative study into molecular processes. **Journal of Hazardous Materials**, v. 411, n. 125068, p. 1-10, 2021.

WOŹNIAK-KARCZEWSKA, M. et al. Isolation of rhamnolipids-producing cultures from faeces: Influence of interspecies communication on the yield of rhamnolipid congeners. **New Biotechnology**, v. 36, p. 17-25, 2017.

WU, J. et al. Fungal chitosan from *Agaricus bisporus* (Lange) Sing. Chaidam increased the stability and antioxidant activity of liposomes modified with biosurfactants and loading betulinic acid. **International Journal of Biological Macromolecules**, v. 123, p. 291-299, 2019.

WU, Y. et al. Adsorption of *Trametes versicolor* laccase to soil iron and aluminum minerals: enzyme activity, kinetics and stability studies. **Colloids and Surfaces B**: Biointerfaces, v. 114, p. 342-348, 2014.

WU, Y. et al. Potential role of polycyclic aromatic hydrocarbons (PAHs) oxidation by fungal laccase in the remediation of an aged contaminated soil. **Soil Biology & Biochemistry**, v. 40, n. 3, p. 789-796, 2008.

WULANDARI, R. et al. Toxicity evaluation and biodegradation of phenanthrene by laccase from *Trametes polyzona* PBURU 12. **3 Biotech**, v. 11, n. 1, p. 1-11, 2021.

XIAO, X. et al. Biosorption of cadmium by endophytic fungus (EF) *Microsphaeropsis* sp. LSE10 isolated from cadmium hyperaccumulator *Solanum nigrum* L. **Bioresource Technology**, v. 101, n. 6, p. 1668-1674, 2010.

XIONG, W. et al. Distinct roles for soil fungal and bacterial communities associated with the suppression of vanilla *Fusarium* wilt disease. **Soil biology & biochemistry**, v. 107, p. 198–207, 2017.

XU, F. et al. Comparative transcriptome analysis reveals the differential response to cadmium stress of two Pleurotus fungi: *Pleurotus cornucopiae* and *Pleurotus ostreatus*. **Journal of Hazardous Materials**, v. 416, n. 125814, p. 1-10, 2021.

XU, P. et al. Cadmium induced oxalic acid secretion and its role in metal uptake and detoxification mechanisms in *Phanerochaete chrysosporium*. **Applied Microbiology and Biotechnology**, v. 99, n. 1, p. 435-443, 2015.

YALÇIN, H. T.; ERGIN-TEPEBAŞI, G.; UYAR, E. Isolation and molecular characterization of biosurfactant producing yeasts from the soil samples contaminated with petroleum derivatives. **Journal of Basic Microbiology**, v. 58, n. 9, p. 782-792, 2018.

YANG, J. et al. Biosorption behavior of heavy metals in bioleaching process of MSWI fly ash by *Aspergillus niger*. **Biochemical Engineering Journal**, v. 46, n. 3, p. 294-299, 2009.

YANG, L. et al. Metabolic profiling and flux distributions reveal a key role of acetyl-CoA in sophorolipid synthesis by *Candida bombicola*. **Biochemical Engineering Journal**, v. 145, p. 74-82, 2019.

YANG, X. et al. Recovery of purified lactonic sophorolipids by spontaneous crystallization during the fermentation of sugarcane molasses with *Candida albicans* O-13-1. **Enzyme and Microbial Technology**, v. 51, n. 6-7, p. 348-353, 2012.

YANG, Z. et al. Enhancement and analysis of Anthracene degradation by Tween 80 in LMS-HOBt. **Scientific Reports**, v. 11, n. 1, p. 1-11, 2021.

YE, M. et al. Feasibility of lettuce cultivation in sophoroliplid-enhanced washed soil originally polluted with Cd, antibiotics, and antibiotic-resistant genes. **Ecotoxicology and Environmental Safety**, v. 124, p. 344-350, 2016.

YELA, A. C. A. et al. A comparison between conventional *Pseudomonas aeruginosa* rhamnolipids and *Escherichia coli* transmembrane proteins for oil recovery enhancing. **International Biodeterioration & Biodegradation**, v. 112, p. 59-65, 2016.

YOSHIDA, S. et al. Effects of biosurfactants, mannosylerythritol lipids, on the hydrophobicity of solid surfaces and infection behaviours of plant pathogenic fungi. **Journal of Applied Microbiology,** v. 119, n. 1, p. 215-224, 2015.

YOUSSEF, N. H. et al. Comparison of methods to detect biosurfactant production by diverse microorganisms. **Journal of Microbiological Methods**, v. 56, n. 3, p. 339-347, 2004.

YU, M. et al. Characteristics of mannosylerythritol lipids and their environmental potential. **Carbohydrate Research**, v. 407, p. 63-72, 2015.

YU, X. et al. Integrated biorefinery process for production of sophorolipids from corn straw. **Biochemical Engineering Journal**, v. 167, n. 107908, p. 1-9, 2021.

YU, Z. et al. Scopularides A and B, cyclodepsipeptides from a marine sponge-derived fungus, *Scopulariopsis brevicaulis*. **Journal of Natural Products**, v. 71, n. 6, p. 1052-1054, 2008.

YUAN, B. et al. Immobilization of lead(II) and zinc(II) onto glomalin-related soil protein (GRSP): Adsorption properties and interaction mechanisms. **Ecotoxicology and Environmental Safety**, v. 236, n. 113489, p. 1-10, 2022.

ZADEH, P. H.; MOGHIMI, H.; HAMEDI, J. Biosurfactant production by *Mucor circinelloides*: Environmental applications and surface-active properties. **Engineering in Life Sciences**, v. 18, n. 5, p. 317-325, 2018.

ZAFRA, G. et al. Construction of PAH-degrading mixed microbial consortia by induced selection in soil. **Chemosphere**, v. 172, p. 120-126, 2017.

ZAIN UL ARIFEEN, M. et al. Anaerobic biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungi isolated from anaerobic coal-associated sediments at 2.5 km below the seafloor. **Chemosphere**, v. 303, n. 2, p. 1-9, 2022.

ZAKY, A. S. et al. Marine yeast isolation and industrial application. **FEMS Yeast Research**, v. 14, n. 6, p. 813-825, 2014.

ZENG, J. et al. Effects of polycyclic aromatic hydrocarbon structure on PAH mineralization and toxicity to soil microorganisms after oxidative bioremediation by laccase. **Environmental Pollution**, v. 287, n. 117581, p. 1-10, 2021.

ZENG, J. et al. Oxidation of benzo[a]pyrene by laccase in soil enhances bound residue formation and reduces disturbance to soil bacterial community composition. **Environmental Pollution**, v. 242, n. Pt A, p. 462-469, 2018.

ZHANG, Q. et al. The effect of heavy metal-induced oxidative stress on the enzymes in white rot fungus *Phanerochaete chrysosporium*. **Applied Biochemistry and Biotechnology**, v. 175, n. 3, p. 1281-1293, 2015.

ZHANG, X. et al. Combined microbial degradation of crude oil under alkaline conditions by *Acinetobacter baumannii* and *Talaromyces* sp. **Chemosphere**, v. 273, n. 129666, p. 1-9, 2021.

ZHANG, Y. et al. Performance and microbial community evolution of toluene degradation using a fungi-based bio-trickling filter. **Journal of Hazardous Materials**, v. 365, p. 642-649, 2019.

ZHANG, Y. et al. Semicontinuous sophorolipid fermentation using a novel bioreactor with dual ventilation pipes and dual sieve-plates coupled with a novel separation system. **Microbial Biotechnology**, v. 11, n. 3, p. 455-464, 2018.

