POLISH POLAR RESEARCH
vol. 38, no. 3, pp. 393–408, 2017 doi: 10.1515/popore-2017-0015

Comparison of the oxidative stress response of two Antarctic fungi to different growth temperatures

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Abstract: Two fungal strains, isolated from Livingston Island, Antarctica (*Penicillium commune* 161, psychrotolerant and *Aspergillus glaucus* 363, mesophilic) were investigated for a relationship between growth temperature and oxidative stress response. Cultivation at temperatures below – (10 and 15°C and 10 and 20°C for *P. commune* and *A. glaucus*, respectively) and above (25°C and 30°C for *P. commune* and *A. glaucus*, respectively) the optimum caused significant difference in growth and glucose uptake in comparison with the control cultures. Enhanced level of reserve carbohydrates (glycogen and trehalose) was determined under cultivation at different temperatures from the optimal one. While the highest content of trehalose was found in the exponential phase, glycogen accumulation was observed in the stationary phase when growth conditions deteriorate. The growth at temperature below– and above–optimum caused strain–dependent changes in two antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). While SOD activity in the psychrotolerant strain increases with decreasing of growth temperature, the mesophilic *A. glaucus* demonstrated marked reduction of it at below– and above–optimal temperature. Decreasing trend of CAT activity was observed in both strains below the optimal temperature indicating a lack of antioxidant protection from this enzyme under the cold stress conditions.

Key words: Antarctic, Livingston Island, fungi, physiological cell response, trehalose, glycogen, antioxidant enzymes.

Introduction

The climate of Antarctic is the coldest and driest known on Earth. The incredibly harsh environment characterized by low temperature, low water availability, frequent freeze–thaw cycles, strong winds, ultraviolet radiation etc., precludes most of the life forms. As a result, the biology of Antarctica, more

than in other continents, is dominated by micro–organisms, with a high level of adaptation and able to withstand severe environmental conditions (Ruisi *et al.* 2007; Onofri *et al.* 2015). The Antarctic micro–organisms capable of growing at low temperatures are referred as psychrophiles and psychrotrophes (also called psychrotolerants) (Russell, 2006; Margesin *et al.* 2009). Psychrophiles grow at or below 0°C and have an optimum growth temperature at 15°C and an upper limit of ≤20°C. In contrast, psychrotolerants, which can also grow close to zero, have optima and upper limits above these temperatures and may well grow at mesophilic temperatures with optima above 30°C; hence they could be considered as being cold–tolerant mesophiles (Russell 2006; Gonçalves *et al.* 2013).

Microscopic fungi are an important part of the polar ecosystem because they mineralize nutrients from soil organic matter and participate in symbiotic associations such as mycorrhiza and lichens (Newsham *et al.* 2009; Park *et al.* 2015; Zhang *et al.* 2015). Moreover, the ecological impact of pathogenic fungi has been reported (Gonçalves *et al.* 2012; Wang *et al.* 2015; Matsumoto and Hsiang 2016). Despite the low number of habitats available for microbial life in the Antarctic continent, a significant number of different fungal species, including new taxa have been isolated and described (see Selbman *et al.* 2014). The major portion of them (99.4%) belongs to phyla of *Chytridiomycota*, *Zygomycota*, *Ascomycota* and *Basidiomycota* (Onofri *et al.* 2005). The new sequencing technologies helped to elucidate the fungal diversity in Antarctica through identifying uncultivable isolates. The molecular methods confirmed domination of *Ascomycota* and *Basidiomycota*, especially in Dry Valley soils (Arenz *et al.* 2006; Cantrell *et al.* 2011; Dreesens *et al.* 2014; Cox *et al.* 2016). The analysis of environmental DNA and RNA (cDNA) for inland Dry Valleys soil showed that approximately half of fungal phylotypes recovered from the RNA–derived library did not affiliate phylogenetically with any known fungus (Rao *et al.* 2012).

During the last years many investigations have been carried out about the floristic, ecophysiological, molecular and, most recently, phylogenetic aspects of Antarctic fungi (Onofri *et al.* 2008). A big deal of results has focussed mainly on the adaptations of protein (enzyme) activity, protein synthesis and membrane lipids (Feller *et al.* 2006; Fenice *et al.* 2012). On the contrary, little is known about their physiological mechanisms responsible for growth and survival at low temperature. Cold-stress response includes synthesis of melanin–like pigments, formation of highly melanized or sterile hyphae, meristematic and flexible morphology (Ruisi *et al.* 2007; Nonzom and Sumbali 2014). Furthermore, there have been reports about changes in fatty acid composition, accumulation of cryoprotective carbohydrates, synthesis of exopolysaccharides, dehydratation ability etc. (Ruisi *et al.* 2007; Silvi *et al.* 2013).

On the other hand, the relationship between low temperature and oxidative stress events as well as the participation of antioxidant defence in cold–stress response has been widely studied in bacteria and plants, but little attention has

been paid to fungi. Cold environment induces enhanced generation of reactive oxygen species (ROS) in all aerobic cells, such as superoxide anion radical ($^{\cdot}O_2^{-}$), hydrogen peroxide (H_2O_2), singlet O_2, and the highly reactive hydroxyl radicals ($^{\cdot}OH$) (see Sharma *et al*. 2012). These strong oxidants are highly reactive towards all biomolecules (DNA, protein, and lipids), and severely harmful for cell survival. As a result, oxidative stress occurs due to the imbalance between oxidants and antioxidants, in favor of the oxidants. To scavenge ROS and prevent damage, all aerobic cells have evolved a complex defence system consisting of both enzymatic and non–enzymatic antioxidants. The main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidases (GPX). SOD are a group of metalloenzymes that catalyse the dismutation of ($^{\cdot}O_2^{-}$) to hydrogen peroxide and O_2. The H_2O_2 resulted from this reaction is subsequently converted to H_2O and O_2 by CAT or GPX (Fridovich 1998; Nimse and Pal 2015). Furthermore, comparison between survival strategies of different thermal classes of micro–organisms (psychrophiles, psychrotolerant and mesophiles) isolated from permanently cold habitats could provide much insight into the adaptations to life in the cold. One hypothesis suggests that responses of Antarctic fungi to different stresses appear similar to those found even in temperate regions (Ruisi *et al*. 2007).

Previously Gocheva *et al*. (2006; 2009) showed the relationship between low temperatures and oxidative stress events in fungal strains isolated from soil samples of different regions of Antarctica: Casey Station, Terra Nova Bay and South Georgia. Also, Kostadinova *et al*. (2009) have isolated fungal strains from permanent Bulgarian Antarctic base "St. Kl. Ohridski" on Livingston Island in the South Shetland Islands (Maritime Antarctica). The short–term treatment with cold temperature induced transient oxidative stress in two Antarctic fungi of different thermal classes, the psychrotolerant *Penicillium commune* 161 and mesophilic *Aspergillus glaucus* 363 (Kostadinova *et al*. 2012). In this study, both strains were tested for the effect of growth temperature on a range of physiological parameters, including growth, glucose consumption and accumulation of reserve carbohydrates. The report demonstrates also the role of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). The experiments were designed to obtain information whether there exists any difference in cell response of the psychrotolerant and the mesophilic strain.

Materials and methods

Fungal strains, culture media and cultivation. — The fungal strains, *P. commune* 161 and *A. glaucus* 363, were isolated from soil samples collected in Livingston Island in the South Shetland Islands (Maritime Antarctica) during the Bulgarian Antarctic expedition 2006/07 and were included in the experiments.

They were isolated at temperature 4 and 25°C for *P. commune* and *A. glaucus*, respectively. The strains belong to the Mycological Collection of the Stephan Angeloff Institute of Microbiology, Sofia, and they are maintained at 4°C on Beer agar, pH 6.3.

For the purpose of temperature characteristic, both Antarctic strains were cultivated individually in plates of 9 cm of diameter with the Beer agar medium (Fassatiová 1986). Triplicates of each plate were incubated in the dark at 0, 4, 10, 12, 18, 20, 25, 30 and 35°C for 3 weeks. The diameter of the colony was measured every 3 days.

The composition of the seed and production media was as described previously (Angelova *et al*. 1995). Cultivation was performed in 3 L bioreactors, ABR–09, equipped with pH and automatic dissolved oxygen (DO) monitoring equipment and a control system. For the inoculum, 80 ml of seed medium was inoculated with 10^7 spores in 500 ml Erlenmeyer flasks. The cultivation was performed on a shaker (220 rpm) for 24 h at 20°C and 25°C for *P. commune* 161 and *A. glaucus* 363, respectively. For bioreactor cultures, 200 ml of the seed culture was brought into the 3 L bioreactor, containing 1800 ml of the productive medium. The cultures were grown for 120 h with a stirred speed of 400 rpm air flow, 1 v.v.m. at 10, 15, 20 and 25°C for *P. commune* 161 and 10, 20, 25 and 30°C for *A. glaucus* 363. In all the experiments the control is represented by culture at the optimal temperature.

Cell–free extract preparation and antioxidant enzyme activity determination. — The cell–free extract was prepared as described earlier (Angelova *et al*. 1995). SOD activity was measured by the nitro–blue tetrazolium (NBT) reduction method of Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme protein required for inhibition of the reduction of NBT by 50% (A_{560}) and was expressed as units per mg protein (U/mg protein). Catalase activity was determined by monitoring the decomposition of 18 mM H_2O_2 at 240 nm (Beers and Sizer 1952). One unit of activity is that which decomposes 1 μmol of H_2O_2 min^{-1} mg $protein^{-1}$at 25°C and pH 7.0. Specific activity is given as U/mg protein.

Other analytical methods. — Glycogen and trehalose contents were determined following the procedure described by Parrou *et al*. (1997). Soluble reducing sugars were determined by the Somogy–Nelson method (Somogy 1952). Protein was estimated by the Lowry procedure (Lowry *et al*. 1951) using bovine serum albumin as standard. The determination of biomass dry weight was performed on samples of mycelia harvested throughout the culture period. The culture fluid was filtered through a Whatman (Clifton, USA) No 4 filter. The separated mycelia were washed twice with distilled water and dried to a constant weight at 105°C.

Other analytical methods. — The results obtained in this investigation were evaluated from at least three repeated experiments using three parallel runs. The statistical comparison between controls and treated cultures was determined by the Student's *t*–test for MIE (mean interval estimation) and by one–away analysis of variance (ANOVA) followed by Dunnet's post test, with a significance level of 0.05.

Results

Effect of temperature on growth and glucose consumption of Antarctic fungal strains. — The effect of temperature on maximum colony growth of *P. commune* 161 and *A. glaucus* 363 is shown in Fig. 1. Presented data demonstrated a big difference in the growth characteristics between both Antarctic strains. Temperature response experiments revealed that *P. commune* 161 was capable of growth on agar medium within a temperature range of 0–25°C, with an optimum at of 15–20°C. *A. glaucus* 363 grew and formed colonies at temperatures between 12–35°C, while the optimum of temperature for growth was 25–30°C. According to the temperature range for growth and optimum, they could be classified as psychrotolerant (*P. commune* 161) and mesophilic (*A. glaucus* 363), respectively.

The temperature profiles of the model strains cultivated under submerged conditions are shown in Fig. 2. Both strains demonstrated growth under all temperature tested with the exception of the mesophilic strain *A. glaucus* at 10°C. Under the optimal temperature conditions, the psychrotolerant strain accumulated

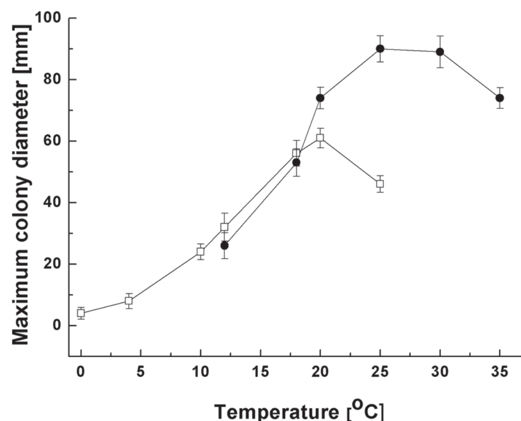

Fig. 1. Mean maximum colony diameter (cm) of *P. commune* 161 (□) and *A. glaucus* 363 (●) grown at different temperature regimes (0–35°C).

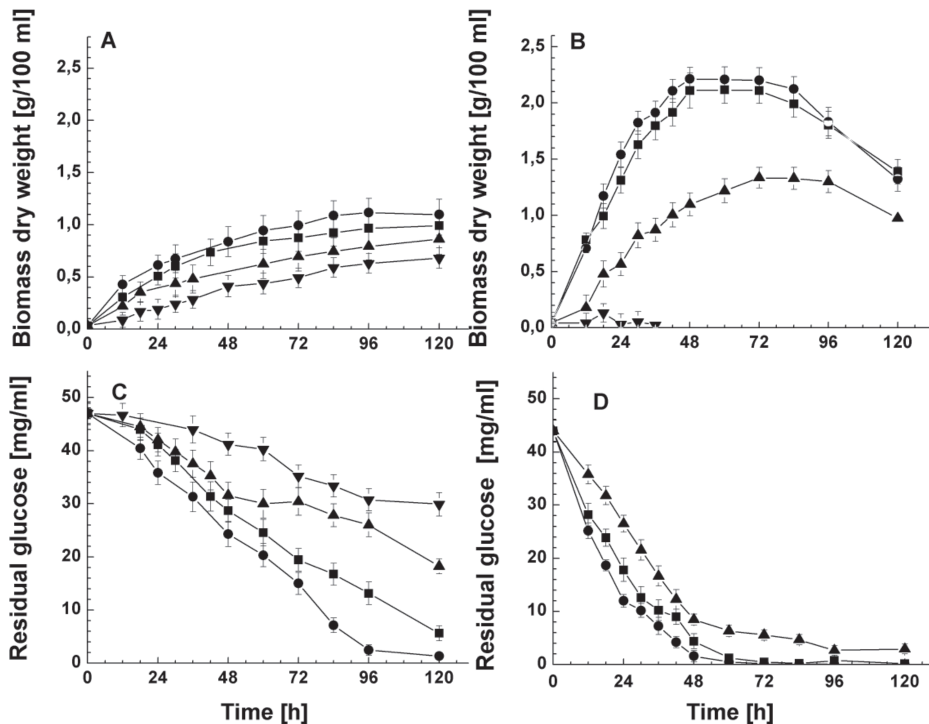

Fig. 2. Biomass (A, B) and glucose (C, D) levels in *P. commune* 161 (A, C) and *A. glaucus* 363 (B, D) under different temperatures: for P. *commune* 161 – (■) – 25°C, (●) – 20°C, (▲) – 15°C, (▼) – 10°C; for *A. glaucus* 363 – (■) – 30°C, (●) – 25°C, (▲) – 20°C, (▼) – 10°C. Bars represent SD of means. The effect of treatment was significant for the temperature treatment ($P \leq 0.05$).

about 2–fold less biomass (Fig. 2A) than the mesophilic fungus (Fig. 2B). The difference was statistically significant ($p \leq 0.05$). While the strain *P. commune* 161 exhibited a good development even at 10°C, mesophilic strain *A. glaucus* 363 showed very fast accumulation of biomass at 25 and 30°C.

Figure 2 also illustrates typical growth curves (exponential growth, stationary phase and decline phase) at all temperatures used for mesophilic strain in shaken liquid cultures. It should be added that upon incubation at lowest temperatures, the time until stationary phase was reached later than under the other temperature conditions and maxima of fungal growth decreased. In contrast, the curves of the psychrotolerant strain outlined continuous (albeit slight) growth until the end of cultivation. Onset of a stationary phase was observed at optimal temperature (20°C) only after 96 hour.

The concentration of glucose in the culture medium was measured throughout the experiment (Fig. 2). Maximum glucose consumption occurred in cultures incubated at optimal growth temperature compared to the lower temperatures.

A comparison of the model strains shows also that the consumption of glucose by mesophilic strain (Fig. 2D) was faster than that of psychrotolerant fungus (Fig. 2C). While the culture of *A. glaucus* 363 consumed about 90–99% in all variants after 72 hour, *P. commune* 161 achieved 98% only at optimum temperature at the end of cultivation. Moreover, the influence of temperature variation on the glucose decay was more sensitive for the psychrotolerant strain. Comparing the curves in the Fig. 2C, it was possible to verify that the downshift of temperature from 20 to 10°C caused significant decrease in glucose consumption. It is noteworthy that the temperature above optimum (25 and 30°C for *P. commune* 161 and *A. glaucus* 363, respectively) resulted in reduction of glucose uptake compared to the control.

Temperature–dependent accumulation of reserve carbohydrates. — As shown in Fig. 3, reserve carbohydrates were detected in both Antarctic strains upon exposure to all the tested temperatures. In the control cultures (at optimal temperature), no significant increase in the level of either carbohydrate was observed during cultivation. A similar trend was observed in the psychrotolerant

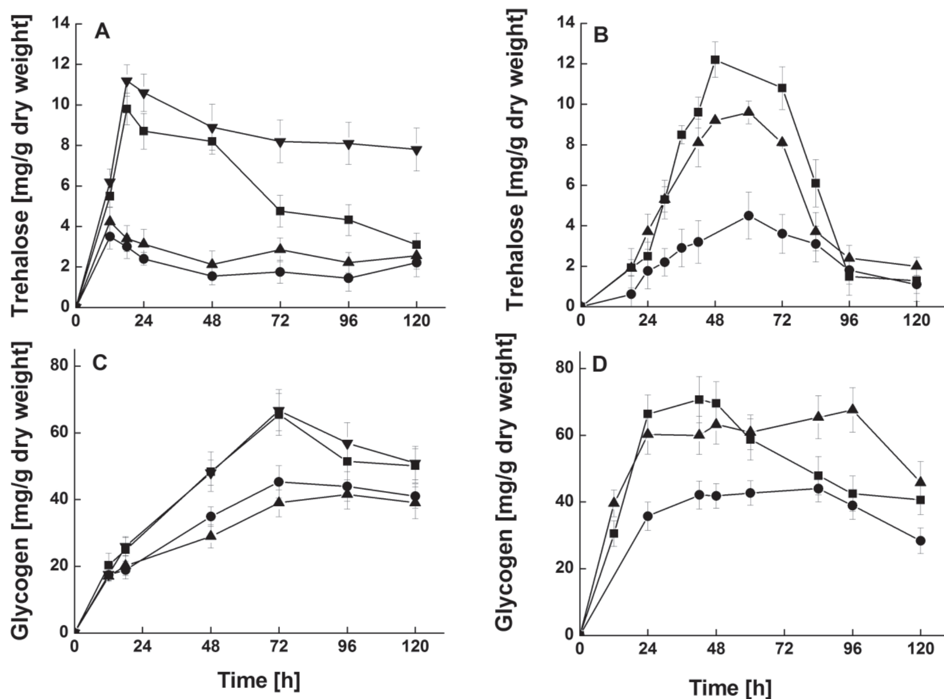

Fig. 3. Trehalose (A, B) and glycogen (C, D) levels in *P. commune* 161 (A, C) and *A. glaucus* 363 (B, D) under different temperatures: for *P. commune* 161 – (■) – 25°C, (●) – 20°C, (▲) – 15°C, (▼) – 10°C; for *A. glaucus* 363 – (■) – 30°C, (●) – 25°C, (▲) – 20°C. Bars represent SD of means. The effect of treatment was significant for the temperature treatment (P ≤ 0.05).

strain *P. commune* 161, grown at 15°C (near to the optimum) (Fig. 3A, 3C), but the maximum level of trehalose and glycogen was about 30% higher than that in the control variants. Each significant change in growth temperature below or above optimum resulted in enhanced content of reserve carbohydrates. The maximum level assessment showed that *P. commune* cells accumulated 4,7– and 4–fold more trehalose and 2–fold more glycogen when cultivated at temperature of 10°C (below optimum) and 25°C (above optimum), respectively, compared with control culture.

Our results indicated that the accumulation of both reserve carbohydrates in the mesophilic strain *A. glaucus* 363 cultivated under above– and below–optimal temperatures (Fig. 3B, 3D) resembled that of the psychrotolerant strain (Fig. 3A, 3C). Furthermore, the highest trehalose content is coincided with the late exponential and early stationary growth phase while the highest glycogen concentrations were found from the exponential phase until the late stationary phase.

Antioxidant enzyme activities at different growth temperature. — To find out whether the growth of Antarctic strains at above– and below–optimal was associated with antioxidant enzyme defence, we determined changes in activities of SOD and CAT (Fig. 4). The SOD activity in cell homogenate from the psychrotolerant strain *P. commune* 161 increased with decreasing growth temperatures (Fig. 4A). Our data demonstrated 2.2– and 1.5–fold higher maximal SOD level in cultures, grown at 10 and 15°C, respectively compared to the control. On the other hand, cultivation at 25°C led to insignificant enhancement of the enzyme activity compared to the control variant. The examinations during the growth cycle at optimal temperature indicated an increase during exponential growth phase, and become constant during the stationary phase. Under conditions of low temperature treatment, the time courses of SOD production show two maxima: at 60 hour (when *P. commune* is still growing) and 96 hour (in early stationary phase).

In marked contrast, SOD activity decreased in cells of mesophilic strain *A. glaucus* 363 grown at temperatures different from optimal 25°C (Fig. 4B). Cultivation at 20°C led to 2–fold reduction in enzyme level, whereas enhanced growth temperature (30°C) caused in activity. Maximum enzyme activity at optimal temperature was measured at 24 hour, (in the active fungal growth), followed by a continuous decline up to 60 hour and a second maximum, which was reached at the end of stationary phase (84 hour). Decrease in the temperature (20°C) caused a delay in reaching of maximum activity.

It is of interest to note that the cultivation of psychrotolerant strain at temperature below optimal lead to opposite cell response concerning CAT activity. As shown in Fig. 4C, temperatures of 15 and 10°C lead to a sharp decrease in enzyme level in comparison to the control. In the experiment with the mesophilic

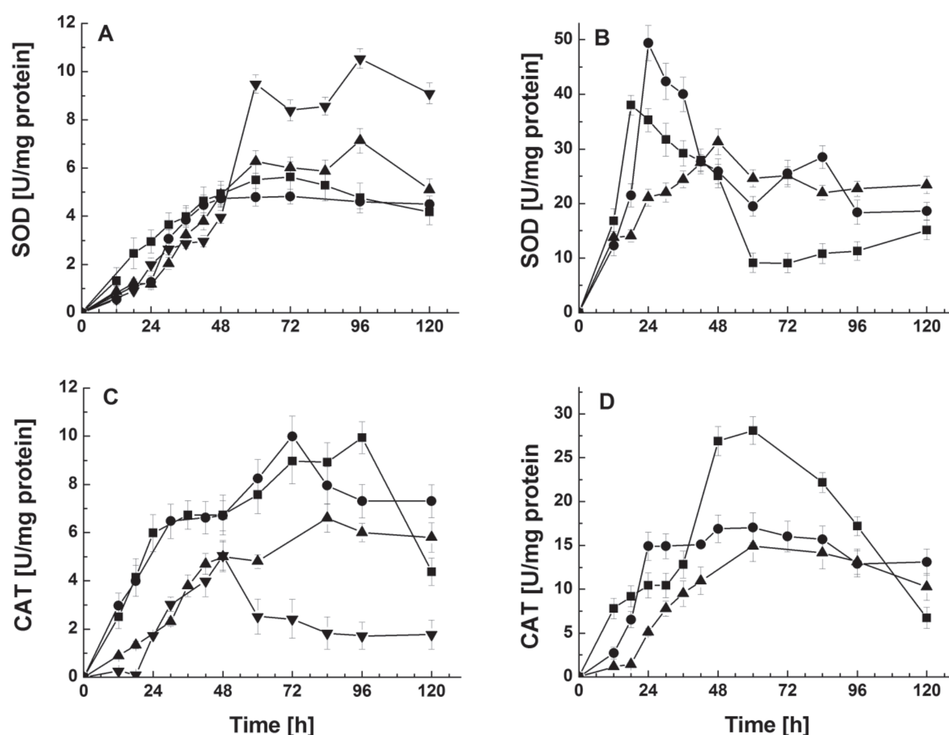

Fig. 4. SOD (A, B) and CAT (C, D) activities in cultures of *P. commune* 161 (A, C) and *A. glaucus* 363 (B, D), cultivated at different temperatures. The treatment of the mycelia was carried out as described in the legend to Fig. 3. Bars represent SD of means. The effect of treatment was significant for the temperature treatment (P ≤ 0.05).

strain a similar trend was observed (Fig. 4D). The maintenance of temperature above the optimal for the growth of *A. glaucus* caused a significant increase in CAT activity (Fig. 4D), while *P. commune* did not demonstrate any alterations compared to the control (Fig. 4C).

Discussion

Despite the fact that so much of our planet is cold, majority of cold–adapted micro–organisms are mesophilic or psychrotolerant (psychrotrophic) rather than psychrophilic (Russell 2006). Our Antarctic strain *P. commune* 161, which was isolated at temperature 4°C, can grow well in the range of temperature between 4 and 25°C and has an optimum at 20°C. According to the recognized definition, this strain is not a true psychrophilic species as it can grow at 25°C. Recently, the terms stenopsychrophile and eurypsychrophile have been favoured for the cold–adapted micro–organisms (psychrophilic and psychrotolerant, respectively)

(Wang *et al*. 2015). Thus, the growth data of *P*. *commune* 161 specify this strain as psychrotolerant or eurypsychrophile. The search for psychrophilic filamentous fungi in Antarctica has been successful in very rare cases (Margesin 2009). In contrast, there appeared many reports on the isolation of psychrotolerant fungal strains (Frisvad 2008). Gonçalves *et al*. (2013) reported on a mesophilic, psychrotolerant fungus *Penicillium solitum* inhabiting marine sediments of Antarctica. The fungus *Lecanicillium muscarium* CCFEE 5003, isolated from continental Antarctica has been characterized as a strain with psychrotolerant behavior (Fenice 2016).

At the same time, the colony growth and growth kinetic data indicate that *A*. *glaucus* 363 is a mesophilic strain since it exhibited optimal growth temperatures around 25°C and was unable to grow at 10°C. As known, Antarctic mycoflora contains also fungi, which are represented by mesophilic species, present as viable propagules but unable to reproduce except in rarely favourable climatic conditions (Ruisi *et al*. 2007). Other species, able to grow actively, at least under Antarctic summer conditions, comprise particular ecotypes of cosmopolitan species showing mesophilic behaviour as an adaptation to the cold Antarctic climate (Onofri *et al*. 2005).

It should be noted that the psychrotolerant strain *P. commune* 161 demonstrated lower biomass productivity than the mesophilic *A*. *glaucus* 363 at optimal temperature. Presumably, cold–adapted micro–organisms have to synthesize a wide range of compounds essential for survival at low temperature, such as reserve carbohydrates, exopolysaccharides, polyoles, melanines *etc*. (Sterflinger 2006). The high metabolic cost for synthesizing all these compounds significantly affects growth velocity of fungi which show a very slow growth rate (Selbman *et al*. 2014). In addition, they have to cope with ROS and oxidative damages in cells.

Glucose uptake illustrated clear dependence on temperature and thermal characteristics of the strains. We detected significant reduction in the glucose consumption in *P. commune* compared to *A*. *glaucus*. These data can be seen as a confirmation of above mentioned suggestion about differences in biomass production between both strains, psychrotolerant and mesophilic. Temperature downshift also resulted in considerable decrease in glucose utilization. Psychrotolerant and mesophilic strain grown at 10 and 20°C, respectively, take up glucose much more slowly. Under temperature induced oxidative stress, fungi reduce the glucose and ammonia uptake (Li *et al*. 2008). In this way, the low temperature–induced stress can cause a dynamic rearrangement of the metabolic flux to the pentose phosphate pathway leading to the generation of the reduced electron carrier NADPH (Dos Reis *et al*. 2013). Fungal cells accelerated the production of glucose by gluconeogenesis and the production of amino acids important to compose the repertoire of molecules to the oxidative stress response (Dos Reis *et al*. 2013). Kostadinova *et al*. (2011) showed a re–routing of carbon metabolism away from glycolysis into the pentose phosphate pathway which serves as a cellular stress–resistance mechanism under cold stress conditions.

Data published in the literature on the effect of low temperatures on glucose uptake have shown contradictory results. Fukunaga & Russell (1990) reported that the glucose uptake in a psychrotrophic *Pseudomonas* spp., isolated from Antarctica was maximal at 20°C but fell at lower temperatures. For two yeast strains belonging to the species *Rhodotorula aurantiaca*, half–saturation constant for glucose uptake for glucose (*Ks*) was relatively constant below optimal temperatures (10°C and 17°C for the psychrophilic and psychrotolerant strain, respectively) but rose when the temperature increased beyond the optimum (Sabri *et al*. 2000). The ability of cold–adapted micro–organisms to grow slowly at low temperatures may actually be an advantage in nutrient–poor environments, where a rapid exhaustion of available resources would lead to starvation (Russell 2006). In contrast, glucose uptake in a psychrotrophic bacterial strain from permanently–cold Antarctic environment was maximal at 0°C and decreased up to 15–20°C (Ellis-Evans and WynnWilliams 1985).

Production of reserve carbohydrates by stressed cells appears to be a critical adaptation that protects micro–organisms against a wide variety of potentially lethal conditions (Feofilova *et al*. 2000; Kanwal *et al*. 2011). Growth and survival strategies by cold–adapted fungi also included accumulation of trehalose and glycogen in the cells (Ruisi *et al*. 2007; Frisvad 2008; Gocheva *et al*. 2009). We demonstrate here that trehalose and glycogen accumulate in both Antarctic cultures during cold shock (*i.e*., 10°C for psychrotolerant strain and 20°C for mesophilic strain). It is noteworthy that a similar induction of trehalose and glycogen was observed at temperatures above the optimum. An accumulation of cryoprotective carbohydrates in response to suboptimal growth temperature was observed in *Humicola marvinii* and *Mortierella elongata*, described from fell–field soils in maritime Antarctica (Weinstein *et al*. 2000) and in yeast strains (Schade *et al*. 2004).

The mechanism by which reserve carbohydrates protects against cold stress still remains unknown. According to Da Costa Morato Nery *et al*. (2008) this mechanism is involved in minimizing the oxidative damage caused to both proteins and lipids, which would require the presence of trehalose on both sides of the lipid bilayer. There are data for up–regulation of the genes, responsible for trehalose– and glycogen–metabolizing enzymes, resulting in their accumulation under conditions of cold stress (Aguilera *et al*. 2007; Kostadinova *et al*. 2009; Iordachescu and Imai 2011). Interestingly, Tsuji (2016) reported that two strains of the Antarctic *Mrakia blollopis* demonstrate difference in cold stress response. *M*. *blollopis* SK–4, which grew well under subzero temperatures, accumulated high levels of TCA–cycle metabolites, lactic acid, aromatic amino acids and polyamines. At the same time, in *M*. *blollopis* TKG1–2, which did not grow efficiently, cold stress strongly induced the metabolites of the TCA cycle, but other metabolites were not highly accumulated in the cell.

Cell response against oxidative stress includes increase in the activities of SOD and CAT which are key enzymes for directly scavenge ROS (Zhang *et al*.

2003, Gocheva *et al*. 2009; Sharma *et al*. 2012). There are only very few reports in published literature about effect of low growth temperature on antioxidant enzyme defense. Chu *et al*. (2016) demonstrated higher activities in SOD, CAT, and APX in arbuscular mycorrhizal fungi under cold–stress conditions. Antioxidants could decrease the accumulation of ROS and reduce the oxidative damage in cells under cold stress. For example, temperature downshift induces antioxidant response in *Saccharomyces cerevisiae* (Zhang *et al*. 2003). Gocheva *et al*. (2006) also showed that the growth at below–optimal temperature was accompanied by typical oxidative stress reaction – an enhanced expression of both antioxidant enzymes. Interestingly, in the present study, a different response was established. Moreover, strain–dependent changes in SOD activity were observed. While SOD activity in the psychrotolerant strain increases with decreasing growth temperature, the mesophilic *A. glaucus* demonstrated marked reduction below and above optimal temperature. Similar decreasing trend was observed for CAT activity in both strains. An increase in SOD activity with a decrease in CAT activity has been reported in plants subjected to abiotic stress (see Lu *et al*. 2008). The differential response of antioxidative enzymes to cold stress in *P. commune* and *A. glaucus* may be attributed to varied level of ROS generation in strains belonging to different thermal classes. The depression in SOD activity in the cold–stressed cells of the mesophilic strain may be a consequence of decreased *de novo* synthesis of SOD proteins or irreversible inactivation of enzyme proteins from increased ROS production resulting from cold–stress metabolism. This situation may be a result of significant increase in the rate of $^{\cdot}O_2^-$ formation and subsequently H_2O_2 accumulation throughout the cold stress. At the same time, excess production of ROS can inactivate CAT activity at higher concentrations, probably by inactivating the enzyme–bound heme group (Willekens *et al*. 1997). Probably, the decrease of catalase activity could be compensated by the up–regulation of peroxidases (Sofo *et al*. 2015).

Moreover, the time courses of SOD production showed two maxima at 10 and 15°C for the *P. commune* and at 25°C for *A. glaucus*. A similar phenomenon, a secondary increase in SOD activity during the late stationary phase, has been observed for SOD production by filamentous fungi and yeasts (Shilova *et al*. 1989; Angelova *et al*. 1996). It can be explained by an intensification of the process of $^{\cdot}O_2^-$ generation when the cells utilize endogenous sources of carbon and nitrogen (organic or amino acids).

Taken together, the present results showed that although the both tested strains were isolated from Antarctic soil samples, cold treatment caused significant differences in physiological cell response depending on their thermal characteristics regarding:

- growth and glucose uptake. The psychrotolerant strain *P. commune* demonstrated growth and glucose uptake retardation compared to the mesophilic strain *A. glaucus*

- participation of the first antioxidant enzyme, SOD in cold survival. While the psychrotolerant strain showed lower basal level of SOD activity that increases with decreasing growth temperature, the higher basal SOD activity in the mesophilic strain demonstrated marked reduction at below and above optimal temperature.

At the same time, both the psychrotolerant and mesophilic strain accumulated glycogen and trehalose at temperature different from the optimum. The results suggested also a lack of antioxidant protection from CAT.

Acknowledgement. — This work was supported by the Bulgarian Scientific Fund (grants VU–B–205 and DO02–172), which is greatly acknowledged.

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Received 24 November 2016
Accepted 8 May 2017