17th International Conference on Environmental Science and Technology

Athens, Greece, 1 to 4 September 2021

Biodegradation of anthracene by an Antarctic fungal strain a member of genus Alternaria

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Abstract By the aim to characterize the ability of an isolated from Antarctic soils fungal strain termed *Alternaria maritima* AL10 to degrade anthracene as sole carbon sources at different temperature conditions (23°C and 10°C), research has performed. The results from GC-MS analysis have shown that the strain *A. maritima* AL10 degraded and utilized anthracene only in mesophilic conditions.

Phenol hydroxylase activity measured in cleared cells' lysate of strain *A. maritima* AL10 was significantly high - 1,123 U/mg P.

Keywords: Alternaria maritima, biodegradation, anthracene, GC-MS

1. Introduction

In order to innovate and improve biodegradation processes, various microorganisms degrading phenol are intensively studied. Of particular interest are microbial strains isolated from areas with extreme climatic conditions, such as Antarctica.

Literature data published on the aromatic compounds' degradation carried out by the representatives of *Alternaria* genus are relatively scarce compared to those of *Aspergillus* and *Penicillium*. Publications have been made on the ability of certain molds belonging to this genus to degrade contaminants of an aromatic or aliphatic nature, as well as those isolated from petroleum substrates. However, in most studies, the *Alternaria* strains studied have been rarely identified to the species. (Jacob and Alsohaili, 2010; Nambudiri et al., 1970; Obire and Anyanwu, 2009; Loretta et al., 2017; Petrujova and Zanora, 1959).

One of the first reactions in lowering the environmental temperature is inhibition of the microbial growth rate. The extended (lag) adaptation phase is observed before the restoration of growth (Yamanaka, 1999). There are publications in which authors affirm that the growth at low temperatures causes cessation and even biomass decrease (Gocheva *et al*. 2006; Gocheva *et al*. 2009).

The objective of the proposed study is to determine the ability of isolated from Antarctic soils strain *A. maritima* AL10 to grow and degrade anthracene at different temperature regimes.

2. Materials and methods

2.1. Microorganisms, Culture media and Cultivation

Alternaria maritima AL10, an Antarctic mold strain isolated from soil samples collected on Livingston Island, Antarctica, has the subject of this study (Kostadinova *et al*., 2009).

The used mineral culture media without carbon source (Czapek Dox broth-CzD) was supplemented with 1% glucose or anthracene (pH=5.5), depended on the purposes of the experiment, and autoclaved for 20` at 110°C. The batch cultivation of the strain was carried out at 23°C, the aeration was performed on a laboratory shaker at 400 rpm in Erlenmeyer flasks, with appropriate volumes of nutrient media.

The cultivation was carried out to a mid-exponential growth phase with the aim to be obtained a vegetative mycelium for inoculation or enzyme activity measuring. Mycelium from the stationary growth phase was used for determing the strain's capacity to degrade a particular compound.

The quantity of the obtained wet or dry biomass of the studied strains was measured at the start and at the end of cultivation on an electronic moisture analyzer KERN DBS Version 1.1 03/2013 (Germany).

2.2. Preparation of molds cell extracts

The harvested cells were mechanically smashed in the presence of potassium phosphate buffer, pH 7.5, and centrifuged (1000 rpm for 10 min at 4°C) to obtain clarified cell extract. The received "clear" supernatant was used for the enzyme activity determination.

2.3. Method for determination of phenol hydroxylase (EC 1.14.13.7) enzyme activity

The phenol hydroxylase activity assay was conducted spectrophotometrically (λ = 340nm) (Neujahr and Gaal, 1973). The specific phenol hydroxylase activity was defined as Enzyme Units per 1mg protein (U/mg Protein).The total protein concentration was established according Bradford method (Bradford, M.M, 1976).

2.4. Anthracene extraction and GCMS analysis

The anthracene was 3 times extracted from the supernatant with dichloromethane (DCM) in an ultrasonic purification apparatus B-3001 (VWR Int., Leuven, Belgium). The extract was mixed with dichloromethane and then concentrated in a rotary vacuum concentrator RVC 2-25 CD plus (Christ, Osterode am Harz, Germany). The GC-MS - analysis was performed on a Hewlett Packard 7890 apparatus connected to MSD 5975 equipment (Hewlett Packard, Palo Alto, CA, USA), operating in EI mode at 70 eV. A MS column HP-5 (30 m × 0.25 mm × 0.25 μm) was used. 1 μL of the probe was introduced by degrees. As a carrier gas was used Helium at a flow rate of 1.0 ml/ min. The analysis was carried out under the following temperature program: 0.4 min at 50° C, a drop to 25° C/min, a rise in temperature to 195° C for 1.5 min, and a second drop to 8° C/min and a subsequent increase to 265° C for 0 min. Finally, a third decrease to 20° C/min and a rise in temperature to 315 ° C was applied for 1.25 minutes. Mass spectra were recorded at 2 scans per second with a scan range m/z 50-550. The ion source and interface temperatures were adjusted to 250° C and 280° C, respectively.

2.5. Processing of results

All results obtained by the analytical methods are presented as arithmetic means of three experiments.