APÊNDICE A

Tabela S1 – Linhagens utilizadas na análise filogenética. Sequências de nucleotídeos foram obtidas/submetidas ao GenBank

Linhagem	**Referência**	**Sequência ITS/ Acesso Genbank**	**Fonte**	**Geografia**	**Número de pb**
Fusarium sp.	AF10D	OQ282829	Solo contaminado com biodiesel de palma	Brasil, Florianópolis	549
Fusarium sp.	AF48D	OQ282830	Solo contaminado com biodiesel de palma	Brasil, Florianópolis	554
Fusarium sp.	AF52DD	OQ282831	Solo contaminado com biodiesel de palma, e enriquecido com óleo diesel	Brasil, Florianópolis	554
Fusarium sp.	AF99PD	OQ282832	Solo contaminado com biodiesel de palma, e enriquecido com borra de petróleo	Brasil, Florianópolis	554
Penicillium sp.	AF38D	OQ282834	Solo contaminado com biodiesel de palma	Brasil, Florianópolis	173
Penicillium sp.	AF41D	OQ282835	Solo contaminado com biodiesel de palma	Brasil, Florianópolis	173
Penicillium sp.	AF42D	OQ282836	Solo contaminado com biodiesel de palma	Brasil, Florianópolis	173
Penicillium brasilianum	AF64PD	OQ282833	Solo contaminado com biodiesel de palma, e enriquecido com borra de petróleo	Brasil, Florianópolis	208
Fusarium proliferatum	PG2-1	MN594807.1	*Malus domestica*	China	527
Fusarium armeniacum	MF-2	OM954338.1	Feijão	Canadá	457
Fusarium acutatum	CBS 402.97	NR_111142.1	Desconhecido	Desconhecido	559
Fusarium concentricum	NRRL 25181	NR_111886.1	Desconhecido	Desconhecido	522
Fusarium oxysporum	A549	KX463005.1	Patchouli	China	509
Fusarium equiseti	F4 NEIST-DRL	HQ332532.1	*Lycopersicon esculentum*	Índia	1156

Fusarium acuminatum	MAFF 236716	AB587002.1	Desconhecido	Japão	1053
Fusarium pseudocircinatum	CBS 449.97	NR_163683.1	Desconhecido	Gana	502
Fusarium beomiforme	NRRL 13606	NR_111885.1	Desconhecido	Desconhecido	536
Fusarium boothii	NRRL 29011	NR_121203.1	Desconhecido	Desconhecido	598
Fusarium pernambucanum	URM 7559	NR_163754.1	Inseto	Brasil, Pernambuco	502
Fusarium fujikuroi	CBS 221.76	NR_111889.1	Arroz	EUA	558
Fusarium robustum	CBS 637.76	NR_159851.1	Desconhecido	Argentina	538
Fusarium aethiopicum	NRRL 46738	NR_138284.1	Desconhecido	Desconhecido	598
Penicillium tropicum	CBS 112584	NR_111485.1	Desconhecido	Desconhecido	544
Penicillium biforme	CBS 297.48	NR_138325.1	Desconhecido	Desconhecido	508
Penicillium Rubens	CBS 129667	NR_111815.1	Desconhecido	Desconhecido	585
Penicillium commune	CBS 311.48	NR_111143.1	Desconhecido	Desconhecido	586
Penicillium solitum	FRR 937	NR_119494.1	Desconhecido	Desconhecido	610
Penicillium ovetense	CBS 163.81	KC411680.1	Desconhecido	Desconhecido	468
Penicillium brasilianum	CMV002C3	MK451584.1	Forragem	África do Sul	642
Penicillium aethiopicum	CBS 484.84	NR_111155.1	Desconhecido	Desconhecido	546
Penicillium halotolerans	CBS 131537	NR_111812.1	Pântano salgado	Egito	558
Penicillium expansum	ATCC 7861	NR_077154.1	Desconhecido	Desconhecido	608
Penicillium citrinum	NRRL 1841	NR_121224.1	Desconhecido	Desconhecido	574
Penicillium chrysogenum	CBS 306.48	NR_077145.1	Desconhecido	Desconhecido	585
Penicillium goetzii	CBS 285.73	NR_111820.1	Solo	Canadá	585
Penicillium castellonense	CBS 170.81	KC411683.1	Desconhecido	Desconhecido	506
Penicillium onobense	CBS 174.81	NR_111497.1	Desconhecido	Desconhecido	549
Penicillium crustosum	FRR 1669	NR_077153.1	Desconhecido	Desconhecido	610
Penicillium oxalicum	NRRL 787	NR_121232.1	Desconhecido	Desconhecido	611
Penicillium daleae	CBS 211.28	NR_111503.1	Desconhecido	Desconhecido	549
Trichoderma reesei	ATCC 13631	NR_120297.1	Tecido de algodão	Papua-Nova Guiné	648

APÊNDICE B

Tabela S2 – Informações com a identificação morfológica de isolados de solos contaminados

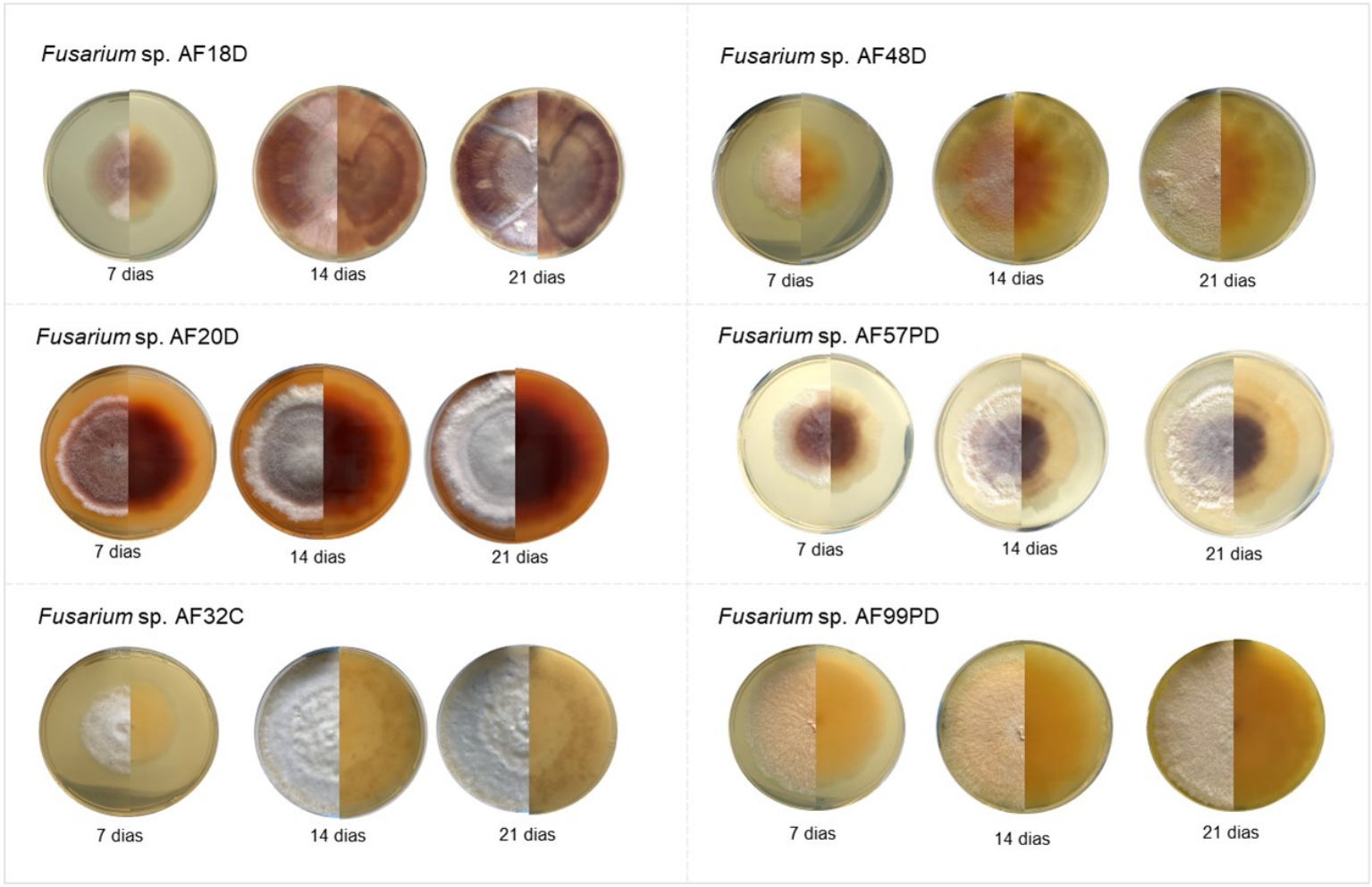
Linhagem	Identificação morfológica	Fonte - solo contaminado com:	Meio de isolamento
AF33A	*Scolecobasidium* sp.	Diesel	BDA
AF34B	*Scopulariopsis* sp.	Gasolina e etanol	BDA
AF46C	*Paecilomyces* sp.	Diesel e biodiesel de soja	Martin
AF47C	*Paecilomyces* sp.	Diesel e biodiesel de soja	Martin
AF40D	*Paecilomyces* sp.	Biodiesel de palma	BDA
AF45D	*Paecilomyces* sp.	Biodiesel de palma	Martin
AF13D	*Penicillium* sp.	Biodiesel de palma	BDA
AF17D	*Penicillium* sp.	Biodiesel de palma	BDA
AF38D	*Penicillium* sp.	Biodiesel de palma	Martin
AF41D	*Penicillium* sp.	Biodiesel de palma	Martin
AF42D	*Penicillium* sp.	Biodiesel de palma	BDA
AF64PD	*Penicillium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	BDA
AF78DD	*Penicillium* sp.	Biodiesel de palma, enriquecido com diesel 1%	BDA
AF98PD	*Penicillium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	BDA
AF5B	*Fusarium* sp.	Gasolina e etanol	Martin
AF31C	*Fusarium* sp.	Diesel e biodiesel de soja	Martin
AF32C	*Fusarium* sp.	Diesel e biodiesel de soja	BDA
AF51C	*Fusarium* sp.	Diesel e biodiesel de soja	Martin
AF52C	*Fusarium* sp.	Diesel e biodiesel de soja	BDA
AF4D	Levedura	Biodiesel de palma	BDA
AF6D	Levedura	Biodiesel de palma	BDA
AF8D	Levedura	Biodiesel de palma	BDA
AF7D	*Fusarium* sp.	Biodiesel de palma	BDA
AF9D	*Fusarium* sp.	Biodiesel de palma	BDA

AF10D	*Fusarium* sp.	Biodiesel de palma	BDA
AF12D	*Fusarium* sp.	Biodiesel de palma	BDA
AF14D	*Fusarium* sp.	Biodiesel de palma	BDA
AF15D	*Fusarium* sp.	Biodiesel de palma	Martin
AF18D	*Fusarium* sp.	Biodiesel de palma	Martin
AF19D	*Fusarium* sp.	Biodiesel de palma	Martin
AF20D	*Fusarium* sp.	Biodiesel de palma	Martin
AF21D	*Fusarium* sp.	Biodiesel de palma	BDA
AF25D	*Ilyonectria* sp.	Biodiesel de palma	BDA
AF27D	*Fusarium* sp.	Biodiesel de palma	BDA
AF35D	*Fusarium* sp.	Biodiesel de palma	Martin
AF36D	*Fusarium* sp.	Biodiesel de palma	Martin
AF39D	*Fusarium* sp.	Biodiesel de palma	Martin
AF43D	*Fusarium* sp.	Biodiesel de palma	BDA
AF44D	*Fusarium* sp.	Biodiesel de palma	BDA
AF48D	*Fusarium* sp.	Biodiesel de palma	BDA
AF49D	*Fusarium* sp.	Biodiesel de palma	BDA
AF52DD	*Fusarium* sp.	Biodiesel de palma, enriquecido com diesel 1%	Martin
AF53DD	*Fusarium* sp.	Biodiesel de palma, enriquecido com diesel 1%	Martin
AF54DD	*Fusarium* sp.	Biodiesel de palma, enriquecido com diesel 1%	BDA
AF55PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	BDA
AF56PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	BDA
AF57PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	BDA
AF58PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	BDA
AF60PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	Martin
AF61PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	Martin
AF62PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	Martin
AF63PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 1%	Martin
AF93DD	*Fusarium* sp.	Biodiesel de palma, enriquecido com diesel 5%	BDA
AF94DD	*Fusarium* sp.	Biodiesel de palma, enriquecido com diesel 5%	BDA

AF95DD	*Fusarium* sp.	Biodiesel de palma, enriquecido com diesel 5%	Martin
AF96PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 5%	BDA
AF97PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 5%	BDA
AF99PD	*Fusarium* sp.	Biodiesel de palma, enriquecido com borra de petróleo 5%	Martin

APÊNDICE C

Figure S3 – Alguns isolados de *Fusarium* crescidos em meio BDA (25°C em 7, 14 e 21 dias) com diferentes características morfológicas (esquerda, vista anversa; e direita, vista reversa)



APÊNDICE D

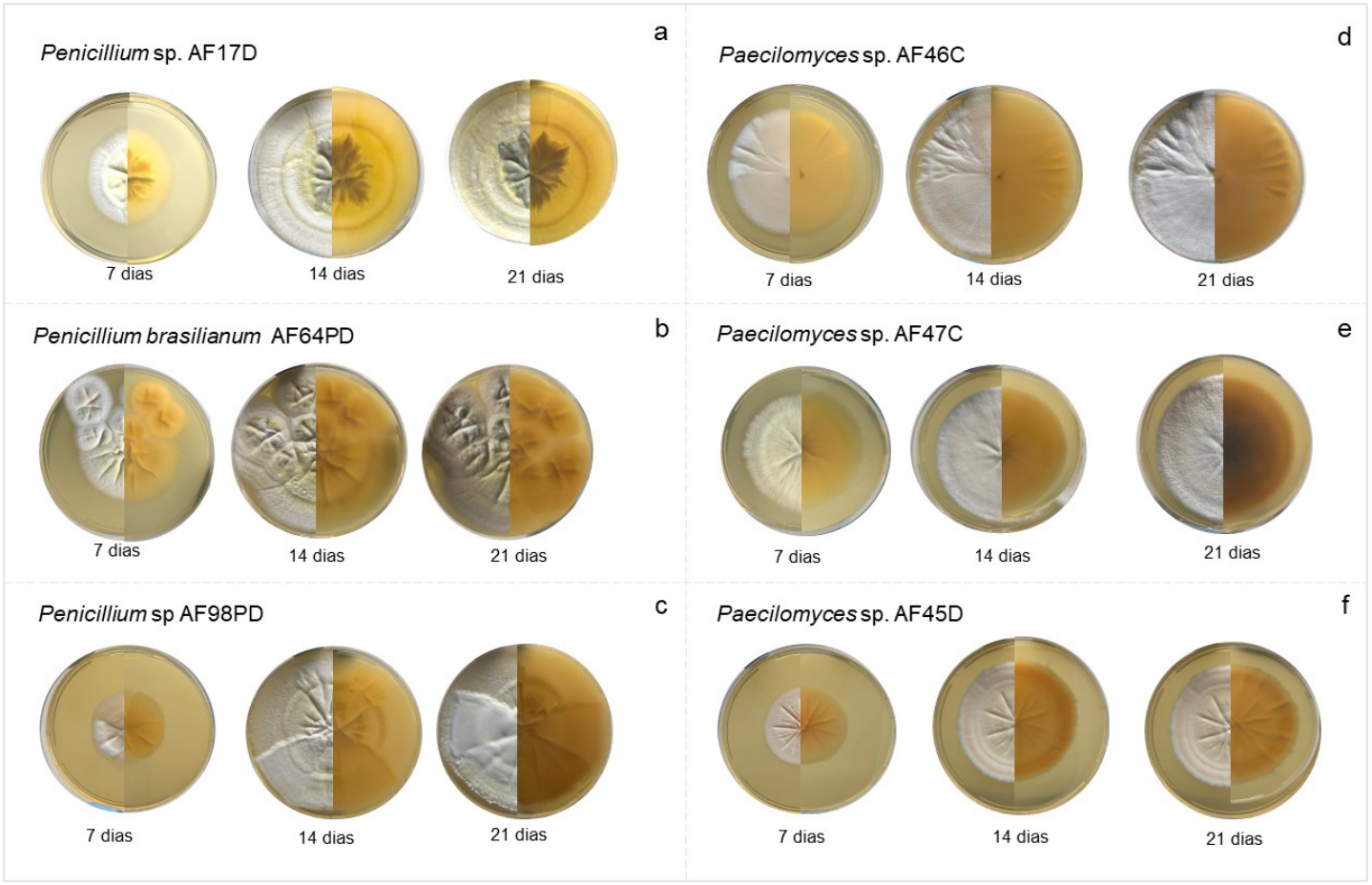
Tabela S4 – Caracterização micromorfológica de isolados de *Fusarium*

Isolado	Macroconídio	Número de septos	Microconídio	Clamidósporo
AF5B	Presente	2	Presente	Ausente
AF7D	Presente	2	Presente	Terminal e Intercalar
AF9D	Presente	1	Ausente	Terminal e Intercalar
AF10D	Presente	3	Presente	Terminal
AF12D	Presente	3	Presente	Terminal e Intercalar
AF14D	Presente	3	Presente	Intercalar
AF15D	Presente	3	Presente	Terminal e Intercalar
AF18D	Presente	4	Presente	Terminal
AF19D	Presente	2	Presente	Terminal e Intercalar
AF20D	Presente	2	Ausente	Ausente
AF21D	Presente	2	Ausente	Ausente
AF27D	Presente	3	Ausente	Terminal
AF31C	Presente	2	Presente	Ausente
AF32C	Presente	2	Presente	Ausente
AF35D	Presente	2	Ausente	Intercalar
AF36D	Presente	2	Ausente	Terminal
AF39D	Presente	3	Presente	Terminal
AF43D	Presente	2	Presente	Terminal
AF44D	Presente	2	Presente	Terminal e Intercalar
AF48D	Presente	2\3	Ausente	Ausente
AF49D	Presente	3	Presente	Terminal e Intercalar
AF51C	Presente	2	Presente	Terminal e Intercalar
AF52C	Presente	1	Presente	Terminal e Intercalar

AF52DD	Presente	3	Presente	Ausente
AF53DD	Presente	3	Presente	Terminal e Intercalar
AF54DD	Presente	2	Ausente	Ausente
AF55PD	Presente	3	Presente	Terminal
AF56PD	Presente	2	Presente	Ausente
AF57PD	Presente	2	Presente	Terminal
AF58PD	Presente	1	Ausente	Terminal
AF60PD	Presente	2	Ausente	Intercalar
AF61PD	Presente	3	Presente	Terminal e Intercalar
AF62PD	Presente	3	Presente	Ausente
AF63PD	Presente	2	Ausente	Terminal e Intercalar
AF93DD	Presente	2	Presente	Intercalar
AF94DD	Presente	3	Presente	Ausente
AF95DD	Presente	1	Ausente	Ausente
AF96PD	Presente	1\2	Presente	Terminal e Intercalar
AF97PD	Presente	3	Ausente	Ausente
AF99PD	Presente	2\3	Ausente	Terminal e Intercalar

APÊNDICE E

Figura S5 – Alguns isolados de *Penicillium* (mostrados em a, b, c) e *Paecilomyces* (mostrado em d, e, f) crescidos em meio BDA (25°C em 7, 14 e 21 dias) com diferentes características morfológicas (esquerda, vista anversa; e direita, vista reversa)



APÊNDICE F

Tabela S6 – Resultados da triagem primária (tensiometria, índice de emulsificação e colapso da gota) para produção de biosurfactantes

Isolado fúngico[a,b,c]	**Local de isolamento**	**Tensão superficial (mNm^{-1})**	**RTS (%)**	**Índice de Emulsificação (%)**	**Colapso da gota (mm)**
Controle negativo (meio de cultura)	-	68,00 ± 1,12	0	0	0
Controle positivo (SDS 1%)	-	36,16 ± 0,24	55,64	80	> 7
Cladosporium sp. NU07[a]	Planta de Mangue	65,58 ± 1,42	3,56	2,5	2
Scolecobasidium sp. AF33A[a]	Solo contaminado com hidrocarbonetos	66,89 ± 0,65	1,62	0	0
Ilyonectria sp. AF25D[a]	Solo contaminado com hidrocarbonetos	67,68 ± 0,19	0,46	5	2,00
Scopulariopsis sp. AF34B[a]	Solo contaminado com hidrocarbonetos	67,87 ± 0,04	0,18	0	0
Curvularia sp. ILRR1A20047[a,b]	Planta de Mangue	47,43 ± 2,28	30,24	2,5	5
Buergenerula spartinae[a,b]	Planta de Mangue	47,73 ± 1,56	29,81	2,5	4
Trichoderma sp. P05R2[a,b]	Solo contaminado com hidrocarbonetos	45,85 ± 2,28	32,56	5	5
Trichoderma sp. RASC1B10122[a,b]	Planta de Mangue	33,17 ± 0,89	51,21	5	5
Aspergillus sp. SC21P3[a]	Solo Antártico	49,30 ± 0,85	27,51	2,5	5
Aspergillus Seção Flavi[a,b]	Planta de Mangue	55,74 ± 1,07	18,03	2,5	5
Thelebolus sp. SC29P3[a,b]	Solo Antártico	52,19 ± 0,24	23,25	5	4
Thelebolus sp. NU11[a]	Solo Antártico	67,06 ± 0,68	1,38	2,5	2
Thelebolus sp. SC16P3[a]	Solo Antártico	65,58 ± 0,67	3,56	2,5	2
Paecilomyces sp. AF40D[a]	Solo contaminado com hidrocarbonetos	67,55 ± 0,38	0,64	2,5	1
Paecilomyces sp. AF46C[a]	Solo contaminado com hidrocarbonetos	67,96 ± 0,81	0,05	2,5	0
Paecilomyces sp. AF47C[a]	Solo contaminado com hidrocarbonetos	67,73 ± 0,76	0,38	0	0
Paecilomyces sp. AF45D[a]	Solo contaminado com hidrocarbonetos	66,36 ± 1,57	2,39	2,5	4

Pseudogymnoascus sp. NU09[a,b]	Solo Antártico	64,60 ± 1,23	5,00	2,5	1
Pseudogymnoascus sp. SC23P3[a]	Solo Antártico	44,74 ± 0,99	34,2	5	5
Pseudogymnoascus sp. IASF2A10008[a]	Planta de Mangue	54,45 ± 0,95	19,92	2,5	4
Pseudogymnoascus sp. SC26P3[a]	Solo Antártico	50,72 ± 1,81	25,41	5	5
Pseudogymnoascus sp. NU04[a]	Solo Antártico	66,62 ± 1,37	2,03	5	2
Pseudogymnoascus sp. NU16[a]	Solo Antártico	67,82 ± 0,59	0,27	0	0
Pseudogymnoascus sp. NU23[a]	Solo Antártico	67,78 ± 0,22	0,32	2,5	2
Pseudogymnoascus sp. NU22[a]	Solo Antártico	66,70 ± 1,25	1,91	2,5	2
Pseudogymnoascus sp. SC04P3[a]	Solo Antártico	59,44 ± 1,05	12,58	0	1
Pseudogymnoascus sp. SC10P3[a]	Solo Antártico	50,56 ± 0,58	25,64	5	4
Pseudogymnoascus sp. SC05P1[a]	Solo Antártico	49,57 ± 1,01	27,1	2,5	4
Pseudogymnoascus sp. NU26[a]	Solo Antártico	66,11 ± 0,27	2,78	5	3
Penicillium sp. AF42D[a,b]	Solo contaminado com hidrocarbonetos	63,95 ± 0,68	5,95	2,5	3
Penicillium sp. AF38D[a,b]	Solo contaminado com hidrocarbonetos	64,60 ± 0,78	5,00	2,5	3
Penicillium sp. AF41D[a,b]	Solo contaminado com hidrocarbonetos	63,71 ± 0,81	6,3	2,5	2
Penicillium sp. AF98PD[a]	Solo contaminado com hidrocarbonetos	67,44 ± 0,62	0,81	2,5	2
Penicillium sp. AF78DD[a]	Solo contaminado com hidrocarbonetos	66,20 ± 0,67	2,63	0	4
Penicillium sp. AF17D[a]	Solo contaminado com hidrocarbonetos	67,69 ± 0,72	0,44	2,5	1
Penicillium sp. AF13D[a]	Solo contaminado com hidrocarbonetos	66,44 ± 0,86	2,28	5	4
Penicillium brasilianum AF64PD[a,b]	Solo contaminado com hidrocarbonetos	64,60 ± 1,26	5,00	5	5
Penicillium sp. SCUV02.P1[a,b]	Solo Antártico	46,47 ± 0,88	31,65	5	4
Penicillium Seção Glabra[a,b]	Planta de Mangue	53,04 ± 2,52	22	2,5	5
Penicillium sp. P05R1[a]	Solo contaminado com hidrocarbonetos	61,48 ± 1,12	9,58	2,5	3
Penicillium sp. P05R3[a]	Solo contaminado com hidrocarbonetos	63,71 ± 1,43	6,32	0	1

Penicillium sp. P10R4[a]	Solo contaminado com hidrocarbonetos	62,10 ± 0,31	8,68	2,5	3
Penicillium sp. P10R5[a]	Solo contaminado com hidrocarbonetos	56,08 ± 1,77	17,53	5	5
Penicillium sp. P10R6[a]	Solo contaminado com hidrocarbonetos	54,63 ± 1,57	19,65	5	5
Penicillium sp. P10R7[a]	Solo contaminado com hidrocarbonetos	55,83 ± 2,43	17,89	5	5
Penicillium sp. SC04P2[a]	Solo Antártico	55,94 ± 0,54	17,73	0	0
Penicillium sp. SC02P2[a]	Solo Antártico	52,39 ± 0,80	22,96	5	4
Fusarium sp. AF10D[a,b]	Solo contaminado com hidrocarbonetos	62,62 ± 1,99	7,91	2,5	3
Fusarium sp. AF48D[a,b]	Solo contaminado com hidrocarbonetos	63,62 ± 1,18	6,44	2,5	3
Fusarium sp. AF52DD[a,b]	Solo contaminado com hidrocarbonetos	63,41 ± 0,81	6,74	2,5	3
Fusarium sp. AF99PD[a,b]	Solo contaminado com hidrocarbonetos	57,35 ± 1,87	15,65	5	5
Fusarium sp. AF95DD[a]	Solo contaminado com hidrocarbonetos	66,53 ± 1,23	2,15	0	2
Fusarium sp. AF35D[a]	Solo contaminado com hidrocarbonetos	68,02 ± 0,27	-0,05	0	0
Fusarium sp. AF49D[a]	Solo contaminado com hidrocarbonetos	65,42 ± 0,38	3,79	0	2
Fusarium sp. AF43D[a]	Solo contaminado com hidrocarbonetos	67,43 ± 1,28	0,82	0	0
Fusarium sp. AF7D[a]	Solo contaminado com hidrocarbonetos	68,45 ± 0,78	-0,66	5	3
Fusarium sp. AF97PD[a]	Solo contaminado com hidrocarbonetos	66,88 ± 0,23	1,64	0	2
Fusarium sp. AF51C[a]	Solo contaminado com hidrocarbonetos	68,03 ± 0,80	-0,04	2,5	1
Fusarium sp. AF12D[a]	Solo contaminado com hidrocarbonetos	68,34 ± 0,74	-0,5	0	1
Fusarium sp. AF18D[a]	Solo contaminado com hidrocarbonetos	67,02 ± 0,58	1,43	0	4
Fusarium sp. AF27D[a]	Solo contaminado com hidrocarbonetos	67,13 ± 0,83	1,26	5	4

Fusarium sp. AF32C[a]	Solo contaminado com hidrocarbonetos	68,08 ± 0,39	-0,11	0	0
Fusarium sp. AF55PD[a]	Solo contaminado com hidrocarbonetos	67,50 ± 0,73	0,73	0	0
Fusarium sp. AF62PD[a]	Solo contaminado com hidrocarbonetos	67,67 ± 0,30	0,47	0	0
Fusarium sp. AF5B[a]	Solo contaminado com hidrocarbonetos	67,25 ± 0,23	1,09	0	0
Fusarium sp. AF44D[a]	Solo contaminado com hidrocarbonetos	64,66 ± 2,59	4,9	2,5	3
Fusarium sp. AF96PD[a]	Solo contaminado com hidrocarbonetos	67,91 ± 1,01	0,13	0	0
Fusarium sp. AF52C[a]	Solo contaminado com hidrocarbonetos	68,95 ± 1,52	-1,4	2,5	3
Fusarium sp. AF19D[a]	Solo contaminado com hidrocarbonetos	65,95 ± 0,53	3	2,5	4
Fusarium sp. AF21D[a]	Solo contaminado com hidrocarbonetos	67,95 ± 0,36	0,06	0	0
Fusarium sp. AF9D[a]	Solo contaminado com hidrocarbonetos	68,18 ± 0,15	-0,27	0	1
Fusarium sp. AF56PD[a]	Solo contaminado com hidrocarbonetos	67,46 ± 0,69	0,79	0	0
Fusarium sp. AF63PD[a]	Solo contaminado com hidrocarbonetos	68,48 ± 0,73	-0,71	0	0
Fusarium sp. AF14D[a]	Solo contaminado com hidrocarbonetos	68,38 ± 0,3	-0,56	0	0
Fusarium sp. AF61PD[a]	Solo contaminado com hidrocarbonetos	68,19 ± 0,20	-0,28	0	0
Fusarium sp. AF20D[a]	Solo contaminado com hidrocarbonetos	66,78 ± 0,89	1,77	0	1
Fusarium sp. AF39D[a]	Solo contaminado com hidrocarbonetos	68,14 ± 0,02	-0,21	0	0
Fusarium sp. AF36D[a]	Solo contaminado com hidrocarbonetos	64,79 ± 0,65	4,71	2,5	3
Fusarium sp. AF53DD[a]	Solo contaminado com hidrocarbonetos	66,56 ± 1,15	2,1	0	0
Fusarium sp. AF57PD[a]	Solo contaminado com hidrocarbonetos	66,20 ± 0,47	2,63	2,5	4

Fusarium sp. AF15D[a]	Solo contaminado com hidrocarbonetos	68,36 ± 0,47	-0,54	0	0,00
Fusarium sp. AF54DD[a]	Solo contaminado com hidrocarbonetos	66,92 ± 0,45	1,58	5	4,00
Fusarium sp. AF58PD[a]	Solo contaminado com hidrocarbonetos	68,46 ± 0,42	-0,68	0	0,00
Fusarium sp. AF94DD[a]	Solo contaminado com hidrocarbonetos	68,18 ± 0,16	-0,27	0	0,00
Fusarium sp. AF93DD[a]	Solo contaminado com hidrocarbonetos	67,07 ± 0,08	1,35	2,5	4,00
Fusarium sp. AF60PD[a]	Solo contaminado com hidrocarbonetos	65,45 ± 1,52	3,8	2,5	4,00
Fusarium sp. AF31C[a]	Solo contaminado com hidrocarbonetos	68,08 ± 1,31	-0,12	2,5	3,00
AF4D[c]	Solo contaminado com hidrocarbonetos	67,46 ± 0,60	0,79	2,5	1,00
AF8D[c]	Solo contaminado com hidrocarbonetos	67,56 ± 0,26	0,64	2,5	2,00
AF6D[c]	Solo contaminado com hidrocarbonetos	66,09 ± 0,20	2,8	2,5	2,00
ACF50[c]	Solo Antártico	66,48 ± 1,46	2,23	2,5	3,00
NU03[c]	Solo Antártico	66,13 ± 1,22	2,76	5	2,00
NU18[c]	Solo Antártico	66,14 ± 1,62	2,73	2,5	3,00
ACF08[c]	Solo Antártico	66,23 ± 1,02	2,61	2,5	2,00
NU12[c]	Solo Antártico	65,75 ± 1,29	3,32	5	4,00
NU02[c]	Solo Antártico	67,80 ± 0,06	0,3	0	1,00
ACF56[c]	Solo Antártico	66,86 ± 0,85	1,67	0	0,00
ACF29[c]	Solo Antártico	67,08 ± 0,74	1,35	0	3,00
NU28[c]	Solo Antártico	66,86 ± 0,38	1,67	2,5	1,00
NU01[c]	Solo Antártico	66,36 ± 0,51	2,42	0	3,00
ACF49[c]	Solo Antártico	65,86 ± 2,00	3,14	5	5,00
NU30[c]	Solo Antártico	65,51 ± 1,91	3,66	0	5,00
NU29[c]	Solo Antártico	65,36 ± 1,26	3,88	2,5	1,00
SC09P3[c]	Solo Antártico	51,65 ± 0,29	24,04	0	4,00

ACF15[c]	Solo Antártico	67,67 ± 0,05	0,48	2,5	3,00
NU27[c]	Solo Antártico	67,26 ± 0,77	1,09	2,5	2,00
NU24[c]	Solo Antártico	67,55 ± 0,37	0,66	0	0,00
NU17[c]	Solo Antártico	67,07 ± 0,83	1,37	2,5	3,00
NU15[c]	Solo Antártico	66,16 ± 0,20	2,7	2,5	2,00
NU25[c]	Solo Antártico	65,60 ± 0,75	3,53	0	4,00
ACF46[c]	Solo Antártico	67,44 ± 0,35	0,83	2,5	1,00
NU14[c]	Solo Antártico	66,77 ± 0,15	1,81	2,5	2,00
ACF77[c]	Solo Antártico	66,88 ± 0,52	1,65	0	3,00
ACF53[c]	Solo Antártico	67,95 ± 0,06	0,07	0	1,00
ACF51[c]	Solo Antártico	67,35 ± 0,15	0,95	5	5,00
ACF92[c]	Solo Antártico	66,95 ± 0,5	1,54	0	2,00
ACF54[c]	Solo Antártico	66,62 ± 1,26	2,03	5	2,00
RSAF3B20271[c]	Planta de Mangue	34,17 ± 1,36	49,75	2,5	4,00

[a] identificado morfologicamente por técnica de microcultivo; [b] identificado molecularmente por ITS; [c] ainda não identificado

APÊNDICE G

Tabela S7 – Resultados da triagem secundária para produção de biosurfactantes com análise de variância (p<0,05) do fator tempo, e regressões polinomiais de 2° ordem

Linhagem	Meio de Cultura	Tempo						Equação; R^2; p-valor
		0h	72h	96h	120h	144h	168h	
Buergenerula spartinae	X	68,00 a	55,29 ± 0,90 a	50,63 ± 2,57 b	49,90 ± 4,07 c	41,99 ± 2,18 c	40,65 ± 1,80 d	$y = 67.9 - 0.18x + 8.66\times10^{-5}x^2$; $R^2 = 0.94$; $P < 0.001$
	Y	68,00 a	52,87 ± 2,88 a	48,80 ± 2,46 b	48,39 ± 1,88 c	44,06 ± 1,90 c	42,55 ± 5,56 d	$y = 67.9 - 0.243x + 0.000561x^2$; $R^2 = 0.92$; $P < 0.001$
	N	68,00 a	53,25 ± 1,89 b	52,84 ± 3,38 b	47,73 ± 3,48 c	48,65 ± 1,06 c	48,51 ± 1,53 c	$y = 68 - 0.256x + 0.000827x^2$; $R^2 = 0.92$; $P < 0.001$
Curvularia sp. ILRR1A20047	X	68,00 a	59,61 ± 4,11 b	53,93 ± 3,93 c	53,18 ± 5,26 c	49,70 ± 5,49 d	51,43 ± 2,37 d	$y = 68.4 - 0.178x + 0.000417x^2$; $R^2 = 0.75$; $P < 0.001$
	Y	68,00 a	54,59 ± 4,73 b	53,03 ± 3,74 b	50,04 ± 5,60 c	47,23 ± 3,43 c	40,20 ± 2,87 d	$y = 67.5 - 0.153x - 2.73\times10^{-6}x^2$; $R^2 = 0.86$; $P < 0.001$
	N	68,00 a	51,90 ± 1,85 b	52,02 ± 1,84 b	43,53 ± 1,15 c	44,24 ± 1,40 c	37,79 ± 3,88 d	$y = 67.7 - 0.213x + 0.000239x^2$; $R^2 = 0.94$; $P < 0.001$
Fusarium sp. AF10D	X	68,00 a	66,79 ± 0,47 a	65,29 ± 1,67 a	66,64 ± 1,14 a	64,97 ± 0,66 a	63,55 ± 0,77 a	$y = 67.9 - 0.0087x - 8.98\times10^{-5}x^2$; $R^2 = 0.64$; $P < 0.001$
	Y	68,00 a	66,99 ± 0,58 a	65,69 ± 1,69 a	63,68 ± 1,15 a	62,25 ± 0,73 b	58,21 ± 2,54 c	$y = 67.9 + 0.0212x - 0.000458x^2$; $R^2 = 0.88$; $P < 0.001$
	N	68,00 a	67,52 ± 0,34 a	67,00 ± 0,39 a	65,90 ± 0,94 a	61,88 ± 1,61 b	60,77 ± 0,66 b	$y = 68 + 0.0306x - 0.000452x^2$; $R^2 = 0.90$; $P < 0.001$
Fusarium sp. AF48D	X	68,00 a	66,49 ± 1,33 a	66,41 ± 1,35 a	65,80 ± 1,22 a	65,44 ± 1,43 a	63,82 ± 0,76 a	$y = 67.9 - 0.00845x - 8.24\times10^{-5}x^2$; $R^2 = 0.62$; $P < 0.001$
	Y	68,00 a	65,86 ± 1,86 a	64,51 ± 1,06 a	62,04 ± 1,38 b	58,93 ± 1,91 c	57,12 ± 2,58 c	$y = 68.1 - 0.00447x - 0.000376x^2$; $R^2 = 0.88$; $P < 0.001$
	N	68,00 a	58,73 ± 1,23 b	56,90 ± 3,28 b	56,00 ± 2,27 b	52,83 ± 1,73 c	49,12 ± 2,31 d	$y = 67.7 - 0.114x + 4.6\times10^{-5}x^2$; $R^2 = 0.91$; $P < 0.001$
Fusarium sp. AF52DD	X	68,00 a	66,84 ± 0,53 a	63,94 ± 1,61 b	62,12 ± 1,24 b	62,28 ± 0,21 b	60,90 ± 1,02 b	$y = 68.2 - 0.0318x - 8.1\times10^{-5}x^2$; $R^2 = 0.84$; $P < 0.001$
	Y	68,00 a	53,90 ± 0,43 b	52,44 ± 0,45 b	50,07 ± 0,36 c	48,84 ± 0,24 c	48,33 ± 1,10 c	$y = 67.9 - 0.236x + 0.000715x^2$; $R^2 = 0.99$; $P < 0.001$

	N	68,00 a	53,61 ± 1,60 b	53,30 ± 1,80 b	51,75 ± 0,56 b	51,65 ± 2,15 b	51,36 ± 1,22 b	$y = 67.8 - 0.242x + 0.000882x^{2;}$ $R^2 = 0.95$; $P < 0.001$
Fusarium sp. AF99PD	X	68,00 a	65,48 ± 1,95 b	63,29 ± 0,90 b	61,64 ± 0,66 c	59,54 ± 1,20 c	58,76 ± 0,56 c	$y = 68.2 - 0.0362x - 0.000135x^2$; $R^2 = 0.92$; $P < 0.001$
	Y	68,00 a	64,20 ± 1,68 b	63,02 ± 0,40 b	59,52 ± 0,85 c	59,75 ± 0,71 c	59,23 ± 1,67 c	$y = 68.2 - 0.0685x + 7.3\times10^{-5}x^2$; $R^2 = 0.88$; $P < 0.001$
	N	68,00 a	58,65 ± 1,00 b	56,68 ± 1,84 b	55,56 ± 2,20 b	53,27 ± 2,34 c	51,35 ± 2,77 c	$y = 67.9 - 0.139x + 0.000256x^2$; $R^2 = 0.91$; $P < 0.001$
Penicillium brasilianum AF64PD	X	68,00 a	67,23 ± 0,40 a	66,47 ± 1,31 a	65,39 ± 1,71 a	64,90 ± 0,89 a	64,37 ± 1,30 a	$y = 68.1 - 0.00987x - 7.92\times10^{-5}x^2$; $R^2 = 0.66$; $P < 0.001$
	Y	68,00 a	65,34 ± 0,91 a	65,24 ± 0,27 a	63,13 ± 1,53 b	62,45 ± 1,60 b	60,21 ± 1,57 b	$y = 67.9 - 0.0186x - 0.000155x^2$; $R^2 = 0.85$; $P < 0.001$
	N	68,00 a	65,39 ± 1,86 b	63,60 ± 1,11 b	60,93 ± 0,73 c	59,36 ± 1,49 c	58,54 ± 3,68 c	$y = 68.2 - 0.0373x - 0.00014x^2$; $R^2 = 0.81$; $P < 0.001$
Penicillium sp. AF38D	X	68,00 a	67,46 ± 0,63 a	67,58 ± 0,93 a	66,78 ± 0,73 a	65,02 ± 2,52 a	64,17 ± 1,53 a	$y = 68 + 0.0144x - 0.000223x^2$; $R^2 = 0.61$; $P < 0.001$
	Y	68,00 a	67,51 ± 0,35 a	66,98 ± 1,46 a	62,75 ± 2,18 b	60,56 ± 0,34 b	59,08 ± 0,79 b	$y = 68.2 + 0.0221x - 0.000481x^2$; $R^2 = 0.89$; $P < 0.001$
	N	68,00 a	64,37 ± 4,10 b	60,78 ± 4,74 c	55,62 ± 2,08 d	48,86 ± 1,20 e	40,19 ± 2,20 f	$y = 67.9 + 0.0441x - 0.00124x^2$; $R^2 = 0.94$; $P < 0.001$
Penicillium sp. AF41D	X	68,00 a	67,27 ± 1,54 a	66,95 ± 0,42 a	66,26 ± 0,29 a	60,83 ± 1,33 b	60,25 ± 0,24 b	$y = 67.9 + 0.0343x - 0.000498x^2$; $R^2 = 0.86$; $P < 0.001$
	Y	68,00 a	67,49 ± 0,40 a	66,19 ± 0,60 a	64,44 ± 1,01 b	63,36 ± 0,80 b	63,36 ± 1,75 b	$y = 68.2 - 0.00958x - 0.000136x^2$; $R^2 = 0.79$; $P < 0.001$
	N	68,00 a	60,35 ± 1,97 b	58,23 ± 1,30 b	54,45 ± 4,89 c	48,17 ± 6,76 d	40,81 ± 5,01 e	$y = 67.7 - 0.0285x - 0.00076x^2$; $R^2 = 0.87$; $P < 0.001$
Penicillium sp. AF42D	X	68,00 a	66,90 ± 0,87 a	65,58 ± 1,80 a	65,13 ± 2,04 a	64,90 ± 0,95 a	63,82 ± 0,65 a	$y = 68 - 0.0181x - 3.93\times10^{-5}x^{2;}$ $R^2 = 0.62$; $P < 0.001$
	Y	68,00 a	66,61 ± 1,30 a	65,17 ± 0,74 b	63,05 ± 0,20 b	58,83 ± 1,90 c	58,88 ± 0,50 c	$y = 68.2 - 0.00055x - 0.000362x^{2;}$ $R^2 = 0.89$; $P < 0.001$
	N	68,00 a	56,87 ± 2,33 b	55,15 ± 2,17 b	52,37 ± 0,62 c	52,68 ± 1,07 c	52,51 ± 0,82 c	$y = 68 - 0.202x + 0.000653x^{2;}$ $R^2 = 0.95$; $P < 0.001$
Penicillium sp. P10R6	X	68,00 a	48,88 ± 4,02 b	47,44 ± 3,10 b	45,48 ± 3,27 b	42,96 ± 2,50 c	39,39 ± 2,71 d	$y = 67.5 - 0.283x + 0.000733x^2$; $R^2 = 0.92$; $P < 0.001$
	Y	68,00 a	48,26 ± 1,59 b	48,08 ± 0,98 b	37,62 ± 2,39 c	35,29 ± 2,73 c	33,52 ± 3,87 c	$y = 68 - 0.292x + 0.000489x^2$; $R^2 = 0.95$; $P < 0.001$
	N	68,00 a	45,19 ± 1,25 b	42,91 ± 1,39 b	37,30 ± 6,99 c	31,81 ± 3,72 d	27,05 ± 3,22 e	$y = 67.6 - 0.315x + 0.00046x^2$; $R^2 = 0.95$; $P < 0.001$

Penicillium sp. SCUV02.P1	X	68,00 a	46,43 ± 0,80 b	45,59 ± 1,85 b	41,43 ± 3,39 c	39,46 ± 3,57 c	39,99 ± 1,20 c	$y = 67.8 - 0.359x + 0.00115x^2$; $R^2 = 0.96$; $P < 0.001$
	Y	68,00 a	47,34 ± 1,17 b	41,78 ± 0,48 c	42,06 ± 1,44 c	39,87 ± 2,89 c	33,72 ± 0,78 d	$y = 67.6 - 0.327x + 0.000818x^2$; $R^2 = 0.96$; $P < 0.001$
	N	68,00 a	45,14 ± 0,53 b	38,63 ± 1,02 c	36,33 ± 1,45 c	34,93 ± 0,76 d	33,17 ± 2,98 d	$y = 68 - 0.415x + 0.00125x^2$; $R^2 = 0.99$; $P < 0.001$
Pseudogymno-ascus sp. NU09	X	68,00 a	63,88 ± 1,73 b	61,84 ± 0,38 c	59,87 ± 0,79 c	59,06 ± 0,37 c	56,56 ± 1,55 d	$y = 68 - 0.0568x - 6.29\times10^{-5}x^2$; $R^2 = 0.94$; $P < 0.001$
	Y	68,00 a	67,43 ± 0,84 a	65,66 ± 1,22 a	60,32 ± 2,02 b	59,32 ± 1,04 b	57,32 ± 0,89 b	$y = 68.3 + 0.00618x - 0.000458x^2$; $R^2 = 0.88$; $P < 0.001$
	N	68,00 a	66,83 ± 0,48 a	58,27 ± 1,03 b	56,33 ± 1,06 b	54,64 ± 2,46 c	52,66 ± 0,78 c	$y = 68.7 - 0.0604x - 0.000245x^2$; $R^2 = 0.86$; $P < 0.001$
Pseudogymno-ascus sp. SC23P3	X	68,00 a	44,35 ± 1,37 b	39,09 ± 0,88 c	37,17 ± 2,25 c	35,01 ± 0,85 c	36,29 ± 1,17 c	$y = 68 - 0.439x + 0.00148x^2$; $R^2 = 0.99$; $P < 0.001$
	Y	68,00 a	41,91 ± 6,69 b	41,25 ± 3,24 b	40,22 ± 3,99 b	35,90 ± 3,26 c	32,46 ± 2,07 d	$y = 67.2 - 0.383x + 0.00111x^2$; $R^2 = 0.90$; $P < 0.001$
	N	68,00 a	42,03 ± 2,46 b	38,59 ± 3,67 c	34,20 ± 3,84 c	31,59 ± 2,95 d	27,13 ± 3,25 e	$y = 67.6 - 0.406x + 0.00102x^2$; $R^2 = 0.96$; $P < 0.001$
Trichoderma sp. P05R2	X	68,00 a	36,23 ± 3,86 b	34,68 ± 2,16 b	32,16 ± 2,70 b	28,97 ± 1,08 c	26,91 ± 0,40 c	$y = 67.4 - 0.507x + 0.00163x^2$; $R^2 = 0.97$; $P < 0.001$
	Y	68,00 a	38,70 ± 3,99 b	36,59 ± 3,95 b	35,09 ± 0,85 b	31,93 ± 2,01 c	27,68 ± 1,51 d	$y = 67.3 - 0.452x + 0.00135x^2$; $R^2 = 0.95$; $P < 0.001$
	N	68,00 a	35,85 ± 2,96 b	31,09 ± 1,11 c	28,92 ± 1,26 c	26,73 ± 0,65 d	26,31 ± 0,60 d	$y = 67.7 - 0.564x + 0.00192x^2$; $R^2 = 0.99$; $P < 0.001$
Trichoderma sp. RASC1B10122	X	68,00 a	51,71 ± 2,21 a	51,01 ± 2,26 b	48,69 ± 1,36 c	47,35 ± 1,71 c	42,38 ± 0,75 d	$y = 67.5 - 0.226x + 0.000509x^2$; $R^2 = 0.94$; $P < 0.001$
	Y	68,00 a	49,66 ± 0,53 b	48,01 ± 1,78 b	44,90 ± 1,41 c	42,24 ± 2,27 d	36,40 ± 1,89 e	$y = 67.5 - 0.25x + 0.000436x^{2;}$ $R^2 = 0.97$; $P < 0.001$
	N	68,00 a	52,85 ± 0,81 b	50,17 ± 1,46 b	44,40 ± 1,39 c	41,80 ± 0,70 c	37,91 ± 3,94 d	$y = 67.9 - 0.219x + 0.000245x^2$; $R^2 = 0.97$; $P < 0.001$

APÊNDICE H

Tabela S8 – Resultados da triagem secundária para produção de biosurfactantes com análise de variância (p<0,05) dos fatores linhagem e meio de cultura

Linhagem/Meio	Tempo														
	72h			96h			120h			144h			168h		
	X	Y	N	X	Y	N	X	Y	N	X	Y	N	X	Y	N
Fusarium sp. AF52DD	66,84 ± 0,53 Aa	53,90 ± 0,43 Bb	53,61 ± 1,60 Cb	63,94 ± 1,61 Ba	52,44 ± 0,45 Bb	53,30 ± 1,80 Db	62,12 ± 1,24 Ba	50,07 ± 0,36 Cb	51,75 ± 0,56 Db	62,28 ± 0,21 Ba	48,84 ± 0,24 Cb	51,65 ± 2,15 Bb	60,90 ± 1,02 Ba	48,33 ± 1,10 Cb	51,36 ± 1,22 Bb
Fusarium sp. AF99PD	65,48 ± 1,95 Aa	64,20 ± 1,68 Aa	58,65 ± 1,00 Bb	63,29 ± 0,90 Ba	63,02 ± 0,40 Aa	56,68 ± 1,84 Cb	61,64 ± 0,66 Ba	59,52 ± 0,85 Ba	55,56 ± 2,20 Cb	59,54 ± 1,20 Ba	59,75 ± 0,71 Ba	53,27 ± 2,34 Bb	58,76 ± 0,56 Ca	59,23 ± 1,67 Ba	51,35 ± 2,77 Bb
Penicillium brasilianum AF64PD	67,23 ± 0,40 Aa	65,34 ± 0,91 Aa	65,39 ± 1,86 Aa	66,47 ± 1,31 Aa	65,24 ± 0,27 Aa	63,60 ± 1,11 Ba	65,39 ± 1,71 Aa	63,13 ± 1,53 Ab	60,93 ± 0,73 Bb	64,90 ± 0,89 Aa	62,45 ± 1,60 Aa	59,36 ± 1,49 Ab	64,37 ± 1,30 Aa	60,21 ± 1,57 Bb	58,54 ± 3,68 Ab
Fusarium sp. AF48D	66,49 ± 1,33 Aa	65,86 ± 1,86 Aa	58,73 ± 1,23 Bb	66,41 ± 1,35 Aa	64,51 ± 1,06 Aa	56,90 ± 3,28 Cb	65,80 ± 1,22 Aa	62,04 ± 1,38 Ab	56,00 ± 2,27 Cc	65,44 ± 1,43 Aa	58,93 ± 1,91 Bb	52,83 ± 1,73 Bc	63,82 ± 0,76 Aa	57,12 ± 2,58 Bb	49,12 ± 2,31 Cc
Penicillium sp. AF38D	67,46 ± 0,63 Aa	67,51 ± 0,35 Aa	64,37 ± 4,10 Aa	67,58 ± 0,93 Aa	66,98 ± 1,46 Aa	60,78 ± 4,74 Bb	66,78 ± 0,73 Aa	62,75 ± 2,18 Ab	55,62 ± 2,08 Cc	65,02 ± 2,52 Aa	60,56 ± 0,34 Bb	48,86 ± 1,20 Cc	64,17 ± 1,53 Aa	59,08 ± 0,79 Bb	40,19 ± 2,20 Dc
Fusarium sp. AF10D	66,79 ± 0,47 Aa	66,99 ± 0,58 Aa	67,52 ± 0,34 Aa	65,29 ± 1,67 Aa	65,69 ± 1,69 Aa	67,00 ± 0,39 Aa	66,64 ± 1,14 Aa	63,68 ± 1,15 Aa	65,90 ± 0,94 Aa	64,97 ± 0,66 Aa	62,25 ± 0,73 Aa	61,88 ± 1,61 Aa	63,55 ± 0,77 Aa	58,21 ± 2,54 Bb	60,77 ± 0,66 Aa
Penicillium sp. AF41D	67,27 ± 1,54 Aa	67,49 ± 0,40 Aa	60,35 ± 1,97 Bb	66,95 ± 0,42 Aa	66,19 ± 0,60 Aa	58,23 ± 1,30 Cb	66,26 ± 0,29 Aa	64,44 ± 1,01 Aa	54,45 ± 4,89 Cb	60,83 ± 1,33 Ba	63,36 ± 0,80 Aa	48,17 ± 6,76 Cb	60,25 ± 0,24 Ba	63,36 ± 1,75 Aa	40,81 ± 5,01 Db
Penicillium sp. AF42D	66,90 ± 0,87 Aa	66,61 ± 1,30 Aa	56,87 ± 2,33 Bb	65,58 ± 1,80 Aa	65,17 ± 0,74 Aa	55,15 ± 2,17 Cb	65,13 ± 2,04 Aa	63,05 ± 0,20 Aa	52,37 ± 0,62 Db	64,90 ± 0,95 Aa	58,83 ± 1,90 Bb	52,68 ± 1,07 Bc	63,82 ± 0,65 Aa	58,88 ± 0,50 Bb	52,51 ± 0,82 Bc
Pseudogymnoascus sp. NU09	63,88 ± 1,73 Aa	67,43 ± 0,84 Aa	66,83 ± 0,48 Aa	61,84 ± 0,38 Bb	65,66 ± 1,22 Aa	58,27 ± 1,03 Cc	59,87 ± 0,79 Ba	60,32 ± 2,02 Ba	56,33 ± 1,06 Cb	59,06 ± 0,37 Ba	59,32 ± 1,04 Ba	54,64 ± 2,46 Bb	56,56 ± 1,55 Ca	57,32 ± 0,89 Ba	52,66 ± 0,78 Bb
Curvularia sp. ILRR1A20047	59,61 ± 4,11 Ba	54,59 ± 4,73 Bb	51,90 ± 1,85 Cb	53,93 ± 3,93 Ca	53,03 ± 3,74 Ba	52,02 ± 1,84 Da	53,18 ± 5,26 Ca	50,04 ± 5,60 Ca	43,53 ± 1,15 Fb	49,70 ± 5,49 Ca	47,23 ± 3,43 Ca	44,24 ± 1,40 Db	51,43 ± 2,37 Da	40,20 ± 2,87 Db	37,79 ± 3,88 Db
Buergenerula spartinae	55,29 ± 0,90 Ca	52,87 ± 2,88 Ba	53,25 ± 1,89 Ca	50,63 ± 2,57 Ca	48,80 ± 2,46 Ca	52,84 ± 3,38 Da	49,90 ± 4,07 Da	48,39 ± 1,88 Ca	47,73 ± 3,48 Ea	41,99 ± 2,18 Db	44,06 ± 1,90 Db	48,65 ± 1,06 Ca	40,65 ± 1,80 Eb	42,55 ± 5,56 Db	48,51 ± 1,53 Ca

Trichoderma sp. RASC1B10122	51,71 ± 2,21 Da	49,66 ± 0,53 Ca	52,85 ± 0,81 Ca	51,01 ± 2,26 Ca	48,01 ± 1,78 Ca	50,17 ± 1,46 Da	48,69 ± 1,36 Da	44,90 ± 1,41 Db	44,40 ± 1,39 Fb	47,35 ± 1,71 Ca	42,24 ± 2,27 Db	41,80 ± 0,70 Eb	42,38 ± 0,75 Ea	36,40 ± 1,89 Eb	37,91 ± 3,94 Db
Penicillium sp. P10R6	48,88 ± 4,02 Ea	48,26 ± 1,59 Ca	45,19 ± 1,25 Db	47,44 ± 3,10 Da	48,08 ± 0,98 Ca	42,91 ± 1,39 Eb	45,48 ± 3,27 Ea	37,62 ± 2,39 Fb	37,30 ± 6,99 Gb	42,96 ± 2,50 Da	35,29 ± 2,73 Fb	31,81 ± 3,72 Fc	39,39 ± 2,71 Ea	33,52 ± 3,87 Fb	27,05 ± 3,22 Fb
Penicillium sp. SCUV02.P1	46,43 ± 0,80 Fa	47,34 ± 1,17 Ca	45,14 ± 0,53 Da	45,59 ± 1,85 Da	41,78 ± 0,48 Db	38,63 ± 1,02 Fb	41,43 ± 3,39 Fa	42,06 ± 1,44 Ea	36,33 ± 1,45 Gb	39,46 ± 3,57 Da	39,87 ± 2,89 Ea	34,93 ± 0,76 Fb	39,99 ± 1,20 Ea	33,72 ± 0,78 Fb	33,17 ± 2,98 Eb
Pseudogymnoascus sp. SC23P3	44,35 ± 1,37 Fa	41,91 ± 6,69 Da	42,03 ± 2,46 Da	39,09 ± 0,88 Ea	41,25 ± 3,24 Da	38,59 ± 3,67 Fa	37,17 ± 2,25 Gb	40,22 ± 3,99 Ea	34,20 ± 3,84 Gb	35,01 ± 0,85 Ea	35,90 ± 3,26 Fa	31,59 ± 2,95 Fb	36,29 ± 1,17 Fa	32,46 ± 2,07 Fb	27,13 ± 3,25 Fc
Trichoderma sp. P05R2	36,23 ± 3,86 Ga	38,70 ± 3,99 Da	35,85 ± 2,96 Ea	34,68 ± 2,16 Fa	36,59 ± 3,95 Ea	31,09 ± 1,11 Gb	32,16 ± 2,70 Ha	35,09 ± 0,85 Fa	28,92 ± 1,26 Hb	28,97 ± 1,08 Fb	31,93 ± 2,01 Ga	26,73 ± 0,65 Gb	26,91 ± 0,40 Ga	27,68 ± 1,51 Ga	26,31 ± 0,60 Fa

APÊNDICE I

Tabela S9- Análise de variância (ANOVA) trifatorial (fungo, meio de cultura e tempo) para os dados de tensão superficial dos isolados submetidos a triagem secundária

		Tensão superficial (mNm^{-1})	
Fator variação	**GL**	**F**	**p-valor**
Fungo	15	990,50	<0,001
Meio de cultura	2	454,66	<0,001
Tempo	5	1747,18	<0,001
Fungo*meio de cultura	30	25,06	<0,001
Fungo*tempo	75	42,89	<0,001
Meio de cultura*tempo	10	26,77	<0,001
Fungo*meio de cultura*tempo	150	3,14	<0,001
Erro	576		
CV %	3,72		