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ORIGINAL PAPER

# Thermal stress responses in Antarctic yeast, *Glaciozyma antarctica* PI12, characterized by real-time quantitative PCR

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**Abstract** Living organisms have some common and unique strategies to response to thermal stress. However, the amount of data on thermal stress response of certain organism is still lacking, especially psychrophilic yeast from the extreme habitat. Therefore, it is not known whether psychrophilic yeast shares the common responses of other organisms when exposed to thermal stresses. In this work, the cold shock and heat shock responses in Antarctic psychrophilic yeast *Glaciozyma antarctica* PI12 which had an optimal growth temperature of 12 °C were determined. The expression levels of 14 thermal stress-related genes were measured using real-time quantitative PCR (qPCR) when the yeast cells were exposed to cold shock (0 °C), mild cold shock (5 °C), and heat shock (22 °C) conditions. The expression profiles of the 14 genes at these three temperatures varied indicating that these genes had their specific roles to ensure the survival of the yeast. Under cold shock condition, the *afp4* and *fad* genes were over-expressed possibly as a way for the *G. antarctica* PI12 to avoid ice crystallization in the cell and to maintain the membrane fluidity. Under the heat shock condition, *hsp70* was significantly up-regulated possibly to ensure the proteins fold properly. Among the six oxidative stress-related genes, *MnSOD* and *prx* were up-regulated under cold shock and heat shock, respectively, possibly to reduce the negative effects caused by oxidative stress. Interestingly, it was found that the trehalase gene, *nth1* that plays a role in degrading excess trehalose, was down-regulated under the heat shock condition possibly as an alternative way to accumulate trehalose in the cells to protecting them from being damaged.

**Keywords** Antarctic yeast · *Glaciozyma antarctica* PI12 · Heat shock · Cold shock · Real-time quantitative PCR

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## Introduction

Antarctica is a continent with extreme low temperature, nutrient, and high ultraviolet (UV) radiation. Temperature is one of the major factors affecting the survival of Antarctic organisms. Antarctic organisms are able to thrive at low temperature and have developed a variety of adaptation strategies to grow and multiply (Morgan-Kiss et al. 2006). By understanding the molecular mechanisms underlying how a range of Antarctic organisms respond to climate change in Antarctica will enable the predictions as how they will adapt to global climate change. Cold and heat adaptation studies have been carried out on some Antarctic organisms such as algae (Hu et al. 2008; Hwang et al. 2008; Chong et al. 2010), plants (Gidekel et al. 2003), fish (Hofmann et al. 2000; Chen et al. 2008), marine bivalve (Park et al. 2008; Kim et al. 2009), insect (Rinehart et al. 2006), fungi (Zucconi et al. 1996; Tosi et al. 2005;

Vishniac 2006; Gocheva et al. 2009), and bacteria (Médigue et al. 2005).

Several reports have indicated that heat shock induces the production of heat shock proteins (HSPs) (Lindquist and Craig 1988; Watson 1990) and the disaccharide trehalose (Attfield 1987; Hottiger et al. 1989). HSPs mainly function as molecular chaperones with respect to protein folding and assembly, whereas trehalose is a thermoprotectant that stabilizes cell membranes and increases the temperature stability of cellular proteins to enable the maintenance of cell structures (Hottiger et al. 1989; Iwahashi et al. 1995). On the other hand, cells are known to respond to cold stress by the elite and rapid production of cold shock proteins (CSPs) (Ermolenko and Makhatadze 2002), cryoprotectant (Borges et al. 2002), antifreeze proteins (AFPs) (Davies et al. 2002) and by activating the desaturase system to maintain membrane fluidity (Deming 2002).

The Antarctic organisms have evolved under the influence of the geological and climatic factors, including geographic isolation of the landmass and the continental shelves, extremely low temperatures, and intense seasonality (Clarke and Crame 1992). Antarctic organisms living in the extreme cold habitats do not always respond in the same way as their temperate or tropical counterparts. Although the heat shock response is thought to be nearly universal among organisms, exception is found in several organisms such as the Antarctic teleost fish, *Trematomus bernacchii* (Hofmann et al. 2000) and Antarctic ciliate, *Euplotes focardii* (LaTerza et al. 2004). Additionally, some Antarctic notothenioids living in subzero temperatures have been shown to lose some of their traits during the process of evolution. For instance, the loss of hemoglobin and myoglobin gene expression abilities in the Antarctic icefish (family Channichthyidae) (Cocca et al. 1997; Sidell et al. 1997; Somero et al. 1998). These reports suggested that some of the living organisms do not response in a similar fashion to temperature shift. Adaptation studies on a diverse population of living organisms from different habitats will help us to better understand the cells response to temperature shifts.

*Glaciozyma antarctica* PI12 (previously known as *Leucosporidium antarcticum*) is a psychrophilic yeast strain which was isolated from a marine environment in Antarctica (Fell et al. 1969). Recently, Turchetti et al. (2011) have proposed to reclassify this yeast from *L. antarcticum* to *G. antarctica*. Besides, *G. antarctica* has also been isolated from various locations in Antarctica (Fell et al. 1969; Donachie 1995; Connell et al. 2008; Law, personal communication). Turkiewicz et al. (2005) reported that *G. antarctica* 171 has an optimum growth temperature at around 15 °C and is able to tolerate higher temperatures up to 20 °C. Thus far, only two thermal stress studies have been carried out on the Antarctic yeasts focusing on the effect of heat shock on the production of HSPs using the proteomic approach (Deegenaars and Watson 1997; Deegenaars and Watson 1998). This project was set out to expand our knowledge on the cold adaptation mechanisms employed by Antarctic yeast by determining the expression patterns of 14 thermal stress-related genes, namely *afp4*, *hsp70*, *hsp90*, *hsp100*, *nth1*, *tps1*, *tps2*, *fad*, *prx*, *gst*, *grxA*, *grxB*, *cat,* and *MnSOD* using the qPCR when *G. antarctica* PI12 is exposed to cold shock (0 °C), mild cold shock (5 °C), and heat shock (22 °C) conditions.

## Materials and methods

### Strain and culture conditions

*Glaciozyma antarctica* PI12 was isolated from sea ice collected in the vicinity of Casey Station (66°21′25″S; 110°37′09″E), Antarctica. It was identified based on its LSU rRNA and ITS sequences which have been deposited in the GenBank database under accession numbers: JX896955 and JX896956. *G. antarctica* PI12 was routinely cultivated in yeast peptone dextrose (YPD) broth (Difco) medium.

### Growth performance under different temperatures

The optimum growth temperature of *G. antarctica* PI12 was pre-determined in this work in order to select temperatures that will trigger cold and heat shock responses. *G. antarctica* PI12 was grown in YPD broth medium at temperatures ranging from 5 to 15 °C (with an interval of 2 or 3 °C) under shaking at 210 rpm. Three replicates were prepared for each incubation temperature. The optical density of the culture at 600 nm ($OD_{600}$) was measured at an interval of 24 h until it reached the stationary phase. The growth rate was calculated using $OD_{600}$ results.

### Exposure of yeast culture to thermal stress

*Glaciozyma antarctica* PI12 was grown in YPD broth medium in 12 conical flasks at the optimal temperature 12 °C to mid log phase. Subsequently, these cultures (3 replicates each) were exposed to 0, 5, and 12 °C for 24 h to give the slow growing yeast sufficient time to response and at 22 °C for 1 h. After the exposure period, the cultures were snap frozen in liquid nitrogen and stored at −80 °C prior to RNA extraction.

### RNA extraction and cDNA preparation

The total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's instructions.

Extracted RNA was treated with RNase-free DNase (Qiagen) to remove traces of genomic DNA. Subsequently, the sample was purified using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. The first-strand cDNA synthesis was performed using the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen) using a reaction mixture comprising, 1 μg total RNA, 50 μM Oligo dT20 primer, and 200 U SuperScript III reverse transcriptase. The mixture was incubated at 50 °C for 50 min. DNA contamination assay was conducted using PCR amplification. The primers used were 5′-CGCACGTA ACCGTGGTATC-3′ and 5′-TAGAGCTGGGTAGCCGA GAA-3′ that targeted the intron of the *hsp70* gene.

