We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

4

Transient Cold Shock Induces Oxidative Stress Events in Antarctic Fungi

Nedelina Kostadinova, Ekaterina Krumova,
Tzvetanka Stefanova, Vladislava Dishliyska and Maria Angelova
The Stephan Angeloff Institute of Microbiology,
Bulgarian Academy of Sciences,
Bulgaria

1. Introduction

The Antarctic biota has evolved under the influence of a suite of geological and climatic factors, including the geographic isolation of the landmass and the continental shelves, extremely low temperatures and intense seasonality (Russo et al., 2010). The isolation and environmental history of Antarctica have led to a unique biota. Many groups of organisms became extinct in Antarctica as a result of the extremely cold conditions. Although this continent is the coldest, highest, windiest, driest, wildest and most pristine of all of the continents, it is full of life. In addition to its well-known inhabitants, such as penguins and seals, it also has a diverse and unique range of microbial diversity (Nichols et al., 1999; Vincent, 2000). Microorganisms successfully colonise cold habitats and play a major role in the processes of nutrient turnover at low temperatures. In recent years, a growing attention in research has been devoted to cold-adapted microorganisms. This interest in Antarctic microorganisms stems from several reasons. Antarctica's environmental extremes present conditions in which microorganisms have evolved unique characteristics for survival, which are of great scientific interest. Moreover, the availability of novel Antarctic species, which are generally isolated from extreme environments, opens the door for biotechnological exploration. Investigations of psychrotolerant and psychrophilic microorganisms are also important for human health because microorganisms can cause food spoilage and food-borne diseases. Research on cold shock raises a number of questions: which cellular function is affected most upon cold shock, what makes cell growth stop, and are there well-conserved or common cold shock proteins as in the case of heat-shock proteins? These questions are no less important than those in the case of heat shock (Inouye, 1999).

Cold-adapted microorganisms include both psychrophilic (organisms with an optimal growth temperature at or below 15°C and a maximum growth temperature below 20°C) and psychrotrophic or psychrotolerant (organisms exhibiting the ability to grow at temperatures below 15°C but exhibiting maximum growth rates at temperature optima above 18°C) organisms, and they are often subjected to other extreme environmental parameters (Morgan-Kiss et al., 2006). Antarctic microflora is also represented by mesophilic and thermophilic bacteria that are isolated from geothermal soils and by mesophilic fungi, which

are present as viable propagules that are able to grow actively, at least under Antarctic summer conditions (Pepi et al., 2005; Ruisi, 2007).

Cold-adapted microorganisms have developed several strategies to adapt to low temperatures (Onofri et al., 2007). This adaptation includes the production of cold-active enzymes (Feller & Gerday, 2003; Collins et al., 2008; Gatti-Lafranconi et al., 2010), the modulation of lipid compositions to maintain the fluidity of the cell membrane (Chintalapati et al., 2004; Russell, 2008), the production of RNA chaperones to suppress the formation of undesired secondary structures of RNA (Kwak et al., 2011) and the synthesis of antifreeze (García-Arribas et al., 2007) and cold shock proteins (Horn et al., 2007).

Additionally, low temperatures can induce oxidative stress due to the enhanced generation of reactive oxygen species (ROS), such as the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide H_2O_2, and the hydroxyl radical •OH (Chattopadhyay, 2002; Gocheva et al., 2009). These ROS are highly damaging to cellular components, including DNA, lipids and proteins (Sies, 1993). To scavenge ROS and prevent damage, all aerobic cells have evolved a complex defence system consisting of both low molecular mass scavengers and high molecular mass antioxidants, particularly, antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) (Fridovich, 1998). Thus, antioxidant defence may play a significant role in the microbial survival mechanism under extremely cold conditions (Chattopadhyay, 2002; Chattopadhyay et al., 2011).

Terrestrial microfungal communities in the Antarctic are rarely investigated (Ruisi et al., 2007). There are even fewer studies of the relationship between low temperatures and the induction of oxidative stress events. The decrease in temperature gives signal to organism for evoking cold-shock response generating molecules that are required for growing at the lower range of growth temperature. These cold-shock response are also critical for survival and growth at lower temperatures (Ray, 2006). Our previous studies indicated that growth at a low temperature induced oxidative stress in fungal strains isolated from soil samples of three regions of Antarctica: Casey Station, Terra Nova Bay and South Georgia (Gocheva et al., 2005, 2006, 2009). In recent years, we have also isolated filamentous fungi from samples taken from another Antarctic region – the permanent Bulgarian Antarctic base "St. Kl. Ohridski" on Livingston Island in the South Shetland Islands (Maritime Antarctica) – during the Bulgarian Antarctic expedition 2006/07 (Tosi et al., 2010). Published data about the filamentous and larger fungi of Livingston Island are scarce (Gray & Smith 1984; Wirtz et al., 2003). Furthermore, the filamentous fungi from the Bulgarian area on Livingston Island have only been investigated by our research team. Moreover, the studies on the specificity of the cell response to cold-induced oxidative stress between two different thermal classes of Antarctic fungi, psychrophilic (psychrotolerant) and mesophilic, are scant (Gocheva et al., 2009).

This paper is an attempt to enlarge our previous investigation focusing on two Antarctic fungi (psychrotolerant and mesophilic strains) that were isolated from the Bulgarian Antarctic area on Livingston Island. This study was designed to compare the effect of short-term cold shock on the growth of mycelia, glucose consumption and the level of oxidative stress biomarkers, including ROS, oxidatively damaged proteins, reserve carbohydrates and trehalose metabolising enzymes. Moreover, the Antarctic strains were used to obtain information concerning changes in the activities of two antioxidant enzymes, SOD and CAT, under a short-term cold treatment.

2. Materials and methods

2.1 Fungal strains, culture media and cultivation

The fungal strains, *Penicillium sp.* 161 and *Aspergillus glaucus* 363 (with optimal growth temperatures of 20°C and 25°C, respectively), isolated from Livingston Island (South Shetlands archipelago, Antarctica) (Tosi et al., 2010) were used for the experiments. The strains belong to the Mycological collection at the Institute of Microbiology, Sofia, and they were maintained at 4°C on beer agar at pH 6.3.

The composition of the seed and production media has been described previously (Angelova et al., 1996). The cultivation was performed in a 3 L bioreactor, which was ABR-09-developed and constructed by the former Central Laboratory for Bioinstrumentation and Automatisation (CLBA) of the Bulgarian Academy of Sciences. The bioreactor was equipped with temperature, pH and automatic dissolved oxygen (DO) monitoring equipment and a control system.

For the submerged cultivation, 74 ml of seed medium was inoculated with 6 ml of spore suspension at a concentration of 2×10^8 spores/ml in 500-ml Erlenmeyer flasks. The cultivation was performed at 20°C for 48 h for the psychrotolerant strain and at 25°C for 24 h for mesophilic strain on a rotary shaker (220 rpm). For the bioreactor cultures, 200 ml of the seed culture was brought into the 3-L bioreactor, which contained 1800 ml of the production medium. The cultures were grown at an optimal temperature with a stirrer speed of 400 rpm and an air flow of 0.5 vvm. During the middle of the exponential phase (24 h for *Penicillium sp.* 161 and 18 h for *A. glaucus* 363), the temperature was reduced to 4 or 10°C. This downshift was reached in approximately 40 min. After an incubation of 6 h under cold stress conditions, the temperature was shifted up to the optimal value. The control variants were grown at their optimal temperature during the entire period.

2.2 Cell-free extract preparation and isolation of the cytosolic and mitochondrial fractions

The cell-free extract, in addition to the cytosolic and mitochondrial fractions, were prepared as previously described (Krumova et al., 2008). All of the steps were performed at 0–4°C.

2.3 Determination of ROS

For the measurement of the $O_2^{\bullet-}$ production rate, the method of superoxide dismutase-inhibitable reduction of cytochrome *c* was used (Hassan & Fridovich, 1979) with some modifications. Briefly, the cell suspensions or mitochondrial fractions, which were taken from the control and cold-stressed cultures, were incubated for 60 min at 30°C on a water bath rotary shaker at 150 rpm. The reaction mixtures contained 50 μM cytochrome *c*, 2% non-autoclaved glucose, 20 mM NADPH in the presence and absence of 50 μg ml^{-1} of the superoxide dismutase from bovine erythrocytes in a 0.05 M potassium phosphate buffer with a pH of 7.8. The reaction was stopped by cooling in an ice-cold water bath. The cells were removed by centrifugation before measuring absorbance at 550 nm to determine the extent of cytochrome *c* reduction. A molar extinction coefficient of $2.11xl0^4$ was used to calculate the concentration of reduced cytochrome C. For measurement of the hydrogen peroxide production, the method of Pick & Mizel (1981) was used. Briefly, fungal cells

treated with temperature were suspended in a 0.05 M potassium phosphate buffer with a pH of 7.8 and containing 50 μg ml^{-1} of horseradish peroxidase type VI-A. After incubation at 30°C for 45 min, the reaction was stopped by the addition of 1N NaOH, and the absorbance was read at 620 nm. For the calculations, a standard curve with H_2O_2 concentrations (from 5 to 50 μM) was used.

2.4 Enzyme activity determination

SOD activity was measured by the nitro-blue tetrazolium (NBT) reduction method of Beauchamp & Fridovich (1971). The reaction mixture contained 56 μM (NBT), 0.01 M methionine, 1.17 μM riboflavin, 20 μM KCN and 0.05 M phosphate buffer with a pH of 7.8. Superoxide was measured by the increase in absorbance at 560 nm at 30° C after 6 min of incubation from the beginning of the illumination. One unit of SOD activity was defined as the amount of enzyme required for the inhibition reduction of NBT by 50% (A_{560}) and was expressed as units per mg protein [U/mg protein]. Cyanide (5 mM) was used to distinguish between the cyanide-sensitive isoenzyme Cu/Zn-SOD and the cyanide-resistant Mn SOD. The Cu/Zn-SOD activity was obtained as the total activity minus the activity in the presence of 5 mM cyanide.

