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Growth of Antarctic fungal strains on phenol at low temperatures

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ABSTRACT

The investigation dedicated to the ability of examined 16 fungal strains isolated from Antarctic soil probes to grow and develop at low temperatures (5°C and 10°C) were carried out. Most of the strains were able to grow at 10°C. Six of the studied strains do not grow at low temperatures. The strains *Penicillium commune* AL 2, *Aspergillus fumigatus* AL 3, *Penicillium commune* AL 5, *Penicillium rugolosum* AL 7, *Lecanicillium* sp. AL 12 and *Aspergillus fumigatus* AL 15 showed a good growth on a rich culture medium at 5°C and could be classified as psichrophyles. The signs of their growth were observed at about the 8th day of cultivation. Five of the strains (*P. commune* AL 2, *A. fumigatus* AL 3, *P. coprobioum* AL 4, *P. rugolosum* AL 5, and *Alternaria maritima* AL 10) were able to grow at 10°C in the presence of 0.3 g/l phenol as well. It was established that *P. commune* AL 2, *A. fumigatus* AL 3 and *P. rugolosum* AL 7 were able to utilize 0.3 g/l phenol as a sole carbon source at 5°C.

Key words: fungi, psichrophyles, phenol

Introduction

Recently, the research on the living organisms of Antarctica cause a growing interest and accumulate more and more data on the mechanisms allowing for the existence of the coldest place on Earth. (Russell et al., 2006). The largest number is the isolated and identified microorganisms, members of Archaea, Bacteria and Eucarya (Friedmann, 1993; Vincent, 2000; Pointing et al., 2009; Teixeira et al., 2010). Many authors report on the valuable biotechnological potential of some of them (Cavicchioli et al., 2002; Gunde-Cimerman et al., 2003; Mojib et al., 2010; Martínez-Rosales et al., 2012).

Livingston Island is located in the North Sea, part of the South Shetland archipelago, located in the western part of Antarctica. Except for some isolated areas, the ground is covered with ice cap, heavily cracked in some areas due to the marked mountain relief of the island. Since 1988 Bulgaria has its own territory - a permanent basis, named "St. Kliment Ohridski". It is located on Hurd Peninsula. This is one of the windiest areas on the planet, the so-called "roaring latitudes." Livingston Island is one of the least studied in terms of mycelia (Stchigel et al., 2001; Romeike et al., 2002).

Phenol and its various derivatives, as well as many other aromatic compounds, are known as some of the most hazardous pollutants (Smith et al., 1988; Sikkema et al., 1995). Many phenol-contaminated environments are characterized by low temperatures (Margesin et al., 2005). It is assumed that cold-adapted hydrocarbon degraders are adapted to grow and thrive under these adverse conditions (Margesin & Schinner, 2001). Laboratory studies have confirmed that hydrocarbon-degrading bacteria typically assigned to the genera *Rhodococcus*, *Sphingomonas* or *Pseudomonas* are present in contaminated polar soils (Aislabie et al., 2006).

Some representatives of fungi in Antarctic areas are mesophilic species present as spores. They are unable to be reproduced except in rare cases when the occurrence of favorable conditions appears. Others are cosmopolitan species showing mesophilic-psihrotolerant behavior adaptation (Zucconi, 1996). Few of them are truly psychrophilic species (including some strains of *Thelebolus*

RESEARCH ARTICLE

microsporus Kimbr. and *Mucor flavus* Bainier and kriptoendolitic strains of black mushrooms), but most are psihrotolerant (Onofri et al., 2007).

Little information is available on the influence of some environmental factors on the growth and metabolic activities of fungi (Ibrahim et al., 2011). Therefore, the aims of this study were to investigate the effect of low temperatures on the growth and phenol degradation ability of a number of fungal strains isolated from Antarctic's soils. This may contribute in developing some environmental biotechnologies related to aromatic pollutants removal in the conditions of unfavorable temperatures.

Materials and Methods

Microbial cultivation

Fungal strains micellium cultures were stored on beer agar medium, pH 6.3 in glass vials with screw caps at 4°C. The strains were grown in a solid or liquid mineral medium Czapek Dox (in %: $NaNO_3$ - 0.2, KH_2PO_4 - 0.1, KCI - 0.05, $MgSO_4.7H_2O$ - 0.05, $FeSO_4.7H_2O$ - 0.001), containing 1 g/l glucose and 0.3 g/l phenol. The YEP - media (Yeast extract – 1% and Peptone – 2%) was used as well through the all experiments. Thereto were also added glucose and phenol in the same concentrations. The used media were sterilized by autoclaving at 110°C for 20 min. The temperature of cultivation was 5°C or 10°C.

Determination of phenol concentration

The phenol concentration was determined by a residual colorimetric method with the basic reagent 3,4-dimethyl amino antipyrine (Hristov, 1997).

Results

Growth ability of studied fungal strains to grow and develop at low temperatures

Previously, all isolates were identified in parallel by classical taxonomy based on morphology and genomic level identification. The comparative analysis of both type of results showed that the obtained fungal isolates were members mostly to the genera *Penicillium*, *Aspergillus* and *Cladosporium* (Kostadinova et al., 2009, Tosi et al., 2010).

Sixteen fungal strains isolated from Livingston Island – Antarctica's soil probes were studied for their ability to grow and develop at low temperatures (5°C and 10°C). Only six of the studied strains (*Aspergillus glaucus* AL 1, *Cladosporium herbarum* AL 6, *Aspergillus fumigatus* AL 8, *Aspergillus fumigatus* AL9, *Penicillium* sp. AL 11 and *Cladosporium cladosporoides* AL16) could not grow at examined low temperatures. All the experiments were carried out in parallel on a salt medium named Chapek Dox and complete medium YEP, both containing glucose as a readily utilized carbon source.

The strains *Penicillium commune* AL 2, *Aspergillus fumigatus* AL 3, *Penicillium commune* AL 5, *Penicillium rugolosum* AL 7, *Lecanicillium* sp. AL 12 and *Aspergillus fumigatus* AL 15 showed a good growth on a rich culture medium at 5°C and could be classified as psichrophyles. Despite the observed growth of both media, it should be noted there are certain differences, mainly in the intensity of the growth and pigmentation of the biomass (Figure 1). Logically, the growth is more considerable at a rich culture medium.

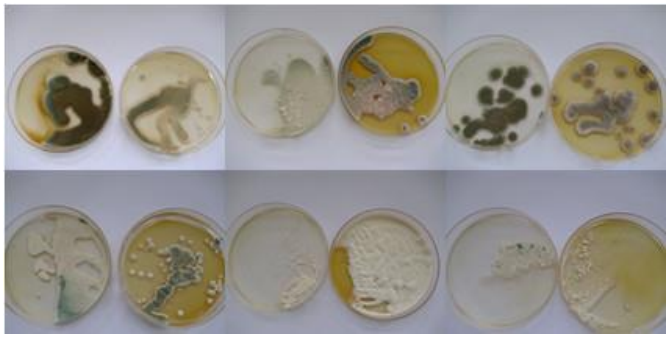

Figure 1. *Growth of strains Penicillium commune AL 2, Aspergillus fumigatus AL 3, Penicillium commune AL 5, Penicillium rugolosum AL 7, Lecanicillium sp. AL 12 and Aspergillus fumigatus AL 15 at 5°C on Chapek Dox mineral medium (left) and YEP complete medium (right), both containing 1% glucose.*

The results from cultivation experiments carried out showed that most of the strains were able to grow at 10°C. In Figure 2 have been demonstrated the data about the needed days for starting the growth of the different strains on both media used for cultivation at both experimental temperatures.

The provided comparative analysis supports the expected conclusion that extremely low temperatures as well as poor nutritional medium contribute to slower growth of the studied cultures, but not when it relates to psychrophiles.

RESEARCH ARTICLE

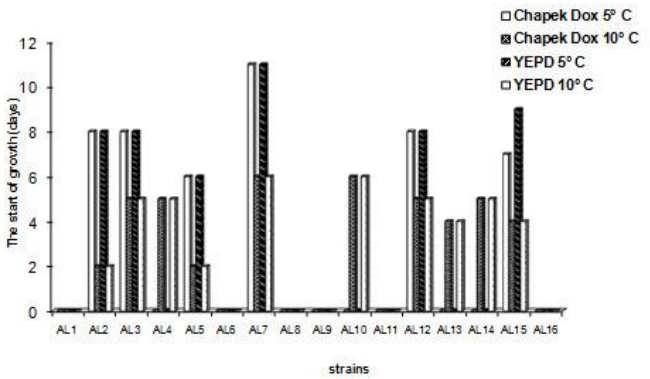

Figure 2. *Comparison of fungal strains' growth at 5°C and 10°C on a complete (YEPD) and salt (Chapek Dox) media comprising 1% glucose.*

Tolerance to the presence of phenol

Six strains were selected on the basis of their good ability to grow at low temperatures on complete and synthetic media. The experiments demonstrating the ability of the selected strains to grow at 10°C on a media comprising 1% glucose and 0.3 g/l phenol were carried out. The first set of these experiments was performed on a rich (YEP) medium. The second set was implemented on a minimal salt medium (Chapek Dox). The obtained results are shown on a Figure 3.

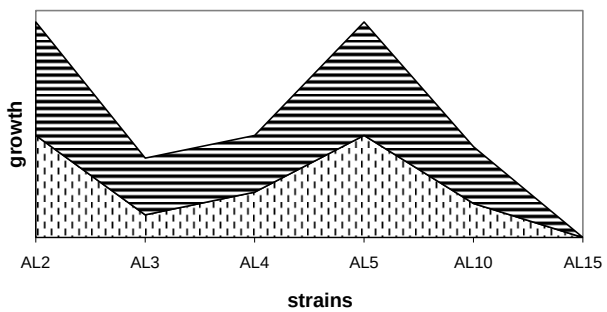

Figure 3. *Tolerance to the presence of 0.3 g/l phenol in the media: YEP – (—); Chapek Dox – (ı), by the fungal strains cultivated at 10°C.*

It may be noted that the presence of phenol in the culture medium has an impact on the selected strains. In the presence of phenol is best developed strains AL2 and AL 5, this and the two test environments. Practically there was no growth noted after the cultivation of strain AL15. The other observation that could be marked was the little slowest growth of the cultures on the mineral medium Chapek Dox. It was established also that sporulation occurs at three of the strains (AL 2, AL 3 and AL 10), when they are grown on a salt medium. These studies can be a good prerequisite for prediction of metabolic properties of the strains.

Fungal strains ability to utilize phenol as a sole carbon source

The last set of experiments was performed to determine whether the tested strains were able to utilize phenol as a sole carbon source. For this purpose we used again the salt's medium Chapek Dox without glucose but with 0.3 g/l phenol, included in it. The studies were conducted with selected strains at 5°C, as well as at 10°C.

We established the ability of the strains *Penicillium commune* AL 2, *Aspergillus fumigatus* AL 3, *Penicillium coprobioum* AL 4, *Penicillium commune* AL 5 and *Alternaria maritima* AL 10 to utilize phenol at 10°C. The cultures grow approximately equally good in both media applied despite of the different number of days needed for the start of growth. For the tested period of 11 days of growth sporulation was observed only with a strain AL 3. Experiments carried out at 5°C have expressed as the most interesting strains AL 2 and AL 3, which showed their unique ability to utilize phenol at such extremely low temperatures. An interesting observation was made, which showed that both strains grew faster during their cultivation on synthetic medium Chapec Dox than on the rich of peptone and yeast extract medium YEP.

Discussion

It is believed that the Antarctic environment is best preserved and clean place on the planet from all sorts of contaminants. There are interesting theories about the spread of various microorganisms and their exceptional survival in harsh conditions (Arenz & Blanchette, 2009; Bridge & Spooner, 2012). Nevertheless, it is believed that the old wooden ships reaching this area could cause local fuel contamination as well as some shipwreck could have left mostly wooden debris, and this in turn created a broth for microorganisms with specific features, such as degradation of aromatic compounds (Hughes et al., 2007). On the other hand, the spores of microorganisms, such as molds, which are extremely resistant to unfavorable living conditions, could be

RESEARCH ARTICLE

transmitted by air and water currents in a variety of directions. In addition, an ornithogenic contribution to soil chemistry must be considered. While our study could not possibly contribute an answer to these questions, it has enriched our knowledge of microbial biodiversity in Antarctica. Conducted research on the growth of fungal strains isolated at low temperatures from Livingston Island, Antarctica indicated that some of it could be determined as real psichrophils. Similar studies on the development and the ability of cold adapted fungal strains to degrade hydrocarbons show the same trend (Bej et al., 2009; Giudice et al., 2010).

The results obtained might create the basis for future developments concerning purification of polluted natural habitats characterized by low temperatures.

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