Research, Society and Development, v. 12, n. 8, e16212842968, 2023
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v12i8.42968

Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by *Diutina mesorugosa* isolated in the Amazon, Brazil

Biodegradação de Hidrocarbonetos Policíclicos Aromáticos (HPAs) por *Diutina mesorugosa* isolada na Amazônia, Brasil

Biodegradación de Hidrocarburos Aromáticos Policíclicos (HAP) por *Diutina mesorugosa* aislada en la Amazonía, Brasil

Received: 08/08/2023 | Revised: 08/24/2023 | Accepted: 08/27/2023 | Published: 08/30/2023

Ferdyanne Beatriz Sabóia Peixoto
ORCID: https://orcid.org/0000-0002-9858-6884
Federal University of Amazonas, Brazil
E-mail: ferdyanne@gmail.com
Jean Charles da Cunha Peixoto
ORCID: https://orcid.org/0000-0002-8584-2009
Federal University of Amazonas, Brazil
E-mail: jean.metagenoma@gmail.com
Adolfo José Mota
ORCID: https://orcid.org/0000-0002-9745-1128
Federal University of Amazonas, Brazil
E-mail: adolfo.mot@gmail.com
Hiléia dos Santos Barroso
ORCID: https://orcid.org/0000-0002-1903-7394
Technological Research Institute of São Paulo, Brazil
E-mail: hileia.barroso@gmail.com
Enedina Nogueira de Assunção
ORCID: https://orcid.org/0000-0002-6929-7813
Federal University of Amazonas, Brazil
E-mail: dinanog@ufam.edu.br
Ieda Hortêncio Batista
ORCID: https://orcid.org/0000-0001-8465-3129
Amazon State University, Brazil
E-mail: iedahbatista@gmail.com
Samára Ferreira Santos
ORCID: https://orcid.org/0000-0001-5397-1052
Federal University of Amazonas, Brazil
E-mail: samara_venture@yahoo.com.br
José Odair Pereira
ORCID: https://orcid.org/0000-0002-0441-381X
Federal University of Amazonas, Brazil
E-mail: joseodairpereira@yahoo.com.br
Spartaco Astolfi-Filho
ORCID: https://orcid.org/0000-0001-7246-6350
Federal University of Amazonas, Brazil
E-mail: spartaco.biotec@gmail.com

Abstract

There is a demand for new microorganisms with the potential to degrade recalcitrant petroleum compounds, such as polycyclic aromatic hydrocarbons (PAHs). The objective of this research was to evaluate the potential of the AM15 strain isolated from water samples from the effluent dike of the Urucu petroleum Province, to degrade a mixture of PAHs. After isolation in culture medium, and initial screening by Gram stain, it was found to be yeast. The characterization of the strain was carried out by analysis based on the sequences of the polymorphic regions ITS1/ITS2 and D1/D2 of the 28S rRNA gene, concomitant with the restriction fragment size polymorphism, generated by the Polymerase Chain Reaction-Fragment Length Polymorphism of Restriction (PCR-RFLP). The ability of yeast to degrade petroleum compounds was determined by testing with the redox indicator 2,6-Dichlorophenol Indophenol (DCPIP), and degradation was confirmed and quantified by gas chromatography and mass spectrometry (GC-MS) analyses. Molecular analyzes indicated that the AM15 strain belongs to the *Diutina mesorugosa* species. The results obtained in the test carried out with DCPIP showed that the AM15 strain has the potential to degrade hydrocarbons present in petroleum and diesel oil. GC-MS analyzes confirmed that the AM15 strain was able to

degrade 79.4% of a mixture of PAHs in 21 days. These results suggest that *D. mesorugosa* AM15 is promising to degrade different types of hydrocarbons, and can be used as a tool in the bioremediation of oil-impacted areas.
Keywords: *Diutina mesorugosa*; PAHs; DCPIP; GC-MS.

Resumo

Existe uma demanda por novos microrganismos com potencial para degradar compostos recalcitrantes do petróleo, como os hidrocarbonetos policíclicos aromáticos (HPAs). O objetivo desta pesquisa foi avaliar o potencial da cepa AM15 isolada de amostras de água do dique de efluente da Província Petrolífera de Urucu, para degradar uma mistura de HPAs. Após o isolamento em meio de cultura, e triagem inicial pela coloração de Gram, constatou-se que se trata de uma levedura. A caracterização da cepa foi realizada por análises baseadas nas sequências das regiões polimórficas ITS1/ITS2 e D1/D2 do gene 28S rRNA, concomitante com o polimorfismo do tamanho do fragmento de restrição, gerado pela Reação em Cadeia da Polimerase-Polimorfismo do Comprimento do Fragmento de Restrição (PCR-RFLP). A capacidade da levedura para degradar compostos do petróleo foi determinada pelo teste com o indicador redox 2,6-Diclorofenol Indofenol (DCPIP), e a degradação foi confirmada por análises de cromatografia gasosa e espectrometria de massa (GC-MS). As análises moleculares indicaram que a cepa AM15 pertence à espécie *Diutina mesorugosa*. Os resultados obtidos no teste realizado com DCPIP demonstrou que a cepa AM15 apresenta potencial para degradar hidrocarbonetos presentes no petróleo e no óleo diesel. As análises de GC-MS confirmaram que a cepa AM15 foi capaz de degradar 79,4% de uma mistura de HPAs em 21 dias. Esses resultados sugerem que *D. mesorugosa* AM15 é promissora para degradar diferentes tipos de hidrocarbonetos, podendo ser utilizada como ferramenta na biorremediação de áreas impactadas com petróleo.
Palavras-chave: *Diutina mesorugosa;* HPAs; DCPIP; GC-MS.

Resumen

Existe una demanda de nuevos microorganismos con el potencial de degradar compuestos de petróleo recalcitrantes, como los hidrocarburos aromáticos policíclicos (HAP). El objetivo de esta investigación fue evaluar el potencial de la cepa AM15 aislada de muestras de agua del dique de efluentes de la Provincia Petrolera de Urucu, para degradar una mezcla de HAP. Después del aislamiento en medio de cultivo y la detección inicial mediante tinción de Gram, se descubrió que se trataba de una levadura. La caracterización de la cepa se realizó mediante análisis basados en las secuencias de las regiones polimórficas ITS1/ITS2 y D1/D2 del gen 28S rRNA, concomitante con el polimorfismo del tamaño del fragmento de restricción, generado por la Polimerasa Chain Reaction-Fragment Length Polymorphism of Restricción (PCR-RFLP). La capacidad de la levadura para degradar compuestos de petróleo se determinó mediante pruebas con el indicador redox 2,6-diclorofenol indofenol (DCPIP), y la degradación se confirmó y cuantificó mediante cromatografía de gases y análisis de espectrometría de masas (GC-MS). Los análisis moleculares indicaron que la cepa AM15 pertenece a la especie *Diutina mesorugosa*. Los resultados obtenidos en la prueba realizada con DCPIP demostraron que la cepa AM15 tiene potencial para degradar los hidrocarburos presentes en el petróleo y el gasóleo. Los análisis de GC-MS confirmaron que la cepa AM15 podía degradar el 79,4 % de una mezcla de HAP en 21 días. Estos resultados sugieren que *D. mesorugosa* AM15 promete degradar diferentes tipos de hidrocarburos y puede usarse como una herramienta en la biorremediación de áreas impactadas por petróleo.
Palabras clave: *Diutina mesorugosa*; HAP; DCPIP; GC-MS.

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are ubiquitous organic compounds, they may have a natural or anthropogenic origin. Some PAHs are highly toxic, mutagenic, carcinogenic, and represent a worldwide concern, due to their harmful potential to human populations, animals and the environment (Haritash & Kaushik, 2009; Hesham et al., 2009; Passarini et al., 2011; Stapleton et al., 1998; Ting et al., 2011). Currently, there are a variety of technologies available, and well established to recover environments impacted with PAHs, however, bioremediation has been chosen, as it is a clean, promising, effective and cost-effective technology (Felisardo & Gonçalves, 2023; Olajire & Essien, 2014; Schippers et al., 2000).

The search for new microbial strains with the potential to degrade recalcitrant compounds of petroleum, such as PAHs, has been recurrent, and a large number of microorganisms have been easily isolated from terrestrial and marine environments, impacted and not impacted (Kanwal et al., 2022; Hesham et al., 2009; Palittapongarnpim et al., 1998; Zinjarde & Pant, 2002). Among these microorganisms, yeasts have been reported to degrade a wide variety of hydrocarbons, however, there are rare strains that are able to degrade PAHs, especially high molecular weight PAHs (MacGillivray & Shiaris, 1993;

Hesham et al., 2006; Gargouri et al., 2015). Biodegradation studies using yeasts belonging to the genera Candida, Cryptococcus, Rhodotorula, Torulopsis, Trichosporon, Yarrowia, and Pichia recorded the biotransformation of PAHs such as Benzo (a) pyrene, Benzo (a) anthracene, phenanthrene, naphthalene, pyrene and chrysene (MacGillivray & Shiaris, 1993, Hesham et al., 2006; Gargouri et al., 2015). However, no records were found related to PAH biodegradation by *Diutina mesorugosa*.

According to Wongsa et al. (2004) and Mishra et al. (2001), PAHs are more resistant to biodegradation compared to aliphatic compounds, which makes them a big problem when establishing a bioremediation model, since the PAHs biodegradation process is related to its hydrophobicity and dissolution in water, because the lower its molecular weight, the more easily they will be degraded, however, the greater the molecular mass, the lower its solubilization, the greater its stability and therefore more recalcitrant (Vasconcelos & França, 2011; Zinjarde & Pant, 2002). In this study was made sample collection, bacterial isolation, analysis of the degradation of PAHs, and finally molecular characterization were carried out to identify the AM15 strain obtained from an effluent lagoon in the state of Amazonas.

2. Methodology

2.1 Study Area

The samples were carried out in the effluent pond (S 04 ° 51'50.4 ”/ W 065 ° 18'00.3”) of the Petroleum Base of Urucu, located in the Coari town, Amazonas state, Brazil, 653 km in a straight line southwest of Manaus city (4º30´S / 64º30´W). the water samples were taken on the edge of the efflux ent pond at a depth of 50 cm and in the middle of the pond at a depth of two meters, in previously sterilized glass bottles, and kept refrigerated in a thermal box, during the transfer from Urucu / Coari Petroleum Province until processing in the DNA Technology Laboratory at the Federal University of Amazonas (UFAM) in the Manaus city.

2.2 Enrichment and isolation

D. mesorugosa AM15 strain was isolated from water samples enriched with Buschnell Haas (BH) broth (Difico ™) and petroleum in the following proportions: 10 mL of water, 90 mL of BH broth and 1% petroleum were added in sterile 250 mL Erlenmeyers, and incubated at 30°C, 180 rpm for 21 days. The isolation was carried out in Petri dishes containing BH medium plus 1% of crude oil. Pure colonies were selected and sown in potato dextrose agar (BDA) (HIMEDIA ™). The crude oil and diesel oil used in the experiments were sterilized by the 0.22 µm and 0.45 µm millipore ™ membrane filtration method.

2.3 Biodegradation assay using DCPIP

The qualitative test of biodegradability of crude oil and diesel oil was carried out in cells cultivation plates (24 wells), incubated at 30 °C. The DCPIP redox indicator [0.01 g L^{-1}] was dissolved in sterile BH broth (Himedia). The evaluations were carried out periodically until the color change from blue (oxidized) to colorless (reduced). The microbial culture was standardized at a wavelength of 600_{nm} (10^9 cells/mL), cell density O.D = 1.0. The experiment was carried out as described by Hanson et al. (1993), Peixoto et al. (2017) and Peixoto et al. (2018).

2.4 Molecular Identification and phylogenetic analysis

Yeast identification was accomplished using restriction fragment length polymorphism (Mota & Nobrega, 2013), followed by confirmation through sequencing of the ITS1/ITS2 polymorphic regions and the D1/D2 polymorphic domains located on the 26S rRNA gene. Genomic DNA was prepared using the Plant/Fungi DNA Isolation Kit (NORGEN BIOTEK CORP™). PCR was performed using the Platinum Taq DNA Polymerase Kit (Invitrogen by Thermo) and PCR products were

purified using PuriLink (R) PCR Purification Kit (Invitrogen). The sequencing reaction was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems by Thermo), and the sequences were determined using the ABI3500 Genetic Analyzer (Applied Biosystems by Thermo). The primers used in both PCR and sequencing were: ITS1 (5'-TCCGTAGGTGAACCTGCGG) (White, et al., 1990), and Uni-R (5' GGTCCGTGTTTCAAGACG) (Fell, 1993). Sequence editing and assembly of the contigs were performed using the SeqManPro™ program (DNAStar(R)). The search for species-level similarity in the INSDC databases was carried out using BLAST program (BLASTn) available at the *National Center for Biotechnology Information* (NCBI) (http://www.ncbi.nlm.nih.gov).

2.5 Sample preparation

Liquid cultures were prepared in 125 mL Erlenmeyer flasks containing 40 mL of BH broth, 1% diesel oil and 1 mL of the inoculum. The control flasks, without microorganisms, were incubated under the same conditions, to estimate the abiotic loss of hydrocarbons. The assay was performed in triplicate and incubated at 30 °C, 150 rpm. Biodegradation was evaluated and quantified after 7, 14 and 21 days of incubation.

2.6 Extraction of polycyclic aromatic hydrocarbons (PAHs)

PAH extraction was performed using methylene chloride and hexane. Briefly, 50 mL of methylene chloride and hexane solution was added to each flask. The material was transferred to a 125 mL separatory funnel and subjected to vigorous agitation for 1 minute. The organic phase was collected and the aqueous phase was extracted twice more by addition of 50 mL methylene chloride and hexane solution. The end of the extraction was monitored by thin layer chromatography (TLC). Anhydrous sodium sulfate was added to the collected organic phase, and after filtration the samples were subjected to vacuum roto-evaporation at 40 °C until sample volume was reduced to approximately 25 mL. The sample was then transferred to 1 mL vial containing 20 μg of the internal standard. One μL of the sample was injected into the GC/MS.

2.7 Analysis of biodegradation data

The internal standard was fluoranthene-d10 (Flu-d10) and surrogate solution was phenanthrene-d10 (Phe-d10) and naphthalene d8 (Nap-d8) with purity ≥ 98 % (Sigma-Aldrich, 98 %), solubilized in hexane grade HPLC. The internal standard (Flu-d10) was inserted into the samples and diluted in a volumetric flask before injection into the GC-MS. The surrogate solution (Phe-d10 and Nap-d8) was added to the samples before beginning the extraction process. These methods are used to realize possible losses during the extraction process. The chromatographic areas of the analytes were corrected by extraction recovery rate, obtained by the following formula: [CAs/CAis] x 100, where CAis is the chromatographic area of the internal standard, and the CAs is the chromatographic area of the surrogate. The fraction analyzed in the diesel were aromatic hydrocarbons (PAH), and the main analytes were: naphthalene, 2-methyl naphthalene, 1-methyl naphthalene, dimethyl naphthalene, trimethyl naphthalene and phenanthrene. Analyte concentrations were obtained from a calibration curve using different concentrations of a mix of PAH standard (Sigma-Aldrich): 50, 55, 60, 65, 70 and 80 μg mL^{-1}. The degradation percentage for PAH in the diesel was calculated by the sum of the analytes (ΣPAH), using the following formula: [(CD - CS) / CD] x 100, where CD is the PAH concentration of the diesel in the control group, and CS is the concentration of PAH of the diesel obtained in the sample after the experiment. Finally, the mean and standard deviation of the biodegradation rate were calculated.

2.8 GC-MS analysis

Analyses were performed on a gas chromatograph (Shimadzu 2010) (Tokyo, Japan) coupled to a mass spectrometer (Shimadzu QP 2010 Plus) and equipped with a Restek fused silica capillary column (RTX-5MS 30 m x 0.25 mm x 0.25 μm) (Bellefonte, PA, USA). The injector temperature was 270 °C and was in the *splitless* injection mode, with helium as a carrier gas at a rate of 1.56 mL min^{-1}. Mass fragments were recorded by selective ion monitoring (SIM). The ions used were as follows: naphthalene (*m/z* 228, 227), 2-methyl naphthalene (*m/z* 142, 141), 1-methyl naphthalene (*m/z* 142, 141), dimethyl naphthalene (*m/z* 156, 141), trimethyl naphthalene (*m/z* 170, 155), and phenanthrene (*m/z* 178, 179). The GC temperature program started at 40 °C for 1 minute, followed by a heating rate of 20 ºC min^{-1} until reaching 60 ºC, then a heating rate of 5 ºC min^{-1} until reaching 260 ºC, and finally 10 ºC min^{-1} until reaching 280 ºC, where it remained for 1 minute.

3. Results

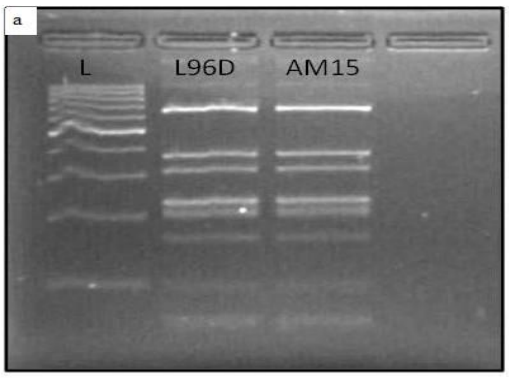
3.1 Biodegradation assay using DCPIP

D. mesorugosa AM15 demonstrated degrading activity by promoting a color change in the medium with DCPIP after 16 hours of incubation with crude oil, and after 10 hours with diesel oil.

3.2 Molecular Identification and phylogenetic analysis

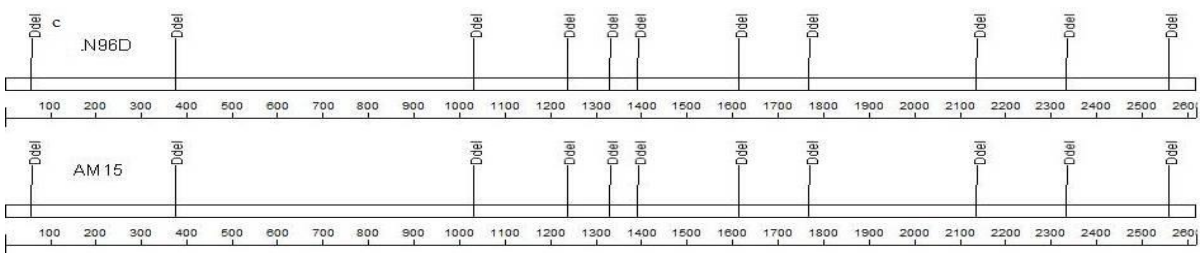
Due to the phenotypic plasticity commonly observed in yeasts, we identified the AM15 isolate using a two-step process. First, we verified the size polymorphism of the fragments generated by the *Dde*I enzyme from 2,622 bp amplicons containing part of the 18S and 28S rRNA genes, the full sequence of 5.8S rRNA gene, and the complete sequence of ITS1 and ITS2. The restriction pattern is identical to the pattern of *Diutina mesorugosa* L96D (Figure 1).

Figure 1 - PCR-RFLP yeast identification method.



b

Site nº.	Nucleotide positions and fragment size (bp)	Fragment size arrangement
01	(0001 – 0030) 030	Fragment 01 – 657
02	(0031 – 0344) 314	Fragment 02 – 368
03	(0345 – 1001) 657	Fragment 03 – 314
04	(1002 – 1206) 205	Fragment 04 – 224
05	(1207 – 1300) 094	Fragment 05 – 222
06	(1301 – 1362) 062	Fragment 06 – 205
07	(1363 – 1584) 222	Fragment 07 – 197
08	(1585 – 1740) 156	Fragment 08 – 156
09	(1741 – 2108) 368	Fragment 09 – 094
10	(2109 – 2305) 197	Fragment 10 – 064
11	(2306 – 2529) 224	Fragment 11 – 062
	(2530 – 2593) 064	Fragment 12 – 030

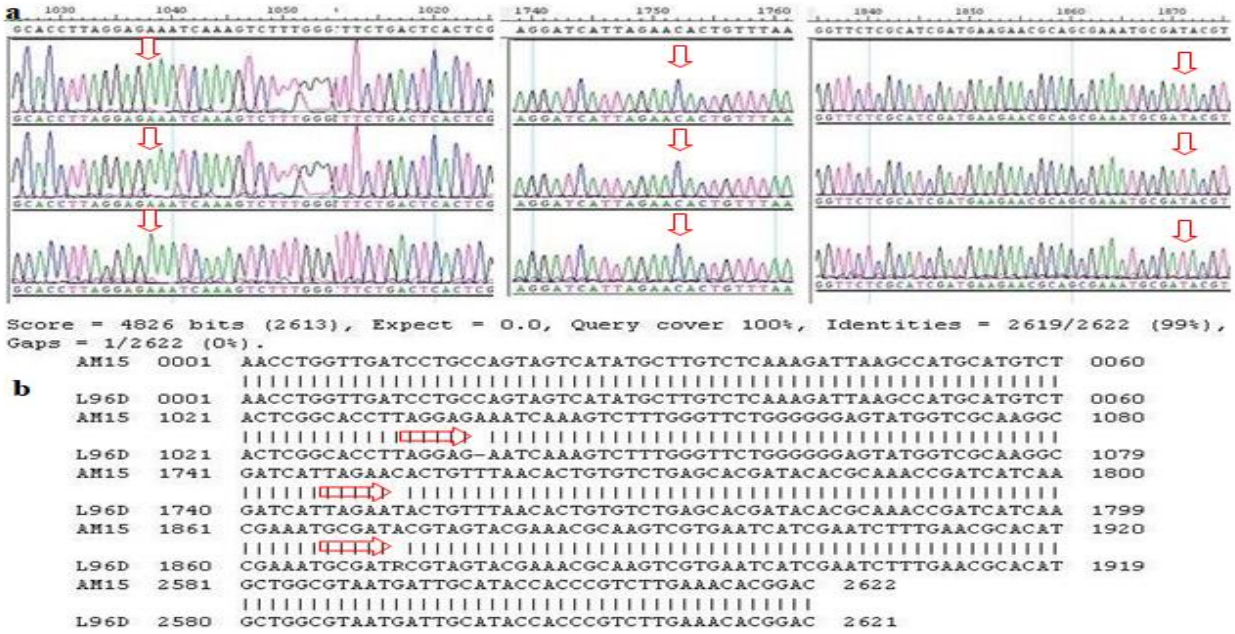


Source: Authors (2023).

Figure 1 shows the 2,622 bp fragment which was digested with the DdeI restriction enzyme and produced the same pattern as *Diutina mesorugosa* L96D **(a)**. The arrangement of restriction fragments in the 2% agarose gel can be compared

with the *in silico* prediction table (**b**). Fragments 222 and 224 are collapsed, as well as fragments 205 and 197 due to having similar sizes. In **(c)** the restriction map shows the position of the 11 cut sites of DdeI. L 100 bp DNA Ladder. Second, the same 2,622 bp amplicon was sequenced and revealed a 99% identity with the L96D sequence (Figure 2). The nucleotide sequence of the AM15 isolate was deposited in GenBank as *Diutina mesorugosa*-AM15 under accession number KY464166.

Figure 2 - Sequence analyses.



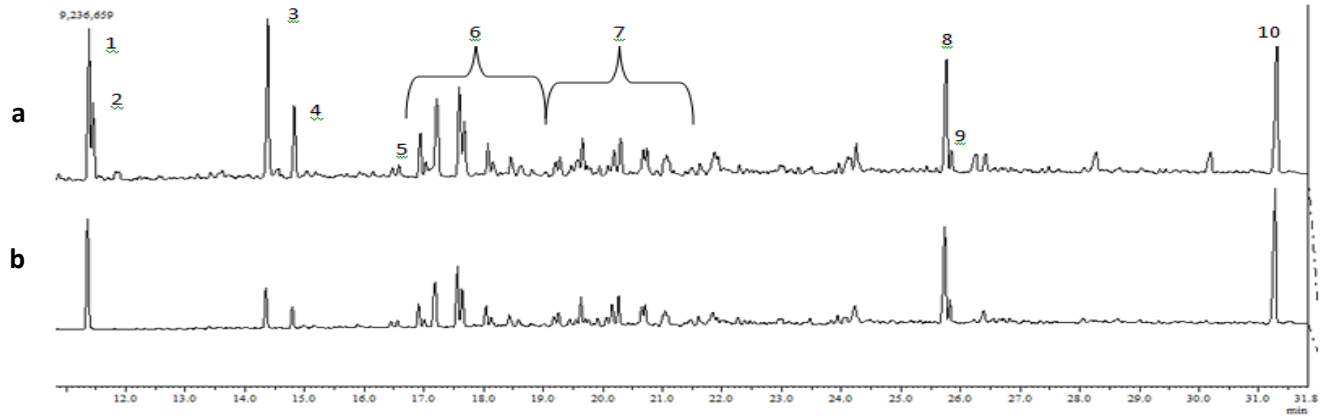
Source: Authors (2023).

Figure 2 shows 2,622 bp fragment amplicon was sequenced using the Sanger method and the sequence alignment showed 3 SNPs, indicated by red arrows. (**a**) Eletropherogram showing the position of the SNP observed in (**b**) the blast2 sequence analyses. Numbers represents the position of each nucleotide in the sequence.

3.3 Quantitative analysis of polycyclic aromatic hydrocarbons (PAHs)

D. mesorugosa AM15 degraded PAHs as naphthalene, 2-methyl naphthalene, 1-methyl naphthalene, dimethyl naphthalene, trimethyl naphthalene and phenanthrene respectively. The degradation spectrum of the PAH's over 14 days is shown in figure 3. The degradation rate obtained was 21.8±3.8% in 7 days, 72.3±6.3% in 14 days, and 79.4±2.2% after 21 days (Figure 4 a-b).

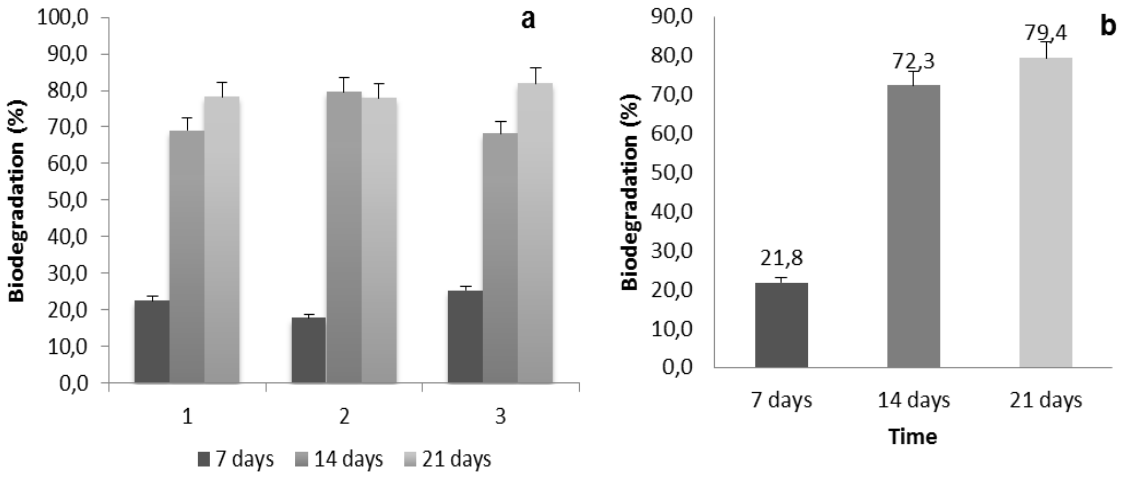
Figure 3 – Degradation spectra obtained by GC-MS.



Source: Authors (2023).

Figure 3 shows in (a) Reaction controls. Numbered chromatographic peaks correspond to the following compounds: 1) Naphthalene D-8 * (Surrogate); 2) Naphthalene; 3) 2-methyl naphthalene; 4) 1-methyl naphthalene, 5) Biphenyl; 6) Alkylnaphthalenes; 7) Trimethylnaphthalenes 8) Phenanthrene D-10 * (Surrogate); 9) Phenanthrene; 10) Fluoranthene D-10 * (Internal Standard). D-8 and D-10 *: Deuterated Compounds. In **(b)** Mass Spectrum demonstrating the degradation of PAHs mixture by *D. mesorugosa* AM15 after 14 days of incubation.

Figure 4 - Biodegradation of PAHs.



Source: Authors (2023).

Figure 4 shows degradation of a PAH's mixture such as 3 – methyl naphthalene, 2 – methyl naphthalene, 1 – methyl naphthalene, dimethyl naphthalene, trimethyl naphthalene, and phenanthrene by the *D. mesorugosa* line, strain AM15. In (a) the percentage of biodegradation containing the mixture of PAHs is shown, in the intervals of 7, 14 and 21 days, in three replicates. In (b) the average degradation percentage of the three replicates in the three time intervals is shown.

4. Discussion

Different yeast species have been widely described as degrading petroleum hydrocarbons and their derivatives (Li et., al 2023; Boz et al., 2015; Das & Chandran, 2011; Farag & Soliman, 2011; Hesham et al., 2009; Junior et al., 2009; Sood et al., 2009; Joo et al., 2008; Miranda et al., 2007; Zinjarde & Pant, 2002). Promising microorganisms for degrading these compounds, when tested with DCPIP indicator, should change the color of the indicator medium from blue (oxidized) to colorless (reduced) in a short period of time. In this case, *D. mesorugosa* showed activity after 14 hours of incubation, using crude oil, and after 10 hours of incubation, using diesel oil. When comparing the results obtained by *D. mesorugosa* AM15, with the results obtained by Ferrari et al. (2011), who mention color change of DCPIP, after three weeks of incubation with fungal isolates, or even those obtained by Lima et. al. (2016), who recorded a change in the color of DCPIP, with amazonian fungi, after 24 hours of incubation, a better degradation performance was observed in the results obtained with *D. mesorugosa* AM15. The degradation percentages (21.8% - 7 days, 72.3% - 14 days, 79.4% - 21 days) obtained in the present study confirm the potential of *D. mesorugosa* AM15 to degrade a mixture of PHAs, which includes naphthalene, 2-methyl naphthalene, 1-methyl naphthalene, dimethyl naphthalene, trimethyl naphthalene and phenanthrene. In this study, the degradation rate was quantified covering a mixture of PAHs, unlike Hesham et al. (2009), Sood et al. (2009), Gargouri et al. (2015), and Farag & Soliman (2011), who obtained degradation efficiency separately for different petroleum compounds. Even so, it can be seen that the degradation rate achieved by *D. mesorugosa* AM15 is of the same order of magnitude as the rates obtained by the strains of *C. viswanathii* (Hesham et al., 2009), *C. digboiensis* (Sood et al., 2009), *Candida sp.* (Gargouri et al., 2015) and *C. tropicalis* (Farag & Soliman, 2015).

Although a wide range of yeasts have been cited as degrading hydrocarbons, this is the first record of *D. mesorugosa* as a potential species to degrade recalcitrant petroleum compounds. *D. mesorugosa* was initially described by Chaves et al. (2013), and classified as belonging to the genus Candida, species *Candida mesorugosa*. The species *C. mesorugosa* was isolated for the first time in a hospital environment (Chaves et al. 2013), diverging from this study, in which the microorganism was obtained from water samples from the effluent pond. However, currently, after phylogenetic and molecular analysis, *C. mesorugosa* has been reclassified to genus Diutina, species *Diutina mesorugosa*, order Saccharomycetales, subphylum Saccharomycotina, phylum Ascomycota (Khunnamwong et al. 2015, Ming et al. 2019). In the present investigation *D. mesorugosa* was identified with 99% identity to the sequence of *D. mesorugosa* L96D. The phylogenetic analysis confirmed that the strain isolated in this research is associated with the phylogenetic group of *Diutina rugosa*, cited by Das & Chandran (2011) as a potent degrader of diesel oil. Thus, considering the degradation rates obtained here and the importance of selecting promising yeasts for bioremediation of environments impacted by petroleum and derivatives, *D. mesorugosa* can be considered a potential species for bioremediation processes.

5. Conclusion

Diutina mesorugosa AM15 strain isolated in the present study proved to be efficient in the degradation of a variety of hydrocarbons, including PAHs. The results provide ample evidence that *D. mesorugosa* AM15 is a promising genetic resource to be exploited as a tool in the bioremediation of environments impacted by petroleum and oil products, especially in the amazon region.

Further studies will be conducted with the AM15 strain, on a microcosm scale, under controlled conditions, aiming at future use in bioremediation processes. The sequencing of the complete genome of the AM15 lineage of *Diutina mesorugosa*, and mining of the genes involved in the processes of biodegradation of PAHs is considered.

Acknowledgments

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the Fundação de Amparo a Pesquisa do Amazonas (FAPEAM) for financial support. We also thank the Universidade Federal do Amazonas (UFAM) /Programa de Pós-Graduação em Biodiversidade e Biotecnologia da Amazônia Legal (Bionorte Netresearch). We are grateful to Petrobras for supporting the collection activities and to Alzira Frota and Vanderlei Sabóia for assistance with the extraction procedures and GC-MS.

References

Boz, D. T., Yalçın,, H. T., Çorbacı, C., & Uçar, F. B. (2015). Screening and molecular characterization of polycyclic aromatic hydrocarbons degrading yeasts. *Turkish Journal of Biochemistry*, *40*(2),105–110. https://doi.org/10.5505/tjb.2015.16023.

Chaves, G. M., Arioli, G. R. T. E. R. Ç., Padovan, A. N. A. C. B., Rosas, R. C., Ferreira, R. C., Melo, A. S. A., & Colombo, A. L. (2013). *Candida mesorugosa sp*. nov., a novel yeast species similar to *Candida rugosa*, isolated from a tertiary hospital in Brazil. *Medical Mycology*, *51*(April), 231–242. https://doi.org/10.3109/13693786.2012.710345.

Li, Chongshu, Cui, Changzheng, Zhang, Jie, Shen, Jing, He, Baoyan, Long, Yan, Ye, Jinshao. (2023) Biodegradation of petroleum hydrocarbons based pollutants in contaminated soil by exogenous effective microorganisms and indigenous microbiome. Ecotoxicology and Environmental Safety, Volume 253. https://doi.org/10.1016/j.ecoenv.2023.114673.

Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology Research International*, *2011*, 941810. https://doi.org/10.4061/2011/941810.

Farag, S., & Soliman, N. a. (2011). Biodegradation of crude petroleum oil and environmental pollutants by Candida tropicalis strain. *Brazilian Archives of Biology and Technology*, *54*(4), 821–830. https://doi.org/10.1590/S1516-89132011000400023.

Fell, J. W. (1993). Rapid identification of yeast species using three primers in polymerase chain reaction.Mol. *Mol Mar. Biology and Biotech.*, *2*(3), 174–180.

Felisardo, R. J. A., & Gonçalves, A. de A. (2023). Biodegradation and biotransformation of petroleum hydrocarbons: progress, prospects, and challenges. *Revista Eletrônica Em Gestão, Educação E Tecnologia Ambiental*, *27*, e1. https://doi.org/10.5902/2236117069288.

Ferrari, B. C., Zhang, C., & van Dorst, J. (2011). 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*, *2*(NOV), 1–14. https://doi.org/10.3389/fmicb.2011.00217.

Gargouri, B., Mhiri, N., Karray, F., Aloui, F., & Sayadi, S. (2015). Isolation and Characterization of Hydrocarbon-Degrading Yeast Strains from Petroleum Contaminated Industrial Wastewater. *BioMed Research International*, *2015*, 1–11. https://doi.org/10.1155/2015/929424.

Hanson, K. G., Desai,, J. D., & Desai, A. J. (1993). a Rapid and Simple Screening Crude Oil Degrading Technique for Potential Microorganisms. *Biotechnology Techniques*, *7*(1), 745–748.

Haritash, A. K., & Kaushik, C. P. (2009). Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. *Journal of Hazardous Materials*, *169*(1–3), 1–15. https://doi.org/10.1016/j.jhazmat.2009.03.137.

Hesham, A. E., Alamri, S. A., Khan, S., Mahmoud, M. E., & Mahmoud, M. (2009). Isolation and molecular genetic characterization of a yeast strain able to degrade petroleum polycyclic aromatic hydrocarbons. *African Journal Od Biotechnology*, *8*(10), 2218–2223. https://doi.org/10.5897/AJB09.016.

Hesham, A. E. L., Wang, Z., Zhang, Y., Zhang, J., Lv, W., & Yang, M. (2006). Isolation and identification of a yeast strain capable of degrading four and five ring aromatic hydrocarbons. *Annals of Microbiology*, *56*(2), 109–112. https://doi.org/10.1007/BF03174990.

Joo, H. S., Ndegwa, P. M., Shoda, M., & Phae, C. G. (2008). Bioremediation of oil-contaminated soil using *Candida catenulata* and food waste. *Environmental Pollution*, *156*(3), 891–896. https://doi.org/10.1016/j.envpol.2008.05.026.

Junior, J. S., Mariano, A. P., & Angelis, D. D. F. De. (2009). Biodegradation of biodiesel/diesel blends by *Candida viswanathii. African Journal of Biotechnology*, *8*(12), 2774–2778. https://doi.org/10.5897/AJB09.238.

Kanwal, Maheen, Ullah,Hayat, Gulzar, Aasma, Sadiq, Tehmina, Gul, Zarif, Ullah, Munzer, Sarfraz, Maliha, Aslam, Muhammad Waseem, Khan, Nida Nasir, Batool, Tayyaba, Maqsood, Saman, Nawaz, Ayesha. (2022). Biodegradation of Petroleum Hydrocarbons and The Factors Effecting Rate of Biodegradation. Am J Biomed Sci & Res. 16(1). https://doi.org/10.34297/AJBSR.2022.16.002182.

Khunnamwong P., Lertwattanasakul N, Jindamorakot S, Limtong S, Lachance MA. (2015). Description of Diutina gen. nov., Diutina siamensis, f.a. sp. nov., and reassignment of *Candida catenulata*, *Candida mesorugosa*, *Candida neorugosa*, *Candida pseudorugosa*, *Candida ranongensis*, *Candida rugosa* and *Candida scorzettiae* to the genus *Diutina*. *Int J Syst Evol Microbiol.*, 65(12):4701-4709. https://doi:10.1099/ijsem.0.000634.

Lima, J. M. (2016). Potential biosurfactant producing endophytic and epiphytic fungi, isolated from macrophytes in the Negro River in Manaus, Amazonas, Brazil. *African Journal of Biotechnology*, *15*(24), 1217–1223. https://doi.org/10.5897/AJB2015.15131.

Luz, C. C., Santos, E. A., Santos, M. O. S., Mussy, M. Y., Yamashita, M., Bastos, W. R., & Reis, M. G. (2011). Estudos de biodegradação de óleo diesel por consorcio microbiano coletado em Porto Velho- Ro, Amazônia. *Quimica Nova*, *34*(5), 775–779. https://doi.org/10.1590/S0100-40422011000500009.

MacGillivray, a. R., & Shiaris, M. P. (1993). Biotransformation of polycyclic aromatic hydrocarbons by yeasts isolated from coastal sediments. *Applied and*

Environmental Microbiology, *59*(5), 1613–1618.

Mariano, A. P., Tomasella, R. C., Oliveira, L. M. De, Conteiro, J., & Angelis, D. D. F. De. (2008). Biodegradability of diesel and biodiesel blends. *African Journal of Biotechnology*, *7*(9), 1323–1328.

Ming CHUNYAN, Huang Jin, Wang Yanyan, Lv Qian Lv, Zhou Bing, Taohua Liu1, Yu Cao, Bert Gerrits van den Ende5, Al-Hatmi Abdullah M. S. Al-Hatmi, Ahmed Sarah A. Ahmed, Huang Guanghua, Bai Fengyan, Hoog Sybren de, Kang Yingqian. (2019). Revision of the medically relevant species of the yeast genus *Diutina. Medical Mycology*,*57, 226-233.* https://doi:10.1093/mmy/mmy001.

Miranda, R. D. C., De Souza, C. S., Gomes, E. D. B., Lovaglio, R. B., Lopes, C. E., & Sousa, M. D. F. V. D. Q. (2007). Biodegradation of diesel oil by yeasts isolated from the vicinity of Suape Port in the State of Pernambuco -Brazil. *Brazilian Archives of Biology and Technology*, *50*(1), 147–152. https://doi.org/10.1590/S1516-89132007000100018.

Mishra, S., Jyot, J., Kuhad, R. C., & Lal, B. (2001). In situ bioremediation potential of an oily sludge-degrading bacterial consortium. *Current Microbiology*, *43*(5), 328–335. https://doi.org/10.1007/s002840010311.

Mota, A. J., & Nobrega, F. G. (2013). Unequivocal Identification of Fungi , Especially Candida and Related Species of Medical Interest. *Journal of Medical Diagnostic Methods*, *2*(5)1-13. https://doi.org/10.4172/2168-9784.1000142.

Olajire, A. A., & Essien, J. P. (2014). Aerobic Degradation of Petroleum Components by Microbial Consortia. *Journal of Petroleum & Environmental Biotechnology*, *5*(5), 1–22. https://doi.org/10.4172/2157-7463.1000195.

Palittapongarnpim, M., Pokethitiyook, P., Upatham, E. S., & Tangbanluekal, L. (1998). Biodegradation of crude oil by soil microorganisms in the tropic. *Biodegradation*, *9*(2), 83–90. https://doi.org/10.1023/A:1008272303740.

Passarini, M. R. Z., Sette, L. D., & Rodrigues, M. V. N. (2011). Improved extraction method to evaluate the degradation of selected PAHs by marine fungi grown in fermentative medium. *Journal of the Brazilian Chemical Society*, *22*(3), 564–570. https://doi.org/10.1590/S0103-50532011000300022

Peixoto, F. B. Sabóia, Peixoto, J. C. C., Assunção, E. N., Peixoto, E. M., Pereira, J. O., & Astolfi-Filho, S. (2017) Biodegradação do petróleo e co-resistência a antibióticos pela cepa Serratia marcescens isolada em Coari, Amazonas. Acta Scientiarum, 39(4), 489-496. https://doi.org/10.4025/actascibiolsci.v39i4.36223.

Peixoto, F. B. Sabóia., Peixoto, J. C. C., Motta, D.C.L, Peixoto, A.T.M , Pereira, J. O., & Astolfi-Filho, S. (2018). Assessment of petroleum biodegradation for *Bacillus toyonensis* by the using redox indicator 2,6 dichlorophenol indophenol. *Acta Scientiarum - BiologicalSciences*, v.40, e35640. https://doi:10.4025/actascibiolsci.v40il.35640.

Romero, M. C., Cazau, M. C., Giorgieri, S., & Arambarri, A. M. (1998). Phenanthrene degradation by microorganisms isolated from a contaminated stream. *Environmental Pollution*, *101*(3), 355–359. https://doi.org/10.1016/S0269-7491(98)00056-6.

Schippers, C., Geßner, K., Müller, T., & Scheper, T. (2000). Microbial degradation of phenanthrene by addition of a sophorolipid mixture. *Journal of Biotechnology*, *83*(3), 189–198. https://doi.org/10.1016/S0168-1656(00)00304-7.

Sood, N., Lal, B., & Asn, T. (2009). Isolation of a novel yeast strain Candida digboiensis TERI ASN6 capable of degrading petroleum hydrocarbons in acidic conditions. *Journal of Environmental Management*, *90*(5), 1728–1736. https://doi.org/10.1016/j.jenvman.2008.11.026.

Stapleton, R. D., Savage, D. C., Sayler, G. S., & Stacey, G. (1998). Biodegradation of aromatic hydrocarbons in an extremely acidic environment. *Applied and Environmental Microbiology*, *64*(11), 4180–4184. https://doi.org/10.1128/AEM.64.11.4180-4184.1998

Ting, W. T. E., Yuan, S. Y., Wu, S. D., & Chang, B. V. (2011). Biodegradation of phenanthrene and pyrene by Ganoderma lucidum. *International Biodeterioration and Biodegradation*, *65*(1), 238–242. https://doi.org/10.1016/j.ibiod.2010.11.007.

Vasconcelos, Ulrich, F. P. de. Françal, Fernando J. S. Oliveira (2011). Removal of high-molecular weight polycyclic aromatic hydrocarbons. *Quimica Nova*, *34*(2), 218–221. https://doi.org/10.1590/S0100-40422011000200009

White, T. J., Bruns, S., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H. and Sninsky, J.J., Eds., PCR Protocols: A Guide to Methods and Applications, Academic Press Inc., New York, 315-322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1.

Wongsa, P., Tanaka, M., Ueno, A., Hasanuzzaman, M., Yumoto, I., & Okuyama, H. (2004). Isolation and characterization of novel strains of *Pseudomonas aeruginosa* and *Serratia marcescens* possessing high efficiency to degrade gasoline, kerosene, diesel oil, and lubricating oil. *Current Microbiology*, *49*(6), 415–422. https://doi.org/10.1007/s00284-004-4347-y.

Zinjarde, S. S., & Pant, A. A. (2002). Hydrocarbon degraders from tropical marine environments, *Marine Pollution Bulletin*, *44*(2002) 118–121. https://doi.org/10.1016/S0025-326X(01)00185-0.