The catalase activity was determined by monitoring the decomposition of 18 mM H_2O_2 at 240 nm (Beers & Sizer, 1952). One unit of activity was that which decomposes 1 μmol of H_2O_2 min^{-1} mg $protein^{-1}$ at 25°C and a pH of 7.0. Specific activity is given as U/mg protein.

The neutral trehalase (NT) and trehalose-6-phosphate synthase (TPS) enzyme activities were assayed in cell-free extracts by the methods of Müller et al. (1992) and Vandercammen et al. (1989), respectively, as described by El-Bashiti et al. (2005).

2.5 Analytical methods

The glycogen and trehalose contents were determined following the procedure of Becker (1978) and Vandecamen et al. (1989) and modified by Parrou et al. (1997). The soluble reducing sugars were determined by the Somogy-Nelson method (Somogy 1952).

The protein oxidative damage was measured spectrophotometrically as the protein carbonyl content using the 2,4-dinitrophenylhydrazine (DNPH) binding assay (Hart et al., 1999) slightly modified by Adachi & Ishii (2000). The cell-free extracts were incubated with DNPH for 1 h at 37°C; the proteins were precipitated in 10% cold TCA and washed with ethanol:ethylacetate (1:1) to remove any excess of DNPH and finally dissolved in 6 M guanidine chloride with a pH of 2. The optical density was measured at 380 nm, and the carbonyl content was calculated using a molar extinction coefficient of 21 mM^{-1} cm^{-1} as nanomoles of DNPH incorporated (protein carbonyls) per mg of protein.

Protein was estimated by the Lowry procedure (Lowry, 1951) using a solution of bovine serum albumin as a standard.

The dry weight determination 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.

2.6 PAGE analyses

Non-denaturing polyacrylamide gel electrophoresis of the extract was followed by activity staining for each of the enzymes tested. A forty μg of total protein was applied to a 10% nondenaturing PAGE. Staining for SOD and CAT was performed as described by Beauchamp and Fridovich (1971) and Woodbury et al. (1971), respectively.

2.7 Statistical evaluation of the results

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

3. Results

Our previous study indicated that the optimal growth temperatures for both Antarctic strains, the psychrotolerant *Penicillium sp.* 161 and the mesophilic *A. glaucus* 363, are 20°C and 25°C, respectively (Tosi et al., 2010). The duration of the temperature shift (6 h) was chosen because this length of time was found to be sufficient to give a clear contrast between the control and the stressed cultures.

3.1 Response of the fungal growth and glucose consumption to temperature shifts

Mycelia of the Antarctic strains, which were grown until the middle of the exponential phase at optimal temperature, were shifted to colder temperatures, i.e., 4°C and 10°C. Figure 1 shows the growth curves of the model strains after the temperature downshift exposure and the subsequent restoration of the normal conditions.

Within the first 4 h of the beginning of the stress, the growth of the psychrotolerant strain *Penicillium sp.* 161 ceased and the biomass as measured by dry weight decreased sharply in comparison to the control (Fig. 1A). In the next 2 h, growth resumed and the return to the optimal 20°C after 6 h allowed the biomass to increase relative to the control levels. A similar trend was demonstrated for the Antarctic mesophilic strain, *A. glaucus* 363, after a shift from 25°C to 10°C or 4°C (Fig. 1B), but the difference in biomass production between the control and treated mycelia was more significant compared to the psychrotolerant strain. Moreover, after 4 h of recovery from either temperature treatment, biomass production was restored and the dry weight reached its pre-stress level.

The concentration of glucose in the culture medium was measured throughout the experiment (Fig. 2). The maximum glucose consumption occurred in cultures incubated at an optimal growth temperature was comparable to the stressed cultures.

A comparison of the model strains also shows that the consumption of glucose by the mesophilic strain (Fig. 2B) was faster than that of the psychrotolerant fungus (Fig. 2A). Comparing the curves in Fig. 2A and Fig. 2B, it was possible to verify that the downshift of temperature from the optimal temperature to 10°C or 4°C caused a significant decrease in glucose consumption. This tendency continued even after the return to the optimal temperature.

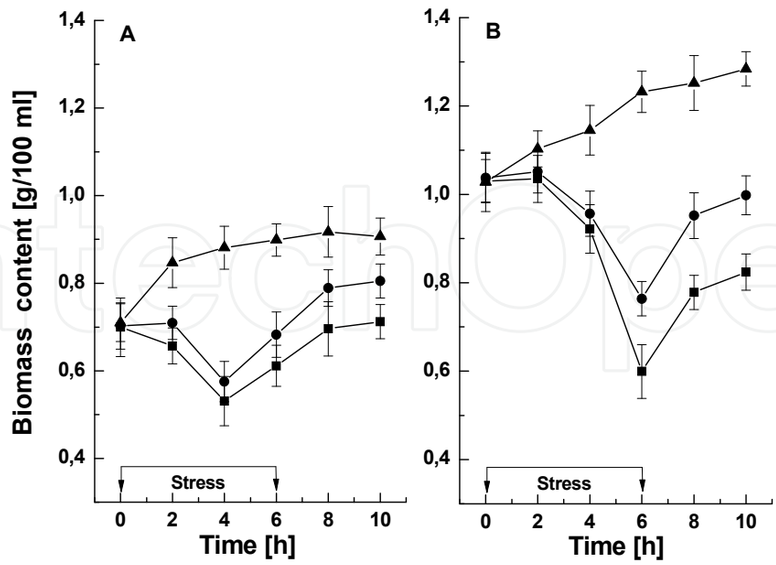


Fig. 1. Effect of cold shock on biomass production by *Penicillium sp.* 161 (A) and *A. glaucus* 363 (B) ▲ – growth at optimal temperature; ■ – downshift from optimal temperature to 4°C; ● - downshift from optimal temperature to 10°C. Bars represent SD of means. The effect of treatment was significant for the temperature treatment ($P \leq 0.05$)

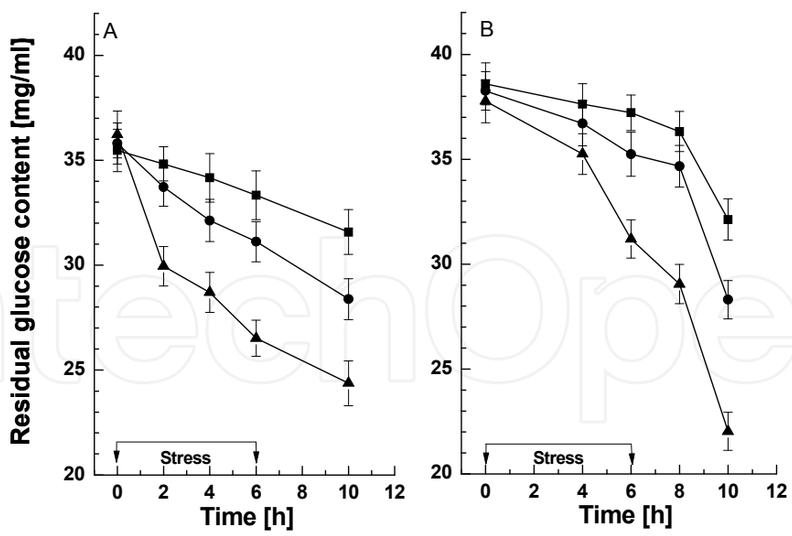


Fig. 2. Glucose consumption in *Penicillium sp.* 161 (A) and *A. glaucus* 363 (B) during cold shock and following recovery period. (▲) – growth at optimal temperature; (■)– downshift from optimal temperature to 4°C; (●) - downshift from optimal temperature to 10°C. Bars represent SD of means. The effect of treatment was significant for the temperature treatment ($P \leq 0.05$)

3.2 Effect of cold stress on ROS generation

To further characterise the fungal cell response to cold stress, the generation of ROS in both intact cells and mitochondrial fractions was determined. Table 1 shows the effect of decreased temperatures on the superoxide anion level in the model Antarctic strains. Elevated $O_2^{\bullet-}$ content was found for all of the temperatures tested. In the psychrotolerant strain, the temperature downshift to 10°C resulted in an increase in the $O_2^{\bullet-}$ level (approximately 116% and 124% for the intact cells and mitochondria, respectively), compared with the control.

The next diminution in the temperature to 4°C caused a more significant increase in the $O_2^{\bullet-}$ content in the intact cells (196%) and the mitochondrial fraction (143%). Furthermore, the mesophilic strain that was treated by cold stress to 10°C and 4°C showed extremely high $O_2^{\bullet-}$ levels, especially in the mitochondria (410% and 660% of the control, respectively). In both of the strains, the exposure to the above-mentioned low temperatures had a less pronounced induction in the H_2O_2 levels compared to the superoxide anion content (Table 2).

Variant	$O_2^{\bullet-}$ [μM/mg d.w. per h]			
	Penicillium sp. 161		*A. glaucus* 363	
	Intact cells	Mitochondrial fraction	Intact cells	Mitochondrial fraction
Control	3.59±0.03	5.24±0.03	4.70±0.02	0.55±0.01
10°C	4.15±0.04	6.50±0.01	6.78±0.01	2.25±0.01
4°C	7.05±0.18	7.47±0.05	11.1±0.07	3.63±0.02

Table 1. Increase in $O_2^{\bullet-}$generation in the intact cells or mitochondrial fractions of *Penicillium* sp. 161 and *A. glaucus* 363 treated by low temperatures

Variant	H_2O_2 [mM/mg d.w. per h]			
	Penicillium sp. 161		*A. glaucus* 363	
	Intact cells	Mitochondrial fraction	Intact cells	Mitochondrial fraction
Control	5.68±0.18	12.11±0.10	11.39±1.10	6.40±0.28
10°C	6.22±0.40	15.00±0.37	13.72±1.09	9.79±0.33
4°C	9.18±0.11	21.57±0.44	14.61±0.56	11.58±0.43

Table 2. Increase in H_2O_2 generation in the intact cells or mitochondrial fractions of *Penicillium* sp. 161 and *A. glaucus* 363 treated by low temperatures

These results also demonstrated a more active process of ROS generation in the mitochondrial fractions than in the intact cells, and in the mesophilic *A. glaucus* 363 compared to the psychrotolerant *Penicillium* sp. 161.

3.3 Cold shock causes protein oxidation

The reaction of proteins with oxygen radicals leads to the appearance of carbonyl groups in polypeptide chains (Davies and Goldberg 1987). Thus, carbonyl formation is a marker for protein oxidation. We investigated whether the exposure of Antarctic strains to a temperature downshift causes oxidative damage to proteins during the stress period and in

the post-stress recovery phase (Fig. 3). The level of protein carbonyl content in the control variants of both Antarctic strains did not change notably during the 6 h of stress. However, after the temperature downshift, the amount of carbonyl groups in the cell proteins increased with the duration of the exposure compared to the control. The increase was dependent on the grade of the temperature shift used in the experiment and less on the temperature requirements of the strains. Both of the strains demonstrated a sharp elevation in oxidatively damaged proteins compared to the control immediately after the start of the treatment.

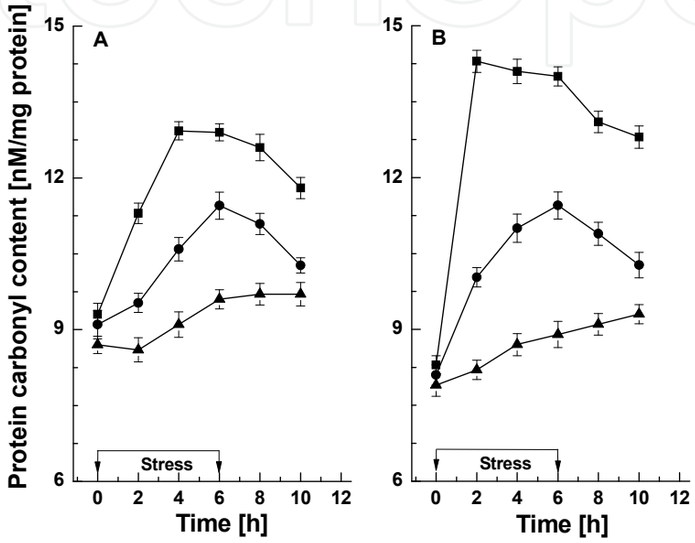


Fig. 3. Oxidative damage of proteins in *Penicillium* sp. 161 (A) and *A. glaucus* 363 (B), upon optimal temperature (▲) and upon temperature downshift from optimal to 4°C (■) or 10°C (●). Bars represent SD of means. The effect of treatment was significant for the temperature treatment ($P \leq 0.05$)

As seen in Fig. 3, the maximum values of the carbonyl content in the mesophilic strain during the downshift to 10°C and 4°C were 1.3- and 1.6-fold higher than in the control, respectively. A similar trend was found for the psychrotolerant strain (1.2- and 1.3-fold, respectively), but it showed a comparably lower sensitivity to cold temperatures. Although the content of the damaged proteins decreased significantly during the recovery phase, its value remained considerably higher than in the control variants.

3.4 Accumulation of reserve carbohydrates under conditions of cold shock

Another physiological consequence of low temperature exposure in both of the Antarctic fungi was the accumulation of reserve carbohydrates through stress. The production of glycogen and trehalose after a temperature downshift to 4°C or 10°C was detected. As is shown in Fig. 4, there was a sharp increase in the glycogen and trehalose content after the beginning of the stress. In the experiments with the psychrotolerant strain, *Penicillium* sp. 161, glycogen was accumulated up to 1.2 - 1.75 times the basal level and was accompanied

by a 1.7-fold increase in trehalose content (Fig. 4A and 4B). It is noteworthy that higher initial levels of both of the reserve carbohydrates were detected in the mesophilic strain, *A. glaucus* 363. The stress conditions (4°C or 10°C) caused an additional sizeable rise in glycogen (1.4- and 1.3-fold, respectively) and trehalose (2.2- and 1.9-fold, respectively) content (Fig. 4C and 4D). The data demonstrated a clear dependence on both the degree of cold shock and the thermal characteristics of the model strain. Four hours after the removal of the temperature stress, the levels of trehalose and glycogen returned to the control values with the exception of glycogen at 4°C. In the recovery phase, both strains maintained the high glycogen amount that had accumulated during the stress conditions.

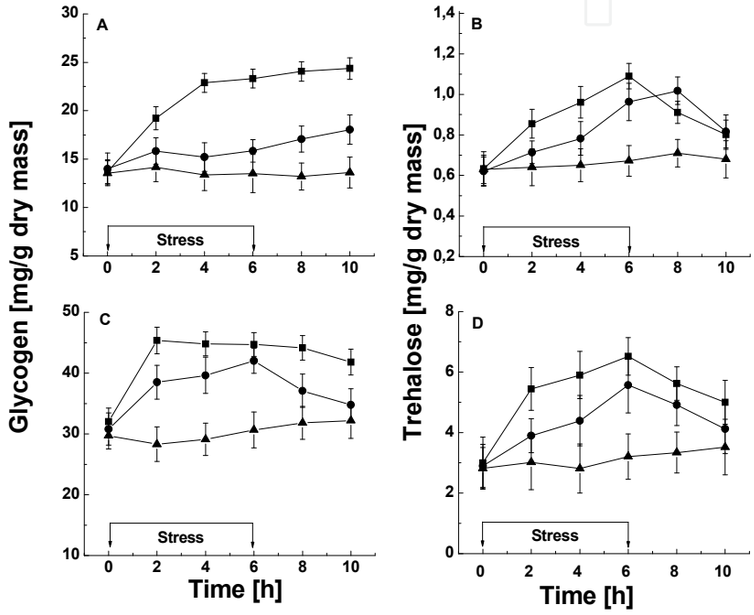


Fig. 4. Glycogen (A, C) and trehalose (B, D) content in *Penicillium* sp. 161 (A, B) and *A. glaucus* 363 (C, D) upon optimal temperature (▲) and upon temperature downshift from optimal to 4°C (■) or 10°C (●). Bars represent SD of means. The effect of treatment was significant for the temperature treatment ($P \leq 0.05$)

3.5 Changes in TPS and NT activities during cold stress conditions

Our results indicated that trehalose metabolism plays an important role in the response to cold stress (Fig. 5). In both strains used, the trehalose accumulation (see Fig. 4) paralleled a significant increase in the activity of TPS for up to 4 h, after which time the activity decreased (Fig. 5A and 5C). When *A. glaucus* 363 cells were exposed to a temperature shift to 4°C or 10°C, the TPS activity rapidly increased approximately 4- and 2.5-fold compared to the control culture, respectively. At the same conditions, *Penicillium* sp. 161 cultures demonstrated 2.4-fold higher activity than the control. The mesophilic strain demonstrated strong temperature shift-dependent changes in TPS activity, while no temperature dependence was observed in the psychrotolerant strain. Curiously, the enhanced levels of TPS in both of the Antarctic fungi coincided with an increase in NT activity (Fig. 5B and 5D). These increases were approximately 1.6- and 1.3-fold in the psychrotolerant fungus and 2.2-

and 1.7-fold in the mesophilic strain after cold shock at 4°C or 10°C, respectively, over the control value. We also found that the exposure to cold stress increased the NT levels in a time- and temperature shift-dependent manner. During the recovery phase, both strains showed a significant reduction in TPS activity, and the enzyme values reached those of the control cultures. In contrast, NT activity was maintained 4 h after the return to the optimal temperature.

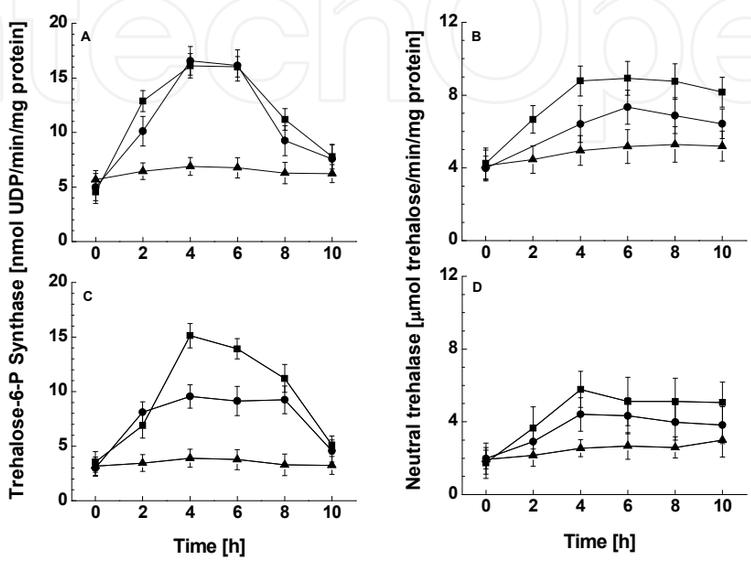


Fig. 5. Cold stress induced trehalose metabolism. TPS (A, C) and NT (B, D) accumulation in *Penicillium* sp. 161 (A, B) and *A. glaucus* 363 (C, D) upon optimal temperature (▲) and upon temperature downshift from optimal to 4°C (■) or 10°C (●). Bars represent SD of means. The effect of treatment was significant for the temperature treatment ($P \leq 0.05$)

3.6 Activation of antioxidant enzyme defence

When the mycelia, taken from middle of the exponential phase at the optimal temperature, were subjected for 6 h to 4°C or 10°C, the antioxidant enzyme defence was activated. The time courses of SOD activities for both of the strains during the cold shock treatment are shown in Fig. 6.

The results showed that transient exposure to 4°C and 10°C resulted in elevated total superoxide scavenging activity compared with the control variant, and the increase was in a dose- and time-dependent manner until 10 h, i.e., 4 h after the temperature recovery. The mesophilic strain had an approximately 7-fold higher SOD activity than the psychrotolerant strain. Moreover, the antioxidant cell response of *Penicillium* sp. 161 included an approximately 1.4- and 1.2- fold increase in SOD activity compared with the control culture at 4°C and 10°C, respectively (Fig. 6A). The fungal cultures of *A. glaucus* 363 also demonstrated a temperature-dependent response that was higher at 4°C compared with the condition at 10°C. The maximum increase was approximately 1.6- and 1.3-fold in the variants that were exposed to 4°C and 10 °C, respectively (Fig. 6B).

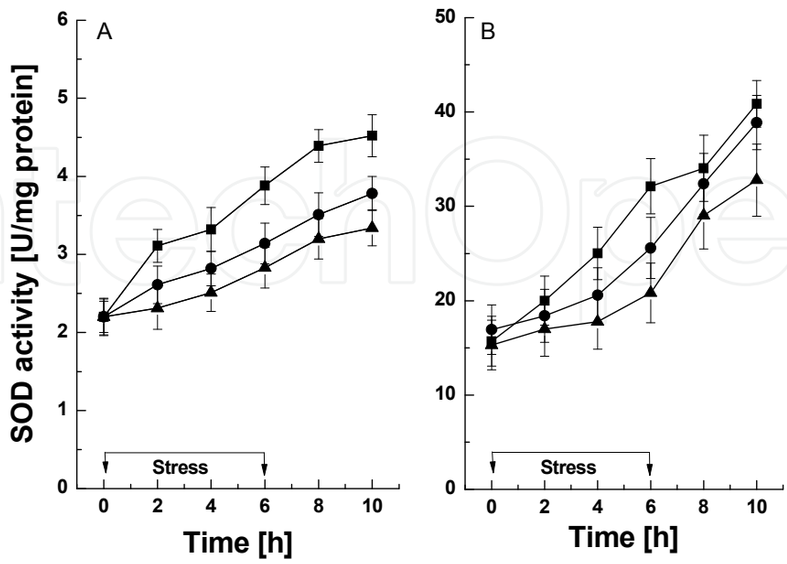


Fig. 6. SOD activities in cultures of *Penicillium* sp. 161 (A) and *A. glaucus* 363 (B) upon optimal temperature (▲) and upon temperature downshift from optimal to 4°C (■) or 10°C (●). Bars represent SD of means. The effect of treatment was significant for the temperature treatment ($P \leq 0.05$)

There were significant differences in the expression of Mn-SOD and Cu/Zn-SOD among strains of both the psychrotolerant and mesophilic thermal classes (Fig. 7). Under optimal conditions, the psychrotolerant strain produced mostly Mn-SOD and less Cu/Zn-SOD (Fig. 7A), while in the mesophilic fungus, the Cu/Zn-SOD content was found to be significantly higher than Mn-containing enzyme (Fig. 7B). The cold shock treatment caused an enhanced level of both isoenzymes, but the increased total SOD activity was due to the Cu/Zn-SOD isoform for both strains.

To confirm the levels of Mn-SOD and Cu/Zn-SOD activity in the fungal cultures, the enzymatic activity of these antioxidant enzymes was determined using the native gel electrophoresis technique (Fig. 8). The non-denaturing PAGE showed that the cell-free extracts of the psychrophilic strain contained two distinct bands with SOD activity, Cu/Zn- and Mn-SOD. At least three clear SOD isoforms were observed after cell-free extract proteins of the mesophilic strain were electrophoresed on a 10% native PAGE.

These isoforms were identified as one Mn-SOD and two Cu/Zn-SOD, which were named I and II in order of increasing migration. The temperature treatment did not change the isoenzyme profile of either strain. At the same time, there was a temperature degree-dependent increase in Cu/Zn- and Mn-containing SOD (Figs. 8B and 8C).

Figure 9 presents the data on the changes in CAT activity. In contrast to SOD, the psychrophilic strain showed a higher CAT level (1.5-fold) than that of the mesophilic strain.

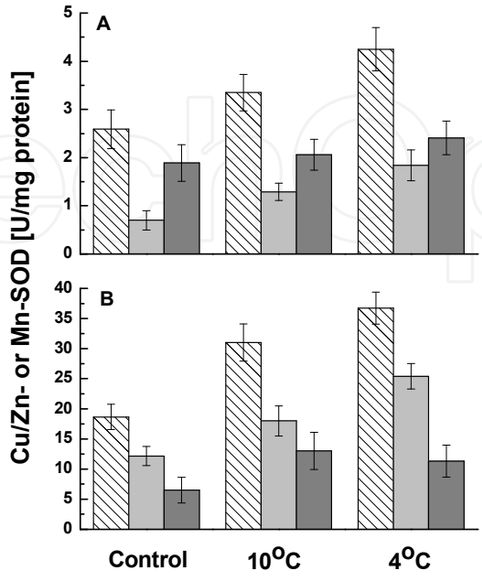


Fig. 7. Effect of low temperatures on isoenzyme profiles of *Penicillium* sp. 161 (A) and *A. glaucus* 363 (B) 4 h after the treatment: total SOD (hatchet bars); Cu/Zn-SOD (light grey bars); Mn-SOD (dark grey bars). Bars represent SD of means. The effect of treatment was significant for the temperature treatment ($P \leq 0.05$).

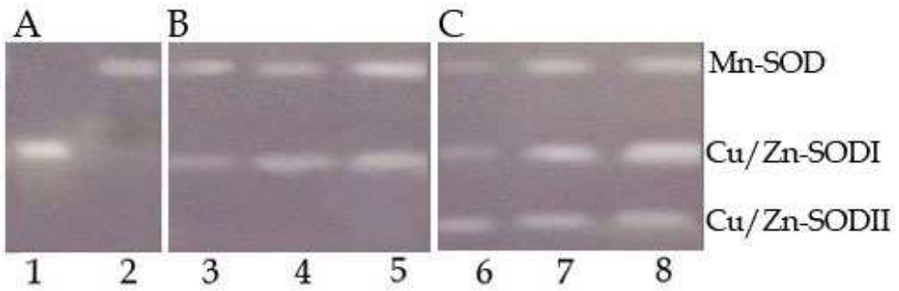


Fig. 8. Isoenzyme profiles of SOD in *Penicillium* sp. 161 and *A. glaucus* 363 cells : standards (A); SOD activity in *Penicillium* sp. 161 (B) and in *A. glaucus* 363 (C) cells evaluated by polyacrylamide gel electrophoresis (10% gel): lane 1, standard Cu/Zn-SOD from bovine erythrocytes; lane 2, standard Mn-SOD from *Escherichia coli*; lanes 3, 4, and 5, SOD in the *Penicillium* sp. 161 sp cells; lanes 6, 7 and 8, SOD in the *A. glaucus* 363 cells, cultivated at control temperatures (lanes 3 and 6), at downshift to 10°C (lanes 4 and 7) and downshift to 4°C.

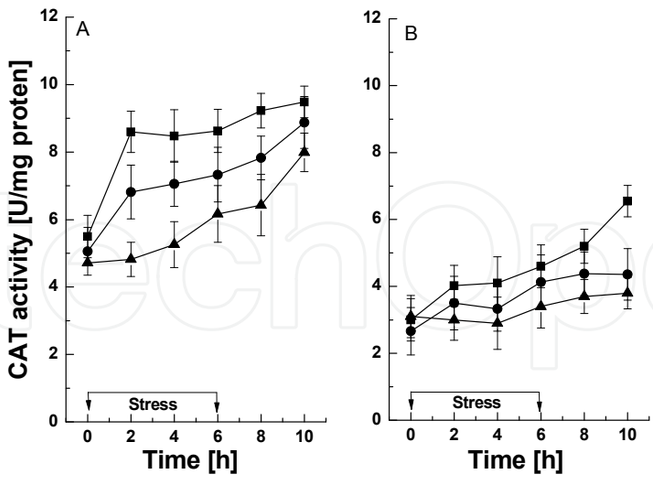


Fig. 9. CAT activities in cultures of *Penicillium* sp. 161 (A) and *A. glaucus* 363 (B) upon optimal temperature (▲) and upon temperature downshift from optimal to 4°C (■) or 10°C (●). Bars represent SD of means. The effect of treatment was significant for the temperature treatment ($P \leq 0.05$)

The low-temperature treatment resulted in a significant increase in CAT activity. The trend of the change was similar to that for SOD (a dose- and time-dependent manner). In all of the variants, the enzyme level exceeded the control value. The highest percentage of increased CAT activity (approximately 2-fold) was found in the psychrotolerant strain after the temperature downshift from 20°C to 4°C. The overexpression of CAT under the cold shock conditions was confirmed by PAGE analysis (Fig. 10). When the protein extracts were separated by native electrophoresis, one CAT isoenzyme was observed in both *Penicillium* sp. 161 and *A. glaucus* 363.

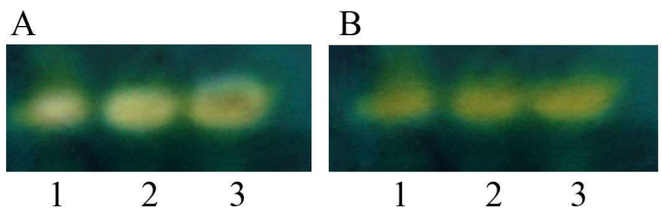


Fig. 10. CAT activity in cultures of *Penicillium* sp. 161 (A) and *A. glaucus* 363 (B) evaluated by polyacrylamide gel electrophoresis (10% gel): lane 1, Control; lane 2, cultures treated with 10°C; lane 3, cultures treated with 4°C

It is noteworthy that the tendency of increased SOD and CAT activities compared to the control variant continued even after the return to the optimal temperature.

4. Discussion

The evaluation of oxidative stress events during low-temperature treatments provides information on the relationship between oxidative stress and cold shock in lower eukaryotes

such as filamentous fungi. Very little information is available on the oxidative cell response of microorganisms from cold habitats (Gocheva et al., 2006, 2009; Tosi et al., 2010; Chattopadhyay et al., 2011; Nichols et al., 1999). Even less is known concerning filamentous fungi from the Bulgarian Antarctic area. We choose to compare the cell responses between fungi belonging to two different thermal classes, psychrotolerant and mesophilic, that are able to grow in a wide range of temperatures. According to many reports on fungal biodiversity, these fungi are prevalent in Antarctica (Onofri et al., 2007, 2008; Arenz & Blanchette, 2011). Our results confirmed that the downshift of temperature induces typical events of oxidative stress. The exposure of both fungal cultures to 4°C or 10°C caused a statistically significant reduction in biomass production and glucose consumption compared to the control. Despite their genetic adaptation to survive in the harsh Antarctic environment, the sharp decrease in temperature resulted in a cessation of growth, which was more pronounced in the mesophilic fungus than in the psychrophilic fungus. A possible explanation or cause for this discrepancy is that most of the Antarctic mycoflora is metabolically active whenever a combination of favourable abiotic conditions occurs during the short growing summer season (Ruisi et al., 2007). However, Hébraud M. & Potier (1999) assumed that, as opposed to mesophiles, one or more regulatory factors exists in cold-adapted microorganisms prior to cold shock that allows for the maintenance of functional translational machinery at low temperatures. However, despite this continuous protein synthesis, the growth of psychrotrophic strains ceases transiently after a cold shock, and additional regulatory mechanisms may exist that allow for growth resumption at low temperatures.

Additionally, the data for all cold shock variants reveal enhanced ROS generation. These ROS can be generated via a variety of extra-environmental conditions, including low temperature exposure (Belozerskaya & Gessler 2007; Gessler et al., 2007; Gómez-Toribio et al., 2009; Gocheva et al., 2009; Krumova et al., 2009). As is known, the unstressed fungal cells produce $O_2^{\bullet -}$ and H_2O_2, presumably due to a single electron reduction of 2% of the consumed oxygen as has been previously suggested (Joseph-Horne et al., 2001). Our direct assay showed a significant increase in $O_2^{\bullet -}$ under cold exposure to 4°C and 10°C in dose-dependent manner. A similar but less-pronounced tendency was found for H_2O_2 levels. This increase was more extreme in experiments with mitochondria than in those with intact cells. Thus, cold stress likely imposes an oxidative burden of which $O_2^{\bullet -}$ is a major component. A similar direct analysis of ROS content in fungal cells has not often been reported. Chattopadhyay et al. (2011) reported that the production of free radicals in the Antarctic bacterium *Pseudomonas fluorescens* MTCC 667 was higher at low temperature compared to that at 22°C. When cells are exposed to low temperature, the rate of enzymatic reactions decreases, leading to a decrease in the demand for ATP and an accumulation of electrons at certain points in the respiratory chain. The situation promotes a sudden increase in the production of a number of ROS, which remove the burden of the excess reducing potential (Chattopadhyay, 2002). According to Zhang et al. (2003), the growth temperature downshift increases ROS levels in *Saccharomyces cerevisiae*. The authors reported that H_2O_2 is a major component in low temperature-exposed yeast cells. In contrast to heterotrophic organisms, most plants possess photosynthetic systems that, when out of control, may produce a lot of ROS and other reactive species (Lushchak, 2011). According to Hu et al.(2008), cold-stress inhibited net photosynthetic rate (P_N) and cytochrome respiratory pathway but enhanced the photosynthetic electron flux to O_2 and over-reduction of respiratory electron transport

chain, resulting in ROS accumulation in cucumber leaves. For fish living at low temperatures, the increased polyunsaturation of mitochondrial membranes should raise rates of mitochondrial respiration which would in turn enhance the formation of ROS, increase proton leak and favour peroxidation of these membranes (Guderley, 2004).

Consistent with previous reports (Şahin & Gümüşlü, 2004; Manfredini et al., 2005; Nyström, 2005; Li et al., 2009), the present study demonstrated the coexistence of enhanced ROS activity and oxidatively damaged proteins in Antarctic fungal models. ROS can modify proteins leading to the formation of additional carbonyl groups caused by the oxidation of specific amino acid residues or the protein backbone (Davies & Goldberg, 1987). The level of proteins with formed additional carbonyl groups, which is thought to be an excellent marker for protein oxidation, has been shown to be related to oxidative stress in various studies. Low temperature stress was shown to induce ROS accumulation in cells (Suzuki & Mittler, 2006; Ouellet, 2007), which leads to a production of oxidised proteins. A similar response was observed in hyper-oxygenated cultures of white-rot fungus *Phanerochaete chrysosporium* (Belinky et al., 2003), heavy metal treatment (Krumova et al., 2009; Belozerskaya et al., 2010), addition of oxidant species (Angelova et al., 2005) and temperature exposure (Kim et al., 2006; Li et al., 2008; Gocheva et al., 2009), for example. In the temperature-induced protein carbonylation, Li et al. (2008) speculated that respiratory enzymes and those within mitochondria may be especially vulnerable because oxygen-derived free radicals from the respiratory chain are generally held to be the main source of the oxidative damage seen during stress.

The psychrotolerant strain *Penicillium* sp. 161 demonstrated a lower level of oxidatively damaged proteins than that in the mesophilic strain *A. glaucus* 363 during the exposure to 4°C or 10°C. At the same time, our unpublished data demonstrate the availability of cold-acclimation proteins (Caps) in *Penicillium* sp. 161 and cold shock proteins (Csps) in *A. glaucus* 363. According to D'Amico et al. (2006), the existing distinctions between the mesophilic and the psychrophilic cold shock response include the lack of repression of housekeeping protein synthesis and the presence of Caps in psychrophiles. The authors considered that many of the Csps involved in various cellular processes, such as transcription, translation, protein folding and the regulation of membrane fluidity and observed in mesophiles (D'Amico et al., 2006, as cited in Phadtare, 1994), act as Caps in psychrophiles, and are constitutively rather than transiently expressed at low temperatures. Furthermore, this differential regulation of expression indicates that a temperature-sensory system exists in psychrophiles, and thermosensors at the cell membrane level, which sense changes in fluidity, have been reported (D'Amico et al., 2006, as cited in Ray et al., 1994).

Trehalose and glycogen accumulation in microbial cells when they are subjected to unfavourable growth conditions could also be used as stress indicators (Parrou et al., 1997; Türkel, 2006; Ocón et al., 2007; Iturriaga et al., 2009; Gonçalves et al., 2011). Both carbohydrates are important storage compounds in fungal vegetative cells and spores (Robinson, 2001; Rúa et al., 2008). In Antarctic fungi, *Penicillium* sp. 161 and *A. glaucus* 363, that were exposed to cold stress, a reduction in glucose consumption coincided with a significant increase in the trehalose and glycogen content (Fig. 4). These results agree with earlier studies regarding the microbial response against different types of abiotic stress (Ostrovskii et al., 2003; Jules et al., 2008; Robinson, 2001). Our previous studies have

confirmed the role of these reserve carbohydrates in the survival strategies of filamentous fungi under stress conditions such as heavy metal toxicity (Krumova et al., 2009), heat shock (Abrashev et al., 2008), oxidative stress-agent treatment (Angelova et al., 2005) and low temperature exposure (Gocheva et al., 2009). In contrast, heat stress led to a significant decrease in the glycogen and trehalose content of *Pichia anomala* (Parrou et al., 1997) and *P. angusta* cells (Türkel, 2006), respectively. However, the nitrogen starvation-induced biosynthesis and the accumulation of trehalose and glycogen in the same strain, *P. angusta*, suggested that both stress responses do not have an overlapping signal transduction pathway in the activation of trehalose and glycogen biosynthesis in this *Pichia* species (Türkel, 2006). Jules et al. (2008) confirmed an interconnection between trehalose and glycogen in yeast cell carbon storage management. They found that glycogen was accumulated earlier and faster, which indicated the presence of a fine-tuning control during periods of stress. In contrast, our results showed that both reserve carbohydrates started to increase simultaneously, immediately after the temperature downshift (Fig. 4), and glycogen maintained its higher level after the recovery to the optimal temperature longer than trehalose did.

Considering that almost all of the genes encoding the enzymes involved in the metabolism of these two reserve carbohydrates contain between one and several copies of the stress-responsive element (STRE), Parrou et al. (1997) suggested the possibility of a link between the potential transcriptional induction of these genes and the accumulation of glycogen and trehalose under different stress conditions. In this study, we evaluated the activities of the trehalose-metabolising enzymes TPS and NT during cold stress conditions. In the used Antarctic strains, cold shock elicits a complex response that involves the activation of both biosynthesis and biodegradation pathways, but not to the same extent. The increase in TPS activity significantly exceeds that of NT activity. This situation leads to a high level of accumulation of trehalose during low temperature exposure. Thus, the intracellular level of trehalose is the result of a well-regulated balance between enzymatic synthesis and degradation. In *Saccharomyces cerevisiae*, an enhanced level of trehalose was the result of the stimulation of trehalose synthase and the inhibition of trehalase by high temperature (Parrou et al., 1997). Moreover, after the stress had ceased, trehalose concentrations in our experiments returned to basal levels, indicating that neutral trehalase activity could have a role in cold shock recovery.

It is well known that the antioxidant systems of microorganisms, including fungi, act as important tolerance mechanisms against abiotic stress. Enhanced SOD and CAT activities have been associated with the induced resistance of fungi to different stress factors (Manfredini et al., 2005; Li et al., 2009; Belozerskaya et al., 2010; Chattopadhyay et al., 2011). Both activities are involved in superoxide radical and hydrogen peroxide scavenging. Our results demonstrated that the exposure to cold shock for 6 h induced the expression of SOD and CAT in a psychrotolerant strain and in a mesophilic strain.

Furthermore, the increase in SOD activity was more pronounced for the mesophilic fungus, while the activation of antioxidant enzyme defence in the psychrotolerant *Penicillium* was primarily due to an increase in CAT activity. One possible explanation for this finding is a much higher level of $O_2^{\bullet -}$ generated upon low temperature stress in the mesophilic strain and higher levels of H_2O_2 in psychrotolerant cultures (Table 1 and Table 2). To antagonise ROS, *A. glaucus* 363 induces SOD synthesis to a much higher degree, while *Penicillium* sp. 161 enhances CAT levels.

The observations by PAGE analysis suggest that in *A. glaucus* 363, Cu/Zn-SODI is primarily involved in the fungal line of defence under the conditions of temperature downshift. In contrast, Mn-SOD activity was considerably higher than Cu/Zn-SODI in *Penicillium* sp. 161, possibly because of a higher level of $O_2^{\bullet -}$, which was generated in mitochondria during the cold stress (Table 1)**.** Moreover, the PAGE results confirmed the significant role of CAT induction in the cold-stress response, especially in the psychrotolerant strain (Fig. 10).

5. Conclusions

Our results provide further evidence indicating a relationship between cold shock and oxidative stress. Growth at low temperature clearly induced oxidative stress events in both of the Antarctic fungal strains tested, which consisted of enhanced levels of oxidatively damaged proteins, the accumulation of reserve carbohydrates and increased activity of the antioxidant enzyme defence. Despite the significant induction of antioxidant enzymes, exposure to low temperature is damaging and appears to exceed the antioxidant defence. Although the tested strains were isolated from Antarctic soil samples, they both demonstrated a different degree of oxidative stress damage and strategy of antioxidant defence. The psychrotolerant strain *Penicillium* sp. 161. more successfully confronted the challenge of low temperature, which slows metabolite flux, than the mesophilic *A. glaucus* 363. As shown by Methé et al. (2005), comparative genome analyses suggests that the psychrophilic lifestyle is most likely conferred not by a unique set of genes, but by a collection of synergistic changes in the overall genome content and amino acid composition.

Analysis of the relationship between oxidative stress and cold stress in different thermal classes of Antarctic microorganisms will improve our understanding, at the molecular level, of the strategies and mechanisms that facilitate fungal cell survival in harsh environments, and the necessary foundation for practical applications. Future study using comparative molecular analysis of genes coding for proteins with antioxidant enzyme activity in psychrotolerant and mesophilic strains by the bioinformatics approach will enhance the knowledge of cold stress signaling mechanisms in eukaryotic microorganisms. From a biotechnological point of view, new information of cold stress response of Antarctic fungi can help further our efforts to develop an effective technology for production of cold-active enzyme SOD. Such SOD can be very important antioxidant in cryopreservation processes (Rossi et al., 2001), cryosurgery and organ transplantation (Sun et al., 2006), *in vitro* fertilization (Agarwal et al., 2006) etc. Cold-active SOD can be used in all cosmetic formulations, to promote, at least, younger looking skin (Lods et al., 2000; Diehl, 2009).

6. Acknowledgment

This work was supported by the NCSI of the Ministry of Education and Science, Bulgaria (grant DO02-172/08 and grant VU-B-205/06) to which sincere thanks are owed.

7. References

Abrashev, R.I., Pashova, S. B., Stefanova, L.N., Vassilev, S.V., Dolashka-Angelova, P.A. & Angelova, M.B. (2008). Heat-shock-induced oxidative stress and antioxidant response in *Aspergillus niger* 26. *Canadian Journal of Microbiology*, Vol. 54, No. 12, (December 2008), pp. 977-983, ISSN 1480-3275

Adachi, H. & Ishii, N. (2000). Effects of tocotrienols on life span and protein carbonylation in Caenorhabditis elegans. *Journals of Gerontology Series A, Biological Sciences and Medical Sciences*, Vol. 55, No. 6, (June 2000), B280–B285, ISSN 1079-5006

Angelova, M., Genova, L., Pashova, S., Slokoska, L. & Dolashka, P. (1996). Effect of cultural conditions on the synthesis of superoxide dismutase by *Humicola lutea* 110. *Journal of Fermentation and Bioengineering*, Vol. 82, No. 5, (July 1996), pp. 464-468, ISSN 0922-338X

Angelova, M.B., Pashova, S.B., Spasova, B.K., Vassilev, S.V. & Slokoska, L.S. (2005). Oxidative stress response of filamentous fungi induced by hydrogen peroxide and paraquat. Mycological Research, Vol. 109, No. 2, (February 2005), pp. 150–158, ISSN 0953-7562

Agarwal A., Gupta S. & Sikka S. (2006). The role of free radicals and antioxidants in reproduction. *Current Opinion in Obstetrics and Gynecology*, 2006, Vol. 18, № 3 (June 2006) pp. 325–332, ISSN 1040872X – 1040-872X

Arenz, B.E. & Blanchette, R.A. **(**2011). Distribution and abundance of soil fungi in Antarctica at sites on the Peninsula, Ross Sea Region and McMurdo Dry Valleys. *Soil Biology and Biotechnology*, Vol. 43, № 2, (February 2011), pp. 308-315, ISSN 0038-0717

Beauchamp, C. & Fridovich, I. (1971). Superoxide dismutase: Improved assay and an assay applicable to polyacrylamide gels. *Analytical Biochemistry*, Vol. 44, No. 1, (November 1971), pp. 276-287, ISSN 0003-2697

Becker, J. (1978). A method of glycogen determination in whole yeast cells. *Analytical Biochemistry*, Vol. 86, No. 1, (May 1978), pp. 56-64, ISSN 0003-2697

Beers, R.F. & Sizer, I.W. (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry*, Vol. 195, No. 1, (March 1952), pp. 133-140, ISSN 0021-9258

Belinky, P.A., Flikshtein, N., Lechenko, S., Gepstein, S. & Dosoretz, C. G. (2003). Reactive oxygen species and induction of lignin peroxidase in *Phanerochaete chrysosporium*. Applied and Environmental Microbiology, Vol. 69, No. 11, (November 2003), pp. 6500–6506, ISSN 0099-2240

Belozerskaya, T.A., & Gessler, N.N. (2007). Reactive Oxygen Species and the Strategy of Antioxidant Defense in Fungi: *A Review. Applied and Biochemistry and. Microbiology*, Vol. 43, No. 5, (September 2007), pp. 506–515, ISSN 0003-6838

Belozerskaya, T., Aslanidi, K., Ivanova, A., Gessler, N., Egorova, A., Karpenko, Yu. & Olishevskaya, S. (2010). Characteristics of Extremophylic Fungi from Chernobyl Nuclear Power Plant. In: *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, Mendez-Vilas A. (*ed*.), 88-94, Vol. 1, Formatex Research Center, *ISBN* 978-84-614-6194-3, Badajoz, Spain

Chattopadhyay, M. K. (2002). Low temperature and oxidative stress. *Current Science*, Vol. 83, No. 2, (July 2002), pp. 109, ISSN 0011-3891

Chattopadhyay, M. K., Raghu, G., Sharma, Y. V. R. K., Biju, A.R., Rajasekharan, M.V. & Shivaji, S. (2011). Increase in Oxidative Stress at Low Temperature in an Antarctic Bacterium. *Current Microbiology*, Vol. 62, No. 2, (February 2011), pp. 544–546, ISSN 1432-0991

Chintalapati, S., Kiran, M.D. & Shivaji, S. (2004). Role of membrane lipid fatty acids in cold adaptation. *Cellular and Molecular Biology*, Vol. 50, No. 5, (July 2004), pp. 631-642, ISSN 1165-158X

Collins, T., Roulling, F., Piette, F., Marx, J. C., Feller, G., Gerday, C. & D'Amico, S. (2008). Fundamentals of Cold-Adapted Enzymes. In: *Psychrophiles: from Biodiversity to Biotechnology,* Margesin R., Schinner F., Gerday C., Marx J.C. (Eds), 211-227, Springer-Verlag, ISBN 978-3-540-74334-7, Berlin, Heidelberg

D'Amico, S., Collins, T., Marx, J., Feller, G., & Gerday, C. (2006). Psychrophilic microorganisms: challenges for life. *European Molecular Biology Organization Reports*, Vol. **7**, No. 4, (April 2006), pp. 385 – 389, ISSN 1469-221X

Davies, K.J.A. & Goldberg, A.L. (1987). Proteins damaged by oxygen radicals are rapidly degraded in extracts of red blood cells. *Journal of Biological Chemistry*, Vol. 262, No. 17, (June 1987), pp. 8227-8234, ISSN 0021-9258

Diehl C. (2009). Use of a Mixture of Superoxide Dismutase and Catalase for Treating Inflammatory Skin Lesions. United States Patent Pub. № US 2009/0092591

El-Bashiti, T., Hamamcı, H., Öktem, H.A. & Yücel, M. (2005). Biochemical analysis of trehalose and its metabolizing enzymes in wheat under abiotic stress conditions. *Plant Science*, Vol. 169, No. 1, (July 2005), pp. 47–54, ISSN 0168-9452

Feller, G. & Gerday, C. (2003). Psychrophilic enzymes: Hot topics in cold adaptation. *Nature reviews. Microbiology*, Vol. 1, No. 3, (December 2003), pp. 200-208, ISSN 1740-1526

Fridovich, I. (1998). Oxygen toxicity, a radical explanation. *The Journal of Experimental Biology*., Vol. 201, No. 8, (April 1998), pp. 1203-1209, ISSN 0022-0949

García-Arribas, O., Mateo, R., Tomczak, M.M., Davies, P.L. & Mateu, M.G. (February 2007). Thermodynamic stability of a cold-adapted protein, type III antifreeze protein, and energetic contribution of salt bridges. In: *Protein Science*, Vol. 16, No. 2, (February 2007), pp. 227–238, ISSN 1469-896x

Gatti-Lafranconi, P., Natalello, A., Rehm, S., Doglia, S.M., Pleiss, J. & Lotti, M. (2010). Evolution of stability in a cold-active enzyme elicits specificity relaxation and highlights substrate-related effects on temperature adaptation. Journal of Molecular Biology, Vol. 395, No. 1, pp. 155-166, ISSN 0022-2836

Gessler, N.N., Averýanov, A.A. & Belozerskaya, T.A. (2007). Reactive oxygen species in regulation of fungal development. *Biochemistry (Mosc),* Vol. 72, No. 10, (October 2007), pp. 1091-109, ISSN 0006-2979

Gocheva, Y., Krumova, E., Slokoska, L., Gesheva, V. & Angelova, M. (2005). Isolation of filamentous fungi from Antarctica. *Comptes-rendus de l'Académie Bulgare des Sciences*, Vol. 58, No. 4, pp. 403-408, ISSN 1310-1331

Gocheva, Y., Krumova, E., Slokoska, L., Miteva, J. & Angelova, M. (2006). Cell response of Antarctic and temperate strains of *Penicillium* spp. to different growth temperature. *Mycological Research*, Vol. 110, No. 11, (November 2006), pp. 1347-1354, ISSN 0953-7562

Gocheva, Y., Tosi, S., Krumova, E., Slokoska, L., Miteva, J., Vassilev, S. & Angelova, M. (2009). Temperature downshift induced antioxidant response in fungi isolated from Antarctica. *Extremophiles,* Vol. 13, No. 2, (March 2009), pp. 273-281, ISSN 1431-0651

Gómez-Toribio, V., García-Martín, A.B., Martínez, M.J., Martínez, Á.T. & Guillén, F. (2009). Induction of Extracellular Hydroxyl Radical Production by White-Rot Fungi through Quinone Redox Cycling. *Applied and Environmental Microbiology,* Vol. 75, No. 12, (June 2009), pp. 3944–3953, ISSN 0099-2240

Gonçalves, R.D., Cupertino, F.B., Freitas, F.Z., Luchessi, A.D. & Bertolini, M.C. (2011). A genome-wide screen for Neurospora crassa transcription factors regulating glycogen metabolism. *Molecular and Cellular Proteomics,* (July 2011) ISSN: 1535-9484 MCP Papers in Press. Published on July 18, 2011 as Manuscript M111.007963 DOI: 10.1074/mcp.M111.007963

Gray, N.F., & Lewis-Smith, R.I. (1984). The distribution of nematophagous fungi in the maritime Antarctic. *Mycopathologia,* Vol. 85, No. 1-2, (March 1984), pp. 81-92, ISSN 0301-486X

Guderley H. (2004). Metabolic responses to low temperature in fish muscle. *Biological Reviews*, Vol. 79, № 2, (May 2004), pp. 409-427, ISSN 1464-7931

Hart, P.J., Belbirine, M.M., Ogihara, N.L., Nersissian, A.M., Weiss, M.S., Valentine, J.S. & Eisenberg, D (1999). A structure-base mechanism for cooper-zinc superoxide dismutase. Biochemistry, 38 (7), (February 1999), pp. 2167-2178, ISSN: 0006-2960.

Hassan, H.M. & Fridovich, I. (1979). Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. *Archives of Biochemistry and Biophysics*, Vol. 196, No. 2, (September 1979), pp. 385-395, ISSN 0003-9861

Hébraud, M. & Potier, P. (1999). Cold Shock Response and Low Temperature Adaptation in Psychrotrophic Bacteria. *Journal of Molecular Microbiology and Biotechnology,* Vol. 1, No. 2, (November 1999), pp. 211-219, ISSN 1464-1801

Horn, G., Hofweber, R., Kremer, W. & Kalbitzer, H.R. (2007). Structure and function of bacterial cold shock proteins. *Cellular and Molecular Life Sciences,* Vol. 64, No. 12, (April 2007), pp. 1457–1470, ISSN 1420-682X

Hu W.H., Song X.S., Shi K., Xia X.J., Zhou Y.H. & Yu J.Q. (2008). Changes in electron transport, superoxide dismutase and ascorbate peroxidase isoenzymes in chloroplasts and mitochondria of cucumber leaves as influenced by chilling. *Photosynthetica*, Vol. *46,* No. 4, (December 2008), pp. 581-588, ISSN 0300-3604

Inouye, M. (1999). Cold-Shock Response and Adaptation. *Journal of Molecular Microbiology and Biotechnology,* Vol. 1, No. 2, (November *1999*), pp. 191, ISSN 1464-1801

Iturriaga, G., Suárez, R. & Nova-Franco, B. (2009). Trehalose Metabolism: From Osmoprotection to Signaling. *International Journal of Molecular Sciences*, Vol. 10, No. 9, (September 2009), pp. 3793-3810, ISSN 1422-0067

Joseph-Horne, T., Hollomon, D.W., & Wood, P.M. (2001). Fungal respiration: a fusion of standard and alternative components. *Biochimica et biophysica acta,* Vol. 1504, No. 2-3, (April 2001), pp. 179-195, ISSN 0006-3002

Jules, M., Beltran, G., François, J. & Parrou, J.L. (2008). New Insights into Trehalose Metabolism by *Saccharomyces cerevisiae*: *NTH2* Encodes a Functional Cytosolic Trehalase, and Deletion of *TPS1* Reveals Ath1p-Dependent Trehalose Mobilization. *Applied and Environmental Microbiology*, Vol. 74, No. 3, (February 2008), pp. 605–614, ISSN 0099-2240

Kim, I. S., Moon, H. Y., Yun ,H. S. & Jin, I. (2006). Heat shock causes oxidative stress and induces a variety of cell rescue proteins in Saccharomyces cerevisiae KNU5377. *Journal of microbiology*, Vol. 44, No. 5, (October 2006), pp. 492-501, ISSN 1225-8873

Krumova, E., Dolashki, A., Pashova, S., Dolashka-Angelova, P., Stevanovic, S., Hristova, R., Stefanova, L., Voelter, W. & Angelova, M. (2008). Unusual location and characterization of Cu/Zn-containing superoxide dismutase from filamentous fungus *Humicola lutea. Archives of Microbiology*, Vol. 189, No. 2, (February 2008), pp. 121-30, ISSN 0302-8933

Krumova, E.Z., Pashova, S.B., Dolashka-Angelova, P.A., Stefanova, T. & Angelova, M.B. (2009). Biomarkers of oxidative stress in the fungal strain *Humicola lutea* under copper exposure. *Process Biochemistry*, Vol. 44 No. 3, (March 2009), pp. 288-295, ISSN 1359-5113

Kwak, K.J., Park, S.J., Han, J.H., Kim, M.K., Han O.S., Han, Y.S. & Kang, H. (2011). Structural determinants crucial to the RNA chaperone activity of glycine-rich RNA-binding proteins 4 and 7 in Arabidopsis thaliana during the cold adaptation process. *Journal of Experimental Botany*, Vol. 62, No. 11, (April 2011), pp. 4003-4011, ISSN 0022-0957

Li, Q., Harvey, L.M. & McNeil, B. (2008). The effects of elevated process temperature on the protein carbonyls in the filamentous fungus, *Aspergillus niger* B1-D. *Process Biochemistry*, Vol. 43, No. 8, (August 2008), pp. 877-881, ISSN 1359-5113

Li, Q., Harvey, L.M. & McNeil, B. (2009). Oxidative stress in industrial fungi. *Critical Review in Biotechnology*, Vol. 29, No. 3, (September 2009), pp. 199-213, ISSN 0738-8551

Lods L.M., Dres C., Johson C., Scholz D.B. & Brooks G.J. (2000). The future of enzymes in cosmetics *International Journal of Cosmetic Science*, Vol. 22, № 2, (April 2000), pp. 85-94, ISSN 0142-5463

Lowry, O.H., Rosenbrough, H.J., Faar, A.L. & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, Vol. 193, No. 1, (November 1951), pp. 265-275, ISSN 0021-9258

Lushchak V.I. (2011). Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comparative Biochemistry and Physiology, Part C* Vol. 153, No. 2, (March 2011), pp. 175–190, ISSN 0742-8413

Manfredini, V., Martins, V.D., do Carmo, M., Peralba, R. & Benfato, M.S. (2005). Adaptative response to enhanced basal oxidative damage in *sod* mutants from *Saccharomyces cerevisiae*. *Molecular and Cellular Biochemistry,* Vol. 276, No. 1-2, (August 2005), pp. 175–181, ISSN 0300-8177

Methé, B.A., Nelson, K.E., Deming, J.W., Momen, B., Melamud, E., Zhang, X., Moult, J., Madupu, R., Nelson, W.C., Dodson, R.J., Brinkac, L.M., Daugherty, S.C., Durkin, A.S., DeBoy, R.T., Kolonay, J.F., Sullivan, S.A., Zhou, L., Davidsen, T.M., Wu, M., Huston, L.M., Weaver, B., Weidman, J.F., Khouri, H., Utterback, T.R., Feldblyum, T.V. & Fraser, C.M. (2005). The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 102, No. 31, (August 2005), pp. 10913-10918, ISSN 0027-8424

Morgan-Kiss, R.M., Priscu, J.C., Pocock, T., Gudynaite-Savitch, L. & Huner, N.P.A. (2006). Adaptation and Acclimation of Photosynthetic Microorganisms to Permanently

Cold Environments. *Microbiology and Molecular Biology Reviews*, Vol. 70, No. 1, (March 2006), pp. 222-252, ISSN 1092-2172

Müller, J., Staehelin, C., Mellor, R., Boller, T. & Wiemken, A. (1992). Partial purification and characterization of trehalose from soybean nodules. *Journal of Plant Physiology*, Vol. 140, No.1, (January 1992), pp. 8–13, ISSN 0176-1617

Nichols, D., Bowman, J., Sanderson, K., Nichols, C., Lewis, T., Mcmeekin, T. & Nichols, P.D. (1999). Development with Antarctic microorganisms: culture collections, bioactivity screening, taxonomy, PUFA production and cold-adapted enzymes. *Current Opinion in Biotechnology*, Vol. 10, No. 3, (June 1999), pp. 240-246, ISSN 0958-1669

Nyström, T. (2005). Role of oxidative carbonylation in protein quality control and senescence, *The EMBO Journal*, Vol. 24, No. 7, (April 2005), pp. 1311–1317 ISSN 0261-4189

Ocón, A., Hampp, R. & Requena, N. (2007). Trehalose turnover during abiotic stress in arbuscular mycorrhizal fungi. *New Phytologist,* Vol. 174, No. 4, pp. 879–891, (June 2007), ISSN 0028- 646X

Onofri, S., Barreca, D., Selbmann, L., Isola, D., Rabbow, E., Horneck, G., de Vera, J.P.P., Hatton, J. & Zucconi, L. (2008). Resistance of Antarctic black fungi and cryptoendolithic communities to simulated space and Martian conditions. *Studies in Mycology*, Vol. 61, No. 1, (December 2008), pp. 99–109, ISSN 0166-0616

Onofri, S., Selbmann, L., de Hoog, G.S., Grube, M., Barreca, D., Ruisi, S. & Zucconi, L. (2007). Evolution and adaptation of fungi at boundaries of life. *Advances in Space Research*, Vol. 40, No. 11, (December 2007), pp. 1657–1664, ISSN 0273-1177

Ostrovskii, D.N., Demina, G.R., Biniukov, V.I., Shashkov, A.S. & Schloter, M. (2003). Free radicals in mercury-resistant bacteria indicate a novel metabolic pathway. *Mikrobilogiia (In Russian*), Vol. 72, No. 5, (September-October 2003), pp. 594-599, ISSN 0026-3656

Ouellet, F. (2007). Cold Acclimation and Freezing Tolerance in Plants. In: *Encyclopedia of life sciences*, (September 2007), John Wiley & Sons, Ltd., http://onlinelibrary.wiley.com/doi/10.1002/9780470015902.a0020093/pdf

Parrou, J.L., Teste, M.A. & François, J. (1997). Effects of various types of stress on the metabolism of reserve carbohydrates in *Saccharomyces cerevisiae*: genetic evidence for a stress-induced recycling of glycogen and trehalose. *Microbiology,* Vol. 143, No. 6, (June 1997) pp. 1891-1900, ISSN 0026-2617

Pepi, M., Agnorelli, C. & Bargagli, R. (2005). Iron Demand by Thermophilic and Mesophilic Bacteria Isolated from an Antarctic Geothermal Soil. *Biometals,* Vol. 18, (October 2005), pp. 529–536, ISSN 0966-0844

Pick, E. & Mizel, D. (1981). Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an autoenzyme immunoassay reader. *Journal of Immunological Methods*, Vol. 46, No. 2, (January 1981), pp. 211–226, ISSN 0022-1759

Ray M. K. (2006). Cold-stress response of low temperature adapted bacteria. In: Stress Response: A Molecular Biology Approach, A.S. Sreedhar & U.K. Srinivas (Eds.), pp. 1-23, Research Signpost, ISBN: 81-308-0109-4, Kerala, India

Robinson, C.H. (2001). Cold adaptation in Arctic and Antarctic fungi. *New Phytologist*, Vol. 151, No. 2, (August 2001), pp. 341-353, ISSN 0028- 646X

Rossi T., Mazzilli F., Delfino M. & Dondero F. (2001). Improved Human Sperm Recovery Using Superoxide Dismutase and Catalase Supplementation in Semen Cryopreservation Procedure. *Cell and Tissue Banking,* Vol. 2, № 12, (March 2001), pp. 9-13, ASSN 1389-9333

Rúa, J., de Cima, S., del Valle, P., Gutiérrez-Larraínzar, M., Busto, F., & ،de Arriaga, D. (2008) Glycogen and trehalose mobilization by acetic acid in *Phycomyces blakesleeanus*: dependence on the anion form. *Research in Microbiology*, Vol. 159, No. 3, (April 2008), pp. 200-206, ISSN 0923-2508

Ruisi, S., Barreca, D., Selbmann, L., Zucconi, L., & Onofri, S. (2007). Fungi in Antarctica. *Reviews in Environmental Science and Biotechnology*, Vol. 6, No. 1-3, (August 2007), pp. 127– 141, ISSN 1569-1705

Russell, N.J. (2008). Membrane components and cold sensing. Fundamentals of Cold-Adapted Enzymes. In: *Psychrophiles: from biodiversity to biotechnology,* Margesin R., Schinner F., Gerday C., Marx J. C. (Eds), 177-190, Springer-Verlag, ISBN 978-3-540-74334-7, Heidelberg

Russo, R., Riccio, A., di Prisco, G., Verde, C. & Giordano, D. (2010). Molecular adaptations in Antarctic fish and bacteria. *Polar Science*, Vol. 4, No. 2, (August 2010), pp. 245-256

Şahin, E., & Gümüşlü, S. (2004). Cold-stress-induced modulation of antioxidant defense: role of stressed conditions in tissue injury followed by protein oxidation and lipid peroxidation. *International journal of biometeorology*, Vol. 48, No. 4, (May 2004), pp. 165-171, ISSN 0020-7128

Sies, H. (1993). Strategies of antioxidant defence. *European Journal of Biochemistry*, Vol. 215, No. 2, (July 1993), pp. 213-219, ISSN 0014-2956

Somogy, M. (1952). Notes on sugar determination. *Journal of Biological Chemistry,* Vol. 195, No. 1, (March 1952), pp. 19-23, ISSN 0021-9258

Sun, H-W., Shen F. & Zhou Y-M. (2006). Influence of perfusion by gaseous oxygen persufflation on rat donor liver. *Hepatobiliary & Pancreatic Diseases International*, Vol. 5, *№ 2, (*May 2006), pp. 195-198, ISSN 1499-3872

Suzuki, N., & Mittler, R. (2006). Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. *Physiologia Plantarum*, Vol. 126, No. 1, (January 2006), pp. 45–51, ISSN 0031-9317

Tosi, S., Kostadinova, N., Krumova, E., Pashova, S., Dishliiska, V., Spassova, B., Vassilev, S. & Angelova, M. (2010). Antioxidant enzyme activity of filamentous fungi isolated from Livingston Island, Maritime Antarctica. *Polar Biology*, Vol. 33, No. 9, (May 2010), pp. 1227-1237, ISSN 0722-4060

Türkel, S. (2006). Comparative Analysis of Glycogen and Trehalose Accumulation in Methylotrophic and Nonmethylotrophic Yeast. *Microbiology*, Vol. 75, No. 6, (December, 2006), pp. 639–643, ISSN 0026-2617

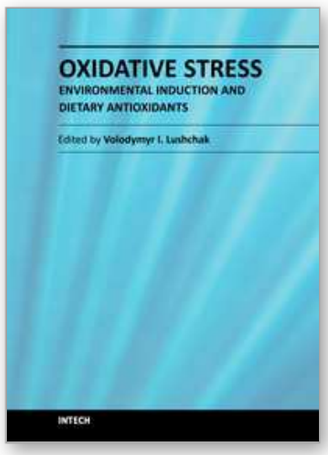
Vandercammen, A., Francois, J. & Her, H. (1989). Characterization of trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase of *Saccharomyces cerevisiae*. *European Journal of Biochemistry*, Vol. 182, No. 5, (July 1989), pp. 613–620, ISSN 0014-2956

Vincent, W.F. (2000). Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. *Antarctic Science*, Vol. 12, No. 3, pp. 374-385, ISSN 0954-1020

Wirtz, N., Thorsten Lumbsch, H., Allan Green, T. G., Türk, R., Pintado, A., Sancho, L. & Schroeter, B. (2003). Lichen fungi have low cyanobiont selectivity in maritime Antarctica. *New Phytologist*, Vol. 160, No. 1, (October 2003), pp. 177-183, ISSN 0028646X - 0028-646X

Woodbury W., Spencer, A.K. & Stahmann, M.A. (1971). Animproved procedure using ferricyanide for detecting catalase isozymes. *Analitical Biochemistry*, Vol. 44, № 1, (November 1971), pp. 301-305, ISSN 0003-2697

Zhang, L., Onda, K., Imai, R., Fukuda, R., Horiuchi, H. & Ohta, A. (2003). Growth temperature downshift induces antioxidant response in *Saccharomyces cerevisiae*. *Biochemical and Biophysical Research Communications*, Vol. 307, No. 2, (July 2003), pp. 308-314, ISSN 0006-291X



Oxidative Stress - Environmental Induction and Dietary Antioxidants

Edited by Dr. Volodymyr Lushchak

ISBN 978-953-51-0553-4
Hard cover, 388 pages
Publisher InTech
Published online 02, May, 2012
Published in print edition May, 2012

This book focuses on the numerous applications of oxidative stress theory in effects of environmental factors on biological systems. The topics reviewed cover induction of oxidative stress by physical, chemical, and biological factors in humans, animals, plants and fungi. The physical factors include temperature, light and exercise. Chemical induction is related to metal ions and pesticides, whereas the biological one highlights host-pathogen interaction and stress effects on secretory systems. Antioxidants, represented by a large range of individual compounds and their mixtures of natural origin and those chemically synthesized to prevent or fix negative effects of reactive species are also described in the book. This volume will be a useful source of information on induction and effects of oxidative stress on living organisms for graduate and postgraduate students, researchers, physicians, and environmentalists.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen