

Article

Biodegradation of Naphthalene and Anthracene by *Aspergillus glaucus* Strain Isolated from Antarctic Soil

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Abstract: Biotechnologies based on microbial species capable of destroying harmful pollutants are a successful way to solve some of the most important problems associated with a clean environment. The subject of investigation is the Antarctic fungal strain *Aspergillus glaucus* AL1. The culturing of the examined strain was performed with 70 mg of wet mycelium being inoculated in a Czapek Dox liquid medium containing naphthalene, anthracene, or phenanthrene (0.3 g/L) as the sole carbon source. Progressively decreasing naphthalene and anthracene concentrations were monitored in the culture medium until the 15th day of the cultivation of *A. glaucus* AL1. The degradation was determined through gas chromatography–mass spectrometry. Both decreased by 66% and 44%, respectively, for this period. The GC-MS analyses were applied to identify salicylic acid, catechol, and ketoadipic acid as intermediates in the naphthalene degradation. The intermediates identified in anthracene catabolism are 2-hydroxy-1-naphthoic acid, o-phthalic acid, and protocatechuic acid. The enzyme activities for phenol 2-monooxygenase (1.14.13.7) and catechol 1,2-dioxygenase (1.13.11.1) were established. A gene encoding an enzyme with catechol 1,2-dioxygenase activity was identified and sequenced (GeneBank Ac. No KM360483). The recent study provides original data on the potential of an ascomycete’s fungal strain *A. glaucus* strain AL 1 to degrade naphthalene and anthracene.

Keywords: biodegradation; PAH; *Aspergillus glaucus*; phenol 2-monooxygenase; catechol 1,2-dioxygenase



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1. Introduction

The availability of sufficient water applicable for drinking, domestic, and agricultural use is a growing problem of global importance. At the same time, the use of water in technological manufacturing leads to huge amounts of wastewater contaminated with various harmful substances and chemical compounds, often polluting the environment. Naturally, it must be concluded that the purification of polluted water is one of the main ways to supply people with usable and drinkable water. Applying a circular economy model regarding wastewater will synchronize the development of modern, legally, and socially supported technologies for water treatment [1,2]. This may lead to new ideas and concepts, as well as the more widespread use of these environmentally friendlier approaches to water use and reuse. More and more new approaches are emerging for the full utilization and recovery of nutrients, wastewater treatment, and removal of heavy metals, such as the design of bioelectrochemical systems (BES) to be used for electricity, fuel production, water recovery, recovery or removal of nitrates, sulfate, sulfides, antibiotics, dyes, etc. BES technologies, which use appropriate microorganisms, are a low-cost option for simultaneously achieving zero waste goals and energy recovery [3,4].

It has been known for a long time that biotechnologies based on microbial species capable of destroying and utilizing harmful pollutants are a successful and cost-effective way to solve some of the most important problems associated with a clean environment.

The microbial capacity to decompose a wide variety of xenobiotics provokes researchers' efforts to explore more opportunities to develop bioremediation technologies. Many efforts are made to achieve the effective microbial utilization of widespread chemical compounds in the environment, such as aromatic and polyaromatic substances, harming humans' and other living organisms' health [5–10].

A large amount of studies on the isolation and selection of active microorganism strains that have the ability to transform or assimilate polyaromatic hydrocarbons (PAHs) have been published by many scientists around the world [11–16].

The influence of a number of basic physico-chemical factors on the microbial elimination of PAHs has been examined. The topic of PAHs' bioavailability and bioaccessibility is being discussed [17–19]. Investigations on microbial strains belonging to all groups of microorganisms, including archaea, cyanobacteria, and algae, have been reported, but it is obvious that the search remains active [20–23]. The ascomycetes nonlignolytic fungi, which often predominate in industrially polluted areas of the environment, are still poorly studied with respect to the decomposition of harmful environmental contaminants, including PAHs [14,24]. Despite significant achievements in real practice, there are still many unsolved complex problems related to the microbial degradation of polyaromatic compounds [25–28].

Many authors attempt to colligate reported data in this field [19,24,29]. The catabolic schemes by which some bacteria carry out the complete or partial degradation of PAHs are described quite well in the available literature, but the same cannot be claimed regarding comes to fungi [30,31]. Most of the authors have focused their attention on lignolytic basidiomycetes fungi and especially "white rot fungi", which are able to transform various polyaromatic compounds with the help of some nonspecific extracellular enzymes [13,32–37]. Other authors suggest that, as a rule, fungi can only convert PAHs to intermediates that are subsequently degraded by bacteria and others [38–40]. Some consider that the degradation of PAHs from fungi is carried out in an identical manner to mammalian cells, which practically excludes the utilization and removal of their degradation products from the body [29,41,42]. All different assertions are corroborated by divers' experimental evidence. However, the results in some published articles clearly show that they do not support the above allegations. It is clear that fungal genetic and functional diversity are very high, but the number of well researched fungal species, therein, is still too narrow. For example, the degradation and utilization of some low-molecular-weight PAHs as a single source of carbon by the strains of *Aspergillus*, *Penicillium*, *Trichoderma*, and *Fuzarium* has been proven [14,43,44]. The published results on the degradation and utilization of PAHs by nonlignolytic fungi are still not enough to clarify the full set of possible degradation mechanisms of these compounds [11,24,45–47]. There is a need to identify and explore new fungal strains capable of degrading PAHs, as well as to gather more experimental data through the use of advanced biochemical and molecular methods [25,48,49].

Logically, the majority of strains active against the degradation of aromatic compounds should be isolated from habitats contaminated with relevant industrial wastes containing such substances. However, there are publications proving the existence of indigenous microorganisms inhabiting areas considered as very clean and practically free from industrial waste, such as Antarctica, that demonstrate the capability to eliminate harmful environmental pollutants [50–56].

The purpose of this study was to investigate the ability of the Antarctic *A. glaucus* AL1 fungal strain to degrade naphthalene and anthracene and to utilize them as the sole source of carbon for its growth.

2. Materials and Methods

2.1. Chemicals

Naphthalene (99.0%), anthracene ($\geq 99.0\%$), phenanthrene ($\geq 97.0\%$), NADPH, and all components of the mineral medium Czapek Dox mentioned below were purchased from

Sigma Aldrich, St. Luis, MO, USA. The monoaromatic hydrocarbons, such as phenol and catechol, as well as acetone, were purchased from Merck-Schuchardt, Hohenbrunn, Germany.

2.2. Microorganism and Culture Conditions

The soil samples were taken during the Bulgarian Antarctic expedition 2006/07 in the vicinity of the permanent Bulgarian Antarctic base “St. Kliment Ohridski” on Livingston Island, West Antarctica (62°38′29″ S, 60°21′53″ W) [57] (Figure S1). The strain *A.glaucus* AL1 grown on the beer agar medium was sub-cultured by a transfer of spores to fresh universal beer agar medium (Sigma-Aldrich, Co., St. Luis, MO, USA). Spores of the studied strain suspended in 6 mL sterile distilled water were used to inoculate the liquid Czapek Dox culture medium (2 g/L NaNO₃, 1 g/L KH₂PO₄, 0.5 g/L KCl, 0.5 g/L MgSO₄·7 H₂O, 0.01 g/L FeSO₄·7 H₂O) supplemented with 1% glucose [45]. The acidity of the medium was fixed to pH 5.5 with NaOH solution. The formed fungal pellets in the culture medium were aseptically filtered and then washed with sterile distilled water to remove residual glucose. A total of 70 mg of mycelium (wet weight) was used to inoculate 20 mL of Czapek Dox medium containing naphthalene, anthracene, or phenanthrene (0.3 g/L) as the sole carbon source in 300 mL flasks.

The flasks were agitated (320 rpm) on a rotary shaker IKA KS 130 Basic, IKA-Werke GmbH&Ko.KG, Germany in the dark at 23 °C. Un-inoculated flasks served as control at each time point.

The YEP medium (10.0 g/L yeast extract, 20.0 g/L peptone) was applied as a control for strain growth.

2.3. Biomass Measurement

The measurement of biomass amount (dry or wet) was in grams per liter of culture medium at the beginning and end of the cultivation process. Dry weight (DW) measurement was performed with an electronic moisture analyzer comprising dryer and scale, which can measure differences of up to 0.0001 ng (KERN DBS Version 1.1 03/2013, Germany). The obtained wet biomass after filtration was placed on the aluminum sample plates at 110 °C to complete dehydration.

2.4. Enzyme Analyses

The vegetative biomass obtained after the strain’s cultivation was centrifuged at 5000 rpm for 10 min at 4 °C. The define cell quantity (approximately 300–500 mg wet cells) was mechanically ground in a sterilized mortar together with quartz sand. A total of 1.5–2 mL potassium phosphate buffer (0.05 M KH₂PO₄/Na₂HPO₄, pH 7.6) was inset, and the mixture was centrifuged at 10,000 rpm for 10 min at 4 °C. The obtained cleared intracellular extracts were used for analytical purposes [43,54,58].

Phenol 2-monooxygenase (EC 1.14.13.7) activity was determined on a spectrophotometer at 340 nm by following NADPH oxidation in the presence of phenol [59]. Catechol 1,2-dioxygenase activity was calculated on the basis of the accumulated amount of cis, cis-muconic acid by the method described by Varga and Neujahr, 1970 [59].

The values of measured enzyme activities were expressed by units (U) per mg protein at 25 °C [43].

The protein concentrations in a clear cell-free lysate were defined by the method of Bradford, 1976 [60]. A sample of 50 µL was inset to 1.5 mL Bradford reagent (Sigma-Aldrich, St. Luis, MO, USA). The absorbance at 595 nm was measured 5 min after the incubation.

The presence of laccase activity was checked by the method of Kiiskinen [61].

The conventional drifts of data from all performed metering are in a diapason of 1–8%.

2.5. Extraction of PAHs and GC-MS Analysis

The PAHs were extracted from 2 mL solution aliquots 3 times by 2 mL dichloromethane (DCM) in an ultrasonic cleaner B-3001 (VWR Int., Leuven, Belgium). The combined

dichloromethane extracts were concentrated in a rotary vacuum concentrator RVC 2-25 CD plus (Christ, Osterode am Harz, Germany) before GC-MS analysis.

Appliance Hewlett Packard 7890 associated with MSD 5975 equipment (Hewlett Packard, Palo Alto, CA, USA) was used to conduct the GC-MS analysis in EI mode—70 eV. The column HP-5 MS used had parameters 30 m × 0.25 mm × 0.25 μm. Helium was used as a carrier gas at a flow rate of 1.0 mL/min [62]. The applied temperature mode included 24 s at 50 °C and was subsequently increased by 25 °C/min to 195 °C and held for 1.5 min. The next increase to 265 °C was at a rate of 8 °C/min, and then the temperature rise rate was changed to 20 °C/min to reach 315 °C, and held at this temperature for 1.25 min. The temperature of the ion source was 250 °C, and the interface temperature was 280 °C.

2.6. DNA Isolation Procedure

A single colony of the test strain of fungi was inoculated into 15 mL of YEP medium with 10 g/L glucose I addition, and was cultured 48 h on a rotary shaker at 180–200 rpm and 23 °C. The precipitate received as a result of the centrifugation (5000 rpm, 15 min) was rinsed with 5 mL 0.98% saline solution and centrifuged once again. The pellet was mashed with quartz sand for approximately 10 min. Two milliliters of 1xTE buffer (10 mM Tris/HCl, pH 8.0, 1 mM EDTA) was added to the cell lysate, and the homogenates were dispensed in 500 mL Eppendorf tubes [43]. According to Maniatis et al. 1982 [63], the samples were centrifuged (5000 rpm, 5 min) at 4 °C, and DNA was isolated from the separated supernatant. The DNA was purified via GFX columns (GE Healthcare, Little Chalfont, Buckinghamshire, England), and its quality and quantity were checked, using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA), from the correlation of the UV absorbance at 260 and 280 nm.

2.7. PCR Conditions and DNA Sequencing

For DNA amplification, a T100TM Thermal Cycler (BIO-RAD, Hercules, CA, USA) was used. PuReTaq™ Ready-To-Go™ PCR beads (Amersham Biosciences, Piscataway, NJ, USA) were inset into the reaction mixture, whose final volume was 25 μL with the 50 ng matrix DNA. The eventual concentration of applied primers was 0.4 pmol/μL (Table 1). The PCR products were analyzed electrophoretically in 1.5% agarose gel. As a buffer, TBE (10.8 g/L Tris base, 5.5 g/L boric acid, 20 mL of 0.05 M EDTA, pH 8.0) was used.

Table 1. The list of the used oligonucleotide primers.

Primers	Sequence (5'→3')	Source
for 18S rDNA Pffor Pffrev	AGGGATGTATTTATTAGATAAAAAATCAA CGCAGTAGTTAGTCTTCAGTAAATC PCR conditions: initial step, 95 °C, 5 min; 35 cycles amplification, 95 °C, 30 s; 58 °C, 30 s; 72 °C, 45 s; extension step, 72 °C, 7 min	[64]
for catechol dioxygenase AFKF1 AFKR1 AFKF2 AFKR2 AFKF3 AFKR3 AFKF4 AFKR4	GCTACTCATCGATTGACC GCGGCCTCATCAGCAAGCTT TTGTCTGTGACGTGATCGGG TGCCACACCTCCACCGTCGC TCATGCACGGCCGGGTGATC GGGTGTCGGTCCATGAGCTC GTCCGACGCCGTATCCTGTT CTACGCCCTGGTCCGCCACCA PCR conditions: initial step, 95 °C, 5 min; 35 cycles amplification, 95 °C, 30 s; 60 °C, 30 s; 72 °C, 60 s; extension step, 72 °C, 5 min	Primers for detection of catechol dioxygenase coding genes were designed in this study on the basis of <i>Aspergillus fumigatus</i> Af293 sequence NCBI Acc. No. XM_744333 [43]

The obtained PCR products were purified using the GFX™ PCR DNA and gel band purification kit (GE Healthcare). The purified PCR products were sent to Macrogen Europe, Amsterdam, Netherlands, for sequencing.

3. Results and Discussion

3.1. Degradation of PAHs

The studied strain *A. glaucus* AL1 originates from soil probes brought from Livingston Island, Antarctica [57]. Its taxonomic affiliation was determined by 18S rDNA analysis. The strain's good biodegradation capacity has been reported earlier toward phenol and other mono-phenolic compounds such as catechol, hydroquinone, o-cresol, m-cresol, and p-cresol [54,65].

There are no published articles about the *A. glaucus* strains' ability to degrade aromatic compounds. To date, there are only a few renowned investigations on the biodegradation of polyethylene by a strain of *A. glaucus* [66,67]. Initially, we checked the strain's ability to utilize naphthalene, anthracene, or phenanthrene in a mineral (CzapekDox) solid media. The strain grew well in the media, incorporating 0.3 g/L of each of the first two compounds. The strain showed a noticeably slower growth in the mineral medium including 0.3 g/L phenanthrene, as well as in a rich organic media with phenanthrene added; thus, these experiments have not been reported in this study.

Our further cultivation and degradation experiments were accomplished in a carbon-less liquid Czapek Dox medium, individually complemented with 0.3 g/L of each of the two investigated PAHs as sole carbon sources. The reductions in concentrations of naphthalene and, respectively, anthracene included in the medium were followed by GC-MS analyses. When tracing the degradation of the two compounds tested within 5 days, it can be seen that, under the same culture conditions, their different amounts are degraded (Figure 1).

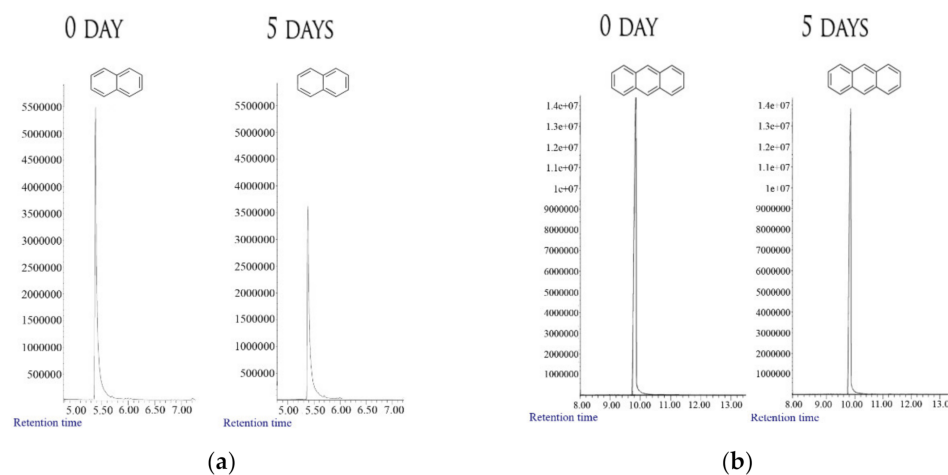


Figure 1. Gas chromatographic analysis of the reduction in the concentration during *Aspergillus glaucus* AL1 culturing: (a) naphthalene—in the beginning (0.3 g/L) and after 5 days (0.2 g/L); (b) anthracene—in the beginning (0.32 g/L) and after 5 days (0.24 g/L).

Naphthalene degraded faster than anthracene. For 5 days, naphthalene's content in the media reduced by 34%, whereas anthracene's content reduced by 25%. Following the concentration decrease in the two studied PAHs up to the 15th day of cultivation confirmed that the strain's degradation effectiveness varied with both compounds. The concentration of naphthalene decreased by over 66%, whereas that of anthracene lowered by approximately 44% for this period (Figure 2 and Table 2).

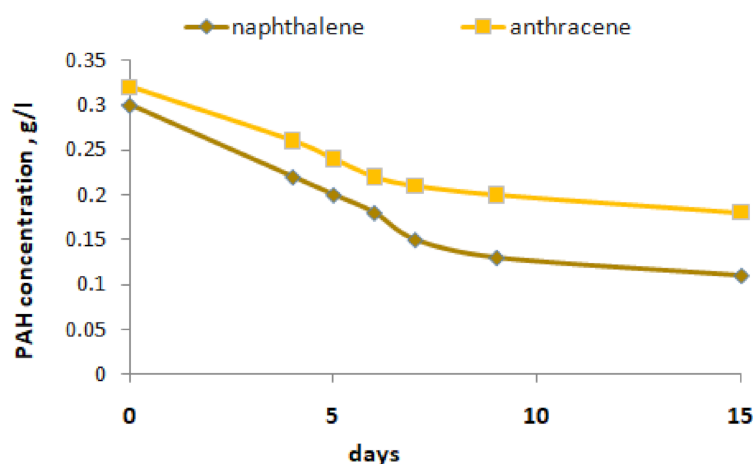


Figure 2. Degradation of naphthalene and anthracene in the Czapek Dox mineral medium by the *Aspergillus glaucus* strain AL1 for 15 days at 23 °C.

Table 2. Mass balance after growth of *Aspergillus glaucus* AL1 on single LMW PAH.

C-Substrate	Initial Biomass, DW (g/L)	Final Biomass, DW (g/L)	Growth %	C ₀ * (g/L)	C ₅ ** (g/L)	Degradation %	C ₁₅ *** (g/L)	Degradation %
Naphthalene C ₁₀ H ₈	0.012	0.05	76	0.3	0.2	33.05	0.11	66.6
Anthracene C ₁₄ H ₁₀	0.011	0.03	64	0.32	0.24	18.75	0.18	43.75

* C₀—initial concentration of C-substrate, ** C₅—a concentration of C-substrate on the 5th day of cultivation, *** C₁₅—a concentration of C-substrate on the 15th day of cultivation.

The spectral peaks on the 5th day show the intermediates' presence. They were not found in the next phases of the degradation process, probably due to the rapid further reduction in their concentrations. The chromatographic specters of identified intermediates, mentioned below, demonstrated some patterns of the naphthalene and anthracene degradation paths of *A. glaucus* AL1.

In the chromatogram demonstrating the naphthalene degradation on the 5th day of the strain's growth, compounds were identified, such as salicylic acid, catechol, and ketoadipic acid (Figure 3). Mass spectral profiles of naphthalene intermediate metabolites are demonstrated in Figure 4. The presence of these compounds correlates well with the second half of the scheme for the catabolic pathway of naphthalene in aerobic bacteria that was proposed earlier by some authors [29,68,69].

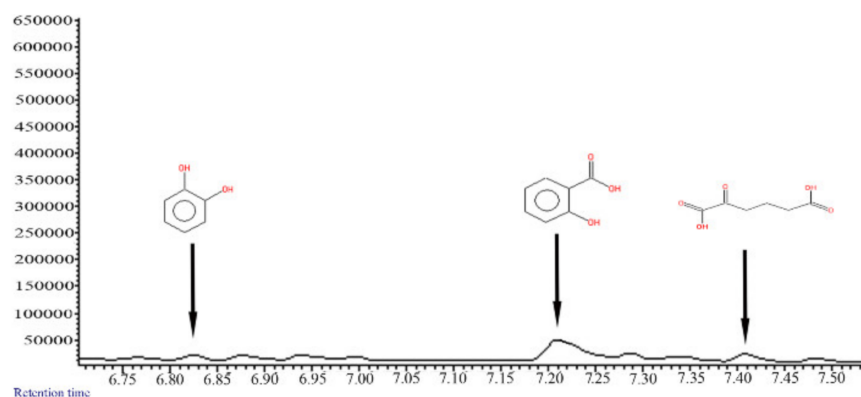


Figure 3. Gas chromatographic analysis of naphthalene metabolites—catechol, salicylic acid, and ketoadipic acid.

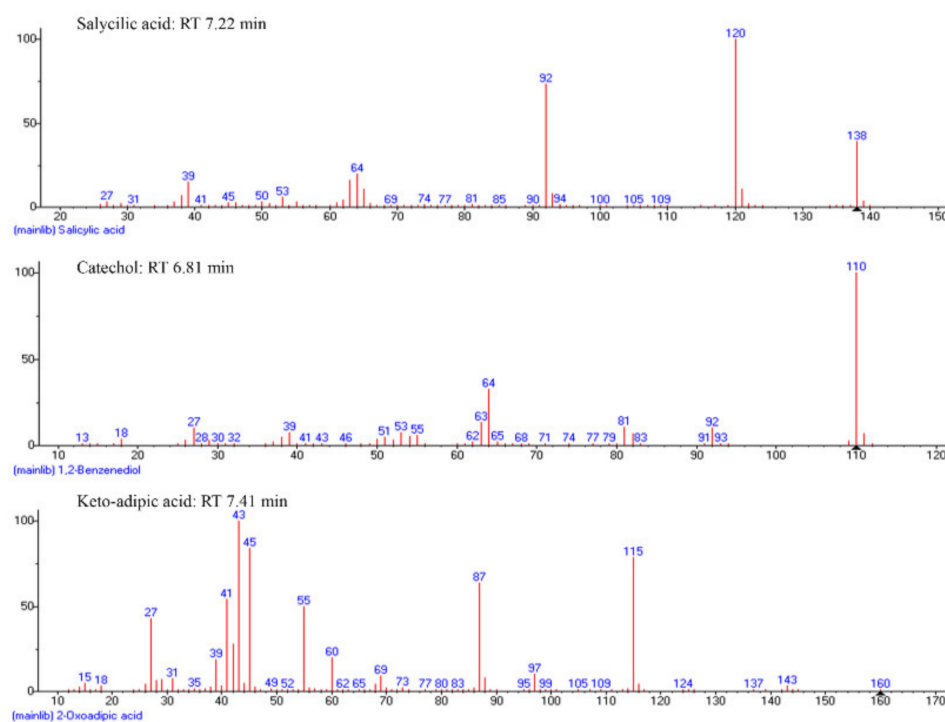


Figure 4. Mass spectral profiles of naphthalene intermediate metabolites.

2-hydroxy-1-naphthoic acid, o-phtalic acid, and protocatechuic acid could be identified in the chromatogram, showing the aroused intermediates during the anthracene catabolism (Figure 5). Mass spectral profiles of anthracene intermediate metabolites are demonstrated in Figure 6. The presence of o-phtalic and protocatechuic acids emphasizes the similarity with a part of the already proposed anthracene catabolic pathway in aerobic bacteria [29].

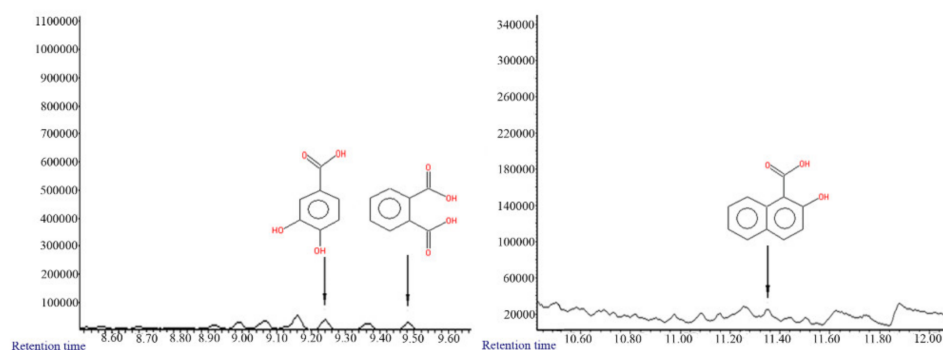


Figure 5. Gas chromatographic analysis of anthracene metabolites—protocatechuic acid, o-phtalic acid, and 2-hydroxy-1-naphthoic acid.

In general, the results obtained by experiments on the biodegradation of naphthalene and anthracene, and subsequent chromatographic analyses in the present study, suggest the presence of some quite similar mechanisms of degradation of PAHs in bacteria and some nonlignolytic fungi, regardless of the different genomic organization and regulation.

The appearance of 2-hydroxy-1-naphthoic acid is somewhat surprising. The availability of this compound was illustrated particularly in the bacterial decomposition of phenanthrene, but has not yet been found in the process of the microbial degradation of anthracene [14,42,70,71].

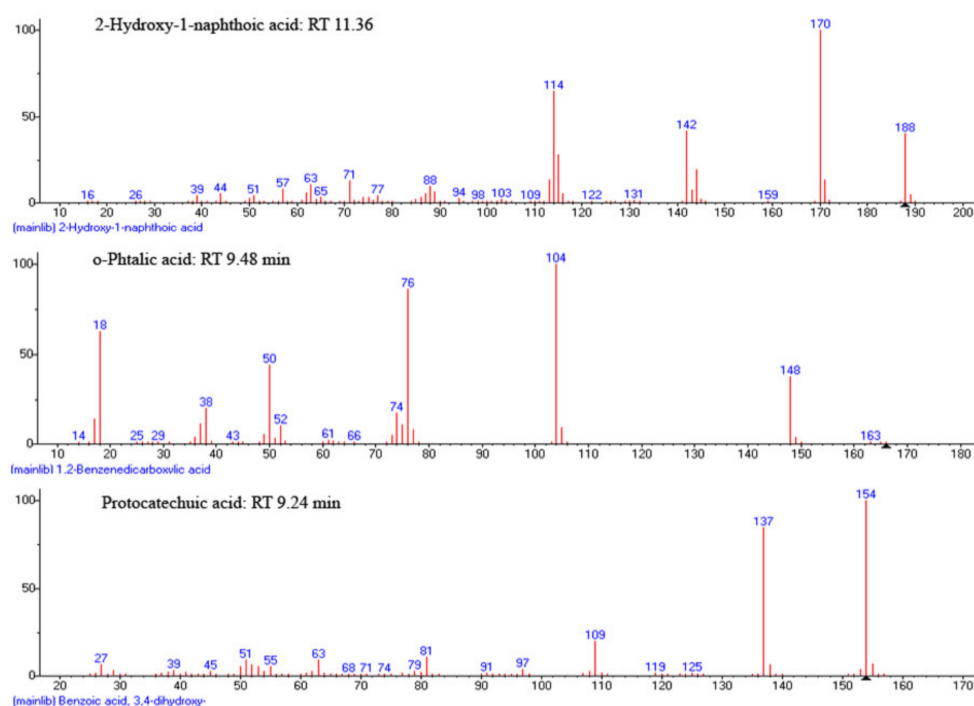


Figure 6. Mass spectral profiles of anthracene intermediate metabolites.

3.2. Enzyme Analyses

Most authors exploring the PAHs' microbial degradation affirm that oxygenases play an essential role in these metabolic pathways. A series of catabolic chains are described in aerobic bacteria and lignolytic fungi, but there is still insufficient information about the enzymes that are substantial for the PAHs catabolism in different groups of nonlignolytic fungi [31,42,47,71].

In our prior investigations on the substrate specificity of phenol 2-monooxygenase in the strain *A. glaucus* AL1, with respect to mono-phenolic compounds, this enzyme activity was examined, and the gene encoding this protein was sequenced [54]. The corresponding oligonucleotide sequence was registered in NCBI—Acc. No KM360482.1. Catechol 1,2-dioxygenase activity was also determined during the degradation of mono-phenols by *A. glaucus* AL1. The highest value was recorded in the process of phenol degradation—2.11 U/mg of protein [54].

In the present study, the activities of both key enzymes for aromatic catabolism were tested in the absence of other carbon sources for growth, except naphthalene or anthracene. The absence of laccase activity proves that the examined strain does not express such extracellular enzyme activity. This verifies that the degradation of the two studied PAH compounds was due to the action of the intracellular enzyme systems.

The rates obtained for the activity of phenol 2-monooxygenase and catechol 1,2-dioxygenase are very comparable when the *A. glaucus* AL1 is cultured in a medium incorporating each of the two compounds individually. For example, in the process of the degradation of naphthalene or anthracene on the fifth day of culture, the activity of phenol 2-monooxygenase is 1.48 U/mg protein or, respectively, 1.12 U/mg protein. The enzyme activity of catechol 1,2-dioxygenase is 0.24 U/mg protein when assimilating naphthalene and 0.16 U/mg protein in the experiments with anthracene (Figure 7). Notwithstanding the detected distinguishable extent of the degradation of the investigated PAHs, contrasting activities of both enzymes under investigation have not been found.

The interesting question that arose as a result of these analyses was the role of the enzyme phenol 2-monooxygenase in the process of the degradation of naphthalene or anthracene when they were used as the sole carbon substrates. Articles focusing on the identity of the cytochrome P450 isoenzyme component(s) with benzene and phenol hydroxylase in rabbit hepatic microsomes have been published [72–74].

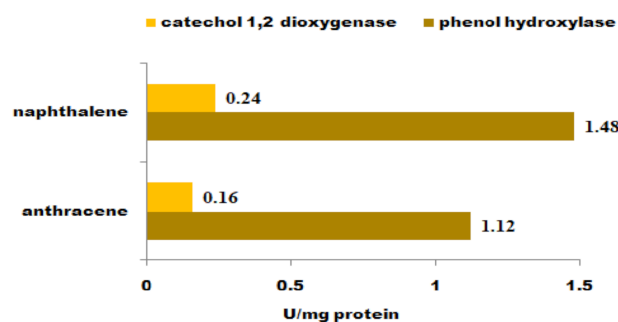


Figure 7. Enzyme activities of phenol 2-monoxygenase and catechol 1,2-dioxygenase in cells of *Aspergillus glaucus* AL1, cultivated in a Czapek Dox mineral medium containing 0.3 g/L naphthalene or 0.3 g/L anthracene as a sole carbon source.

Based on these studies and the fact that P450 oxygenases play a major role in the first phase of PAH degradation in fungi, it can be considered reasonable to establish a relatively high phenol 2-monoxygenase activity in the described experiments [12]. At the same time, we could assume that phenol 2-monoxygenase can be induced directly by the two polyaromatic substances or by other metabolites obtained in the course of their transformation.

The presence of catechol dioxygenase activity has confirmed the fact that catechol is a central metabolite in the degradation pathway of aromatic compounds [75–77]. The ability of dioxygenase enzymes to cleave 1,2-carbon bonds suggests that catechol 1,2-dioxygenase may be involved not only in the final phase of catechol degradation but also in the formation and transformation of other previously occurring intermediate metabolites such as, for example, 2-hydroxy-1-naphthoic acid [30].

The variety of microorganisms, including fungi, is enormous, as well as the variety of metabolic schemes, which permits them to adapt and manage to survive in exclusively extreme circumstances from a human point of view [78]. The diversity of genes encoding proteins with enzymatic activity involved in the PAH decomposition increases by the mechanisms of so-called horizontal transfer, protoplast and cellular fusion, and other mechanisms of genetic transfer [41,69].

3.3. Sequence Analyses of Putative Catechol 1,2-dioxygenase Gene

To identify and sequence the gene encoding protein with catechol 1,2-dioxygenase activity in the *A. glaucus* AL1 strain, four pairs of oligonucleotide primers were created (Table 1). The oligonucleotide primer pairs were arranged using the sequence of gene coding for catechol dioxygenase in *A. fumigatus* Af293 (NCBI Acc. No. XM744333.1). The sequence of the putative gene was formed by sequencing and combining the obtained overlapping PCR fragments. Upon application of the first pair of primers, no acceptable fragment was obtained. The catechol 1,2-dioxygenase gene's partial sequence consisted of 843 bp. The 644 bp coding region organized in four exons was identified. The found sequence also contained three introns with lengths of 75 bp, 71 bp, and 53 bp, respectively. Both sequences were registered in the NCBI Gene Bank under accession numbers as follows: KM360483.1 (for nucleotide) and AIY27718.1 (for protein).

The 644 bp oligonucleotide fragment of the putative catechol 1,2-dioxygenase gene of *A. glaucus* AL1 was compared by BLAST analysis with the analogous sequences in the NCBI gene bank. An over 99% similarity was found regarding the sequences coding for catechol 1,2-dioxygenase in *A. fumigatus* Af293, *A. fumigatus* AL3 (KT781126.1), and *A. fumigatus* AL15 (KT371935.1). The similarity of this sequence with *Neosartorya fischeri* NRRL 181 dioxygenase, putative (NFIA_035270) partial mRNA was 96% (XM001265855).

The corresponding protein sequence (213 amino acids) showed a 99% similarity between both the reference protein sequence, i.e., the catechol 1,2-dioxygenase of *A. fumigatus* Af293, and the respective protein sequence of *A. glaucus* AL1. The same percent of similarity was observed in comparison to catechol 1,2-dioxygenase protein sequences of

A. fumigatus var. RP-2014 (KEY75569.1), *A. fumigatus* AL15 (AMA07788.1), and *A. fumigatus* Z5 (KMK57661.1). The similarity of the investigated partial protein sequence with a putative dioxygenase of *N. fischeri* NRRL 181 (XP001265856.1), and catechol 1,2-dioxygenase of *A. lentulus* (GAQ07244.1) was 98% and, with catechol 1,2-dioxygenase of *N. udagawa* (GAO90728.1), was 96%. As a result, a cladogram was created (Figure 8) that reflects the phylogenetic proximity of the resulting sequence, with proteins expressing a similar function in fungi belonging to the genus *Aspergillus* [79].



Figure 8. Phylogenetic tree of homologous catechol 1,2-dioxygenase amino acid sequences in fungi (NCBI). The analyses were performed by the maximum likelihood method using the MEGA 11 software.

4. Conclusions

The recent study provides original data about the potential of an ascomycete's fungal strain *A. glaucus* strain AL 1, isolated from Antarctic soil, to degrade naphthalene and anthracene. These species are a representative of the nonlignolytic fungi. *A. glaucus* AL1 has been found to possess a significant capacity to remove naphthalene and anthracene from media as a single carbon source using its intracellular enzyme capacity.

The enzymes phenol 2-monoxygenase and catechol 1,2-dioxygenase of the investigated strain exhibited different expressions associated with the type of PAHs consumed as a carbon substrate. A comparative analysis of the DNA sequence found in the current study and the available ones in the NCBI database revealed a significant similarity with a few other earlier reported DNA sequences for catechol 1,2-dioxygenase genes found in *Aspergillus* strains. As a result, we could assume that closely similar genes coding for catechol 1,2-dioxygenase considerably spread in that genus. There have not been many other fungal nucleotide or amino acid sequences found that possess a high similarity with those obtained in the present study.

The carried out analyses of the Antarctic's strain *A. glaucus* AL1 give us a solid reason to value its potential and good outlook in enhancing the biotechnological processes related to environment remediation. We claim that the current study extends knowledge of the degradation of PAH by fungi from the Ascomycota division and provides information that can be useful in developing and improving advanced bioremediation technologies aimed at purifying PAH-contaminated water and soil. Such elaborations will contribute both to a cleaner living environment and to an increased access to purified and safe water through cyclical use and reuse.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/pr10050873/s1>, Figure S1: The permanent Bulgarian Antarctic base "St. Kliment Ohridski" on Livingston Island, West Antarctica (62°38'29" S, 60°21'53" W).

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References

1. Voulvoulis, N. Water Reuse from a Circular Economy Perspective and Potential Risks from an Unregulated Approach. *Cur. Opin. Environ. Sci. Health* **2018**, *2*, 32–45. [[CrossRef](#)]
2. Morris, J.; Georgiou, I.; Guenther, E.; Caucci, S. Barriers in Implementation of Wastewater Reuse: Identifying the Way Forward in Closing the Loop. *Circ. Econ. Sust.* **2021**, *1*, 413–433. [[CrossRef](#)]
3. Bratkova, S.; Alexieva, Z.; Angelov Nikolova, A.K.; Genova, P.; Ivanov, R.; Gerginova, M.; Peneva, N.; Beschkov, V. Efficiency of microbial fuel cells based on the sulfate reduction by lactate and glucose. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 6145–6156. [[CrossRef](#)]
4. Apollon, W.; Rusyn, I.; González-Gamboa, N.; Kuleshova, T.; Luna-Maldonado, A.I.; Vidales-Contreras, J.A.; Kamaraj, S.-K. Improvement of zero waste sustainable recovery using microbial energy generation systems: A comprehensive review. *Sci. Total Environ.* **2022**, *817*, 153055. [[CrossRef](#)]
5. Zhang, X.; Cheng, S.; Zhu, C.; Sun, S. Microbial PAH-degradation in soil: Degradation pathways and contributing factors. *Pedosphere* **2006**, *16*, 555–565. [[CrossRef](#)]
6. Alexieva, Z.; Yemendzhiev, H.; Zlateva, P. Cresols utilization by *Trametes versicolor* and substrate interactions in the mixture with phenol. *Biodegradation* **2010**, *21*, 625–635. [[CrossRef](#)]
7. Hamdi, H.; Benzarti, S.; Aoyama, I.; Jedidi, N. Rehabilitation of degraded soils containing aged PAHs based on phytoremediation with alfalfa (*Medicago sativa* L.). *Int. Biodeterior. Biodegrad.* **2012**, *67*, 40–47. [[CrossRef](#)]
8. Sun, G.-D.; Jin, J.-H.; Xu, Y.; Zhong, Z.-P.; Liu, Y.; Liu, Z.-P. Isolation of a high molecular weight polycyclic aromatic hydrocarbon degrading strain and its enhancing the removal of HMW-PAHs from heavily contaminated soil. *Int. Biodeterior. Biodegrad.* **2014**, *90*, 23–28. [[CrossRef](#)]
9. Liu, Y.-Q.; Maulidiany, N.; Zeng, P.; Heo, S. Decolourization of azo, anthraquinone and triphenylmethane dyes using aerobic granules: Acclimatization and long-term stability. *Chemosphere* **2021**, *263*, 128312. [[CrossRef](#)]
10. Malhotra, M.; Gupta, D.; Sahani, J.; Singh, S. Microbial Degradation of Phenol and Phenolic Compounds. In *Recent Advances in Microbial Degradation*; Inamuddin, Ahamed, M.I., Prasad, R., Eds.; Environmental and Microbial Biotechnology; Springer: Singapore, 2021. [[CrossRef](#)]
11. Potin, O.; Rafin, C.; Veignie, E. Bioremediation of an aged polycyclic aromatic hydrocarbon (PAHs)-contaminated soil by filamentous fungi isolated from the soil. *Int. Biodeterior. Biodegrad.* **2004**, *54*, 45–52. [[CrossRef](#)]
12. Haritash, A.; Kaushik, C. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. *J. Hazard. Mater.* **2009**, *169*, 1–15. [[CrossRef](#)] [[PubMed](#)]
13. Cerniglia, C.; Sutherland, J. Degradation of polycyclic aromatic hydrocarbons by fungi. In *Handbook of Hydrocarbon and Lipid Microbiology*; Timmis, K.N., Ed.; Springer: Berlin/Heidelberg, Germany, 2010; pp. 2079–2110.
14. Ye, J.-S.; Yin, H.; Qiang, J.; Peng, H.; Qin, H.-M. Biodegradation of anthracene by *Aspergillus fumigatus*. *J. Hazard. Mater.* **2011**, *185*, 174–181. [[CrossRef](#)] [[PubMed](#)]
15. Lee, H.; Jang, Y.; Choi, Y.; Kim, M.; Lee, J.; Lee, H.; Hong, J.; Lee, Y.M.; Kim, G.; Kim, J. Biotechnological procedures to select white rot fungi for the degradation of PAHs. *J. Microbiol. Methods* **2014**, *97*, 56–62. [[CrossRef](#)] [[PubMed](#)]
16. Marchand, C.; St-Arnaud, M.; Hogland, W.; Bell, T. Petroleum hydrocarbon capacity of bacteria and fungi isolated from petroleum-contaminated soil. *Int. Biodeterior. Biodegrad.* **2017**, *116*, 48–57. [[CrossRef](#)]
17. Leonardi, V.; Šašek, V.; Petruccioli, M.; D’Annibale, A.; Erbanova, P.; Cajthaml, T. Bioavailability modification and fungal biodegradation of PAHs in aged industrial soils. *Int. Biodeterior. Biodegrad.* **2007**, *60*, 165–170. [[CrossRef](#)]
18. Szczepaniak, Z.; Cyplik, P.; Juzwa, W.; Czarny, J.; Staninska, J.; Piotrowska-Cyplik, A. Antibacterial effect of the *Trichoderma viride* fungi on soil microbiome during PAH’s biodegradation. *Int. Biodeterior. Biodegrad.* **2015**, *104*, 170–177. [[CrossRef](#)]
19. Lawal, A.T. Polycyclic aromatic hydrocarbons. A review. *Cogent Environ. Sci.* **2017**, *3*, 1339841. [[CrossRef](#)]
20. García-Díaz, C.; Ponce-Noyola, M.; Esparza-García, F.; Rivera-Orduña, F.; Barrera-Cortés, J. PAH removal of high molecular weight by characterized bacterial strains from different organic sources. *Int. Biodeterior. Biodegrad.* **2013**, *85*, 311–322. [[CrossRef](#)]

21. Thion, C.; Cébron, A.; Beguiristain, T.; Leyval, C. Inoculation of PAH-degrading strains of *Fusarium solani* and *Arthrobacter oxydans* in rhizospheric sand and soil microcosms: Microbial interactions and PAH dissipation. *Biodegradation* **2013**, *24*, 569–581. [[CrossRef](#)]
22. Ferradji, F.; Mnif, S.; Badis, A.; Rebbani, S.; Fodil, D.; Eddouaouda, K.; Sayadi, S. Naphthalene and crude oil degradation by biosurfactant producing *Streptomyces* spp. isolated from Mitidja plain soil (North of Algeria). *Int. Biodeterior. Biodegrad.* **2014**, *86*, 300–308. [[CrossRef](#)]
23. Pandey, P.; Pathak, H.; Saurabh, D. Microbial ecology of hydrocarbon degradation in the soil: A Review. *Res. J. Environ. Toxicol.* **2016**, *10*, 1–15. [[CrossRef](#)]
24. Aranda, E. Promising approaches towards biotransformation of polycyclic aromatic hydrocarbons with *Ascomycota* fungi. *Curr. Opin. Biotechnol.* **2016**, *38*, 1–8. [[CrossRef](#)] [[PubMed](#)]
25. Isaac, P.; Lozada, M.; Dionisi, H.; Estevez, M.; Ferrero, M. Differential expression of the catabolic *nahAc* gene and its effect on PAH degradation in *Pseudomonas* strains isolated from contaminated Patagonian coasts. *Int. Biodeterior. Biodegrad.* **2015**, *105*, 1–6. [[CrossRef](#)]
26. Kuppasamy, S.; Thavamani, P.; Venkatesvarlu, K.; Lee, Y.; Naidu, R.; Megharaj, M. Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. *Chemosphere* **2017**, *168*, 944–968. [[CrossRef](#)] [[PubMed](#)]
27. Sharma, M.; Nandy, A.; Taylor, N.; Venkatesan, S.; Ozhukil, K.; Vinayaraj, O.; Karan, K.; Thangadurai, V.; Tsesmetzis, N.; Gieg, L. Bioelectrochemical remediation of phenanthrene in a microbial fuel cell using an anaerobic consortium enriched from a hydrocarbon-contaminated site. *J. Hazard. Mater.* **2019**, *389*, 121845. [[CrossRef](#)]
28. Mauger, S.; Monard, C.; Thion, C.; Vandenkoornhuysse, P. Contribution of single-cell omics to microbial ecology. *Trends Ecol. Evol.* **2022**, *37*, 67–78. [[CrossRef](#)]
29. Peng, R.-H.; Xiong, A.-S.; Xue, Y.; Fu, X.-Y.; Gao, F.; Zhao, W.; Tian, Y.-S.; Yao, Q.-H. Microbial biodegradation of polyaromatic hydrocarbons. *FEMS Microbiol. Rev.* **2008**, *32*, 927–955. [[CrossRef](#)]
30. Seo, J.; Keum, Y.; Hu, Y.; Lee, S.; Li, Q. Degradation of phenanthrene by *Burkholderia* sp. C3: Initial 1,2- and 3,4-dioxygenation and meta- and ortho-cleavage of naphthalene-1,2-diol. *Biodegradation* **2007**, *18*, 123–131. [[CrossRef](#)]
31. Brzeszcz, J.; Kaszycki, P. Aerobic bacteria degrading both n-alkanes and aromatic hydrocarbons: An undervalued strategy for metabolic diversity and flexibility. *Biodegradation* **2018**, *29*, 359. [[CrossRef](#)]
32. Hammel, K.; Green, B.; Gai, W. Ring fission of anthracene by eucaryote. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 10605–10608. [[CrossRef](#)]
33. Wang, C.; Sun, H.; Li, J.; Li, Y.; Zhang, Q. Enzyme activities during degradation of polycyclic aromatic hydrocarbons by white rot fungus *Phanerochaete chrysosporium* in soils. *Chemosphere* **2009**, *77*, 733–738. [[CrossRef](#)] [[PubMed](#)]
34. Harms, H.; Schlosser, D.; Wich, L. Untapped potential: Exploiting fungi in bioremediation of hazardous chemicals. *Nat. Rev. Microbiol.* **2011**, *9*, 177–192. [[CrossRef](#)] [[PubMed](#)]
35. Nakamura, T.; Ichinose, H.; Wariishi, H. Flavin-containing monooxygenases from *Phanerochaete chrysosporium* responsible for fungal metabolism of phenolic compounds. *Biodegradation* **2012**, *23*, 343–350. [[CrossRef](#)] [[PubMed](#)]
36. Adenipekun, C.; Ipeaiyeda, A.; Olayonwa, A.; Egbewale, S. Biodegradation of polycyclic aromatic hydrocarbons (PAHs), in spent and fresh cutting fluids contaminated soils by *Pleurotus pulmonarius* (Fries). Quelet and *Pleurotus* *treatus* (Jacq.) Fr. P. Kumm. *Afr. J. Biotechnol.* **2015**, *48*, 661–667. [[CrossRef](#)]
37. Kadri, T.; Rouissi, T.; Brar, S.; Cledon, M.; Sarma, S.; Verma, M. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review. *J. Environ. Sci.* **2017**, *51*, 52–74. [[CrossRef](#)]
38. Cerniglia, C. Fungal metabolism of polycyclic aromatic hydrocarbons: Past, present and future applications in bioremediation. *J. Ind. Microbiol. Biotechnol.* **1997**, *19*, 324–333. [[CrossRef](#)]
39. Schmidt, S.; Christensen, J.; Johnsen, A. Fungal PAH-metabolites resist mineralization by soil microorganisms. *Environ. Sci. Technol.* **2010**, *44*, 1677–1682. [[CrossRef](#)]
40. Zang, S.; Lian, B.; Wang, J.; Yang, Y. Biodegradation of 2-naphtol and its metabolites by coupling *Aspergillus niger* with *Bacillus subtilis*. *J. Environ. Sci.* **2010**, *22*, 669–674. [[CrossRef](#)]
41. Tortella, G.; Diez, M. Fungal diversity and use in decomposition of environmental pollutants. *Crit. Rev. Microbiol.* **2005**, *31*, 197–212. [[CrossRef](#)]
42. Ghosal, D.; Ghosh Sh Dutta, T.; Ahn, Y. Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. *Front. Microbiol.* **2016**, *7*, 1837. [[CrossRef](#)]
43. Gerginova, M.; Manasiev, J.; Yemendzhiev, H.; Terziyska, A.; Peneva, N.; Alexieva, Z. Biodegradation of phenol by Antarctic strains of *Aspergillus fumigatus*. *Z. Nat.* **2013**, *68C*, 384–393. [[CrossRef](#)]
44. Mineki, S.; Suzuki, K.; Iwata, K.; Nakajima, D.; Goto, S. Degradation of polyaromatic hydrocarbons by fungi isolated from soil in Japan. *Polycycl. Aromat. Compd.* **2015**, *35*, 120–128. [[CrossRef](#)]
45. Mohammed, D.; Khudeir, S.; Al-Jubouri, M. The optimum conditions for naphthalene biodegradation by filamentous fungi. *Iraqi J. Sci.* **2014**, *55*, 1780–1791.
46. Reyes-Cesar, A.; Absalon, A.; Fernandez, F.; Gonzalez, J.; Cortes-Espinosa, D. Biodegradation of a mixture of PAHs by non-ligninolytic fungal strains isolated from crude oil-contaminated soil. *World J. Microb. Biot.* **2014**, *30*, 999–1009. [[CrossRef](#)] [[PubMed](#)]

47. Marco-Urrea, E.; García-Romera, I.; Aranda, E. Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. *N. Biotechnol.* **2015**, *32*, 620–628. [[CrossRef](#)] [[PubMed](#)]
48. Martínez-Rosales, C.; Fullana, N.; Musto, H.; Castro-Sowinsk, S. Antarctic DNA moving forward: Genomic plasticity and biotechnological potential. *FEMS Microbiol. Lett.* **2012**, *331*, 1–9. [[CrossRef](#)]
49. Amrani, A.; Dumas, A.-S.; Wick, L.; Yergeau, E.; Berthomé, R. “Omics” Insights into PAH degradation toward improved green remediation biotechnologies. *Environ. Sci. Technol.* **2015**, *49*, 11281–11291. [[CrossRef](#)]
50. Giudice, A.; Bruni, V.; De Domenico, M.; Michaud, L. Psychrophiles-cold-adapted hydrocarbon degrading microorganisms. In *Handbook of Hydrocarbon and Lipid Microbiology*; Timmis, K.N., Ed.; Springer: Berlin/Heidelberg, Germany, 2010; pp. 1897–1921. [[CrossRef](#)]
51. Okere, U.; Cabrerizo, A.; Dachs, J.; Jones, K.; Kirk, T. Simple Biodegradation of phenanthrene by indigenous microorganisms in soils from Livingstone Island, Antarctica. *FEMS Microbiol. Lett.* **2012**, *329*, 69–77. [[CrossRef](#)]
52. Cury, J.; Jurelevicius, D.; Villela, H.D.M.; Jesus, H.; Peixoto, R.; Schaefer, C.E.G.R.; Bicedo, M.; Seldin, L.; Rosado, A. Microbial diversity and hydrocarbon depletion in low and high diesel-polluted soil samples from Keller Peninsula, South Shetland Islands. *Antarct. Sci.* **2015**, *27*, 263–273. [[CrossRef](#)]
53. Litova, K.; Manasiev, J.; Gerginova, M.; Peneva, N.; Alexieva, Z. Sequence analysis of gene coding key enzymes responsible for degradation of phenolic compounds by *Aspergillus fumigatus* strain AL3. *J. BioSci. Biotechnol.* **2015**, *SE*, 195–203.
54. Gerginova, M.; Litova, K.; Manasiev, J.; Peneva, N.; Bibova, R.; Krastanov, A.; Alexieva, Z. Studies on biodegradation of phenolic compounds by *Aspergillus glaucus* strain. In *Microbes in the Spotlight: Recent Progress in the Understanding of Beneficial and Harmful Microorganisms*; Méndez-Vilas, A., Ed.; BrownWalker Press: Boca Raton, FL, USA, 2016; pp. 135–139.
55. Elster, J.; Margesin, R.; Wagner, D.; Häggblom, M. Polar and Alpine Microbiology—Earth’s cryobiosphere. *FEMS Microbiol. Ecol.* **2017**, *93*, fiw221. [[CrossRef](#)] [[PubMed](#)]
56. Ogaki, M.B. Antarctic Marine Fungi and Their Potential Application in Bioremediation. In *Marine Microbial Bioremediation*; Vala, A., Dudhagara, D., Dave, B., Eds.; CRC Press: Boca Raton, FL, USA, 2021. [[CrossRef](#)]
57. Tossi, S.; Kostadinova, N.; Krumova, E.; Pashova, S.; Dishliiska, V.; Spassova, B.; Vassilev, S.; Angelova, M. Antioxidant enzyme activity of filamentous fungi isolated from Livingston Island, Maritime Antarctica. *Polar Biol.* **2010**, *33*, 1227–1237. [[CrossRef](#)]
58. Neujahr, H.; Gaal, A. Phenol Hydroxylase from Yeast. Purification and properties of the enzyme from yeast *Trichosporon cutaneum*. *Eur. J. Biochem.* **1973**, *35*, 386–400. [[CrossRef](#)] [[PubMed](#)]
59. Varga, J.; Neujahr, H. Purification and Properties of Catechol 1,2-Oxygenase from *Trichosporon cutaneum*. *Eur. J. Biochem.* **1970**, *12*, 427–434. [[CrossRef](#)] [[PubMed](#)]
60. Bradford, M. A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [[CrossRef](#)]
61. Kiiskinen, L.-L.; Ratto, M.; Kruus, K. Screening for novel laccase-producing microbes. *J. Appl. Microbiol.* **2004**, *97*, 640–646. [[CrossRef](#)]
62. Dincheva, I.; Badjakov, I.; Kondakova, V.; Batchvarova, R. Metabolic profiling of red raspberry (*Robus idaeus*) during fruit development and ripening. *Int. J. Agric. Sci. Res.* **2013**, *3*, 81–88.
63. Maniatis, T.; Fritsch, E.; Sambrook, J. *Molecular Cloning: A Laboratory Manual*; Sambrook, J., Ed.; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, USA, 1982.
64. Jaeger, E.; Carroll, N.; Choudhury, S.; Dunlop, A.; Towler, H.; Matheson, M.; Adamson, P.; Okhravi, N. Rapid Detection and Identification of *Candida*, *Aspergillus*, and *Fusarium* Species in Ocular Samples Using Nested PCR. *J. Clin. Microbiol.* **2000**, *38*, 2902–2908. [[CrossRef](#)]
65. Alexieva, Z.; Yemendzhiev, H.; Tossi, S.; Krumova, E.; Angelova, M.; Terziyska, A.; Peneva, N.; Gerginova, M. Growth of fungal strains isolated from Livingston Island on phenolic compounds—Biodegradation potential. In *Microbes in Applied Research: Current Advances and Challenges*; Mendez-Vilas, A., Ed.; World Scientific Publishing Co.: Singapore, 2012; pp. 131–134. [[CrossRef](#)]
66. Kathiresan, K. Polythene and plastic-degrading microbes in an Indian mangrove soil. *Rev. Biol. Trop.* **2003**, *51*, 629–633.
67. Prabhat, S.; Bhattacharyya, S.; Vishal, V.; Kalyan, R.; Vijai, K.; Pandey, K.; Singh, M. Studies on isolation and identification of active microorganisms during degradation of polyethylene/starch film. *Int. Res. J. Environ. Sci.* **2013**, *2*, 83–85.
68. Habe, H.; Omori, T. Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 225–243. [[CrossRef](#)] [[PubMed](#)]
69. Mallick, S.; Chatterjee, S.; Dutta, T. A novel degradation pathway in the assimilation of phenanthrene by *Staphylococcus* sp. strain PN/Y via meta-cleavage of 2-hydroxy-1-naphthoic acid: Formation of trans-2,3-dioxo-5-(29-hydroxyphenyl)-pent-4-enoic acid. *Microbiology* **2007**, *153*, 2104–2115. [[CrossRef](#)] [[PubMed](#)]
70. Mallick, S.; Chakraborty, J.; Dutta, T. Role of oxygenases in guiding diverse metabolic pathways in the bacterial degradation of low-molecular-weight polycyclic aromatic hydrocarbons: A review. *Crit. Rev. Microbiol.* **2011**, *37*, 64–97. [[CrossRef](#)] [[PubMed](#)]
71. Kunihiro, M.; Ozeki, Y.; Nogy, Y.; Hamamura, N.; Kanaly, R. Benz[a]anthracene biotransformation and production of ring fission products by *Sphingobium* sp. strain KK22. *Appl. Environ. Microbiol.* **2013**, *79*, 4410–4420. [[CrossRef](#)]
72. Coon, M.; Persson, A.; French, J. On the possible relationship of cytochrome P-450 to alcohol metabolism: Fundamental aspects of the microsomal hydroxylation system, including properties and interactions of the components. *Adv. Exp. Med. Biol.* **1980**, *132*, 11–22. [[CrossRef](#)]

73. Koop, D. Hydroxylation of p-nitrophenol by rabbit ethanol-inducible cytochrome P-450 isozyme 3a. *Mol. Pharmacol.* **1986**, *29*, 399–404.
74. Koop, D.; Laethem, C.; Schnie, G. Identification of ethanol-inducible P450 isozyme 3a (P450IIE1) as a benzene and phenol hydroxylase. *Toxicol. Appl. Pharmacol.* **1989**, *98*, 278–288. [[CrossRef](#)]
75. Evans, W. The microbiological degradation of aromatic compounds. *J. Gen. Microbiol.* **1963**, *32*, 177–184. [[CrossRef](#)]
76. Guo, C.; Dang, Z.; Wong, Y.; Tam, N. Biodegradation ability and dioxygenase genes of PAH-degrading *Sphingomonas* and *Mycobacterium* strains isolated from mangrove sediments. *Int. Biodeterior. Biodegrad.* **2010**, *64*, 419–426. [[CrossRef](#)]
77. Fuchs, G.; Boll, M.; Heider, J. Microbial degradation of aromatic compounds—From one strategy to four. *Nat. Rev. Microbiol.* **2011**, *9*, 803–816.
78. Pugazhendi, A.; Qari, H.; Al-Badry Basahi, J.; Godon, J.; Dhavamani, J. Role of a halothermophilic bacterial consortium for the biodegradation of PAHs and the treatment of petroleum wastewater at extreme conditions. *Int. Biodeterior. Biodegrad.* **2017**, *121*, 44–54. [[CrossRef](#)]
79. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [[CrossRef](#)]