## Primer design

Specific primers were designed based on the annotated expressed sequence tag (EST) of *G. antarctica* PI12 generated by Izwan and Munir (personal communication). Nineteen pairs of specific primers were designed to target the reference genes and target genes (Table 1) using Primer3 software, with the length of the amplicons varying between 90 and 150 bp. The primers were synthesized by First-Base Laboratories (Singapore).

## qPCR

The iScript™ One-Step RT-PCR kit with SYBR® Green (Bio-rad) was used for qPCR. qPCR was carried out in 25 μl mixture containing, 12.5 μl 2× SYBR Green RT-PCR reaction mix, 5 μM primers, 500 ng total RNA, 0.5 μl iScript reverse transcriptase, and 10 μl nuclease-free water. The PCR conditions were 50 °C for 10 min and 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Data were collected at 60 °C. The melt curve was determined under the following conditions: 95 °C for 1 min, 55 °C for 1 min followed by 80 cycles at 55 °C for 10 s at an increment of 0.5 °C per cycle. All reactions were performed in triplicate using the iCycler (Bio-rad). A set of negative control was included in each run. The qPCR products were sequenced and aligned against the sequences in the NCBI for annotation purposes.

**Table 1** Description, primer sequences, amplicon length, and PCR efficiency for five candidate reference genes and 14 target genes

| Name | Description | Forward primer (5′ → 3′) | Reverse primer (5′ → 3′) | Amplicon length (bp) | PCR efficiency (%) |
|---|---|---|---|---|---|
| *tub*[a] | Alpha tubulin | GGACACTACACCGTTGGCAA | CGAAGAATCCCTGGAGACCA | 94 | 90.6 |
| *act3*[a] | Actin-binding protein | CCCCGATGGACGAGTTATCA | CGTGTTGAAGAGCATTTCTGC | 118 | 90.9 |
| *act4*[a] | Actin-like protein 3 | GAGCACTCAGTTACGGGCAA | GGAAGTCGGATGAGGCGAT | 103 | 90.4 |
| *gdh*[a] | Glyceraldehyde-3-phosphate dehydrogenase | GAGTCGACCGGTGTCTTCA | GTTGACACCGCAAACGTACAT | 120 | 97.7 |
| *idhD*[a] | D-Lactate dehydrogenase 2, mitochondrial precursor | GGACAACACGGGTTACGATC | AGTCGGGAACGCACAAAACG | 134 | 92.1 |
| *afp4* | Antifreeze protein | CGGTTCCTACTGACCTCGT | GCGAGAGAGAAAGCGACAC | 103 | 96.5 |
| *nth1* | Neutral trehalase | CGGCACTTACATGCTCTCCA | GTTCTCGCTGAGTCGAGCTT | 100 | 108.1 |
| *tps1* | Trehalose-6-phosphate synthase | CATCGAGCCCAGTCAGTTC | CGTGAAGCTTCTGAGGGACT | 149 | 99.0 |
| *tps2* | Trehalose-phosphatase | CATCGTCTCGGAATTCACTGG | TCGTGAGGCAGTGGTCGAT | 107 | 100.3 |
| *hsp70* | Heat shock protein 70 | GAACCCTCACAACACCGTCT | CGGTACTCGACCTGGATGAT | 141 | 94.7 |
| *hsp90* | Heat shock protein 90 | CCATGAAGGCCTTGGTTCCA | CTCTTCATCCGCATCACTCC | 133 | 101.6 |
| *hsp100* | Heat shock protein 100 | CATCACGGAGTAACCGTCCT | TCCCTCGAGACGTTGAGCT | 140 | 106.2 |
| *fad* | Oleate delta-12 desaturase | CTCCCTCGTACACCCAGAA | GGCGAAGACGTAGCTGAAAC