Results and Discussion

The use of mold strains for degradation is still a relatively intact area. There is even less scientific information about Antarctic eukaryotic microorganisms and their industrial potential, although their presence there has long been established. Most Antarctic fungi are cosmopolitan. Some of them have been transferred to Antarctica, but despite the survival of their spores, they are unable to grow in Antarctic conditions. Others, called indogens, are well adapted and able to grow and reproduce even at low temperatures.

A previous study by the laboratory team reported the ability of *A. maritima* AL10 to grow in rich and poor anthracene-containing media as the sole carbon source or in combination with glucose [Gerginova *et al*., 2013].

Gas chromatographic - mass spectral analysis of the supernatant after the strain *A. maritima* AL10 cultivation with 0.3 g/l anthracene as a sole carbon and energy source was performed to determine the residual compound's concentration. The strain was cultured at both 23° C and 10° C. The strain degradation activity toward anthracene was found under mesophilic growth conditions (23 ° C) only.

It could be seen that the area of the peak shown in Fig. 1a has significantly larger than demonstrated one on Figs. 1b. The corresponding measured anthracene concentrations were 0.3065 g/l at baseline and 0.2283 g / l on the sixth day of the experiment.

A strain of *A. maritama* AL10 was able to degrade 0.3 g/l anthracene over a period of 20 days of cultivation. The degradation curve was characterized by two distinct phases - rapid assimilation of the substrate in the first 10 days and delay of degradation after the 10^{th} day of cultivation. That indicated the strong toxic effect of anthracene on the cells of this strain. Nevertheless, the substrate was completely eliminated, which has confirmed the strong degradation activity of *A. maritima* AL10 against that polyaromatic compound.

Data from the growth analysis of the strain *A. maritima* AL10 revealed that it was unable to utilize anthracene as a carbon source at low temperatures.

Phenol hydroxylase activity measured in the intracellular cleared extracts of strain *A. maritima* AL10 was significantly high - 1,123 U / mg P.

A number of authors have reported that fungi have not able to degrade PAHs as carbon source without the presence of appropriate cometabolites [Sutherland, 1992; Keck et al. 1989; Leitão, 2009]. In the present study, complete degradation of 0.3 g/l anthracene has achieved by *A. maritima* AL10, as the sole source of carbon and energy. Meléndez-Estrada reported a high rate of degradation of 0.2 g/l

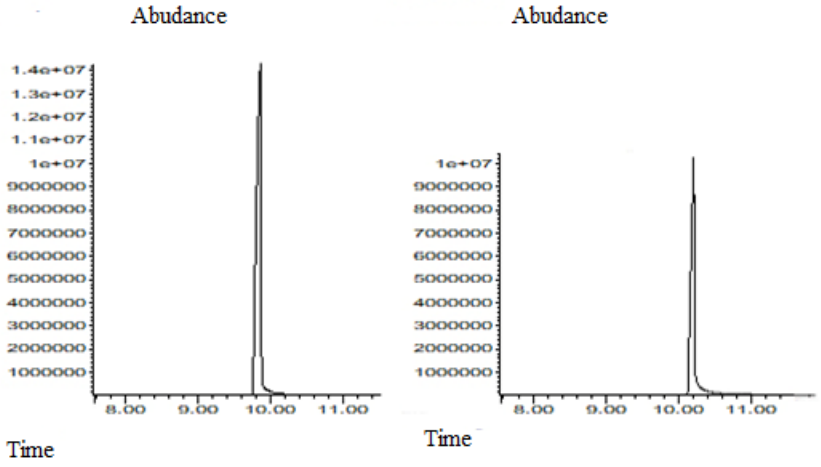

Figure 1. Gas chromatographic-mass spectrometric analysis of anthracene concentration measured at the starting point (A) and after 6 days (B) cultivation of *A. maritima* AL10 in Czapeck Dox mineral medium, at 23 ° C.

phenanthrene by *Penicillium frequentans*, which in 17 days of cultivation under optimal conditions managed to eliminate 52% of the substrate [Meléndez-Estrada al., 2006].

As a result of the analysis, an interesting question arises about the role of phenol hydroxylase in the process of degradation of PAH. Studies have been published on P450 isozymes, substantially identical to benzene, and phenol hydroxylase in rabbit hepatitis microsomes [Coon et al., 1980; Buy 1986; Koop et al., 1989]. Based on those studies and the fact that cytochrome P450 oxygenases play important roles in the fungal degradation of polyaromatics it can be assumed that phenol hydroxylase is induced either by the tested substrate or by one of the metabolites that might arise in the process of its transformation [Haritash and Kaushik, 2009].

The microbial diversity, including that of fungi, is enormous, hence the great diversity in the mechanisms by which these organisms adapt and manage to exist in extremely extreme human living conditions [Pugazhendi et al., 2017].

Our report on the biodegradation of anthracene by *A. maritima* AL10, as the only carbon source in the culture medium, is the first published data in the scientific literature on this genus of fungi.

Based on the obtained results, it can be concluded that the representative of the genus *Alternaria* studied by us shows high activity in terms of mineralization and utilization of anthracene.

Acknowledgments This work was supported by the Project BG05M2OP001-1.002-0019: „Clean technologies for sustainable environment – water, waste, energy for circular economy“ (Clean&Circle), for development of a Centre of Competence, is financed by the Operational programme “Science and Education for Smart Growth”2014-2020, co-funded by the European union through the European structural and investment funds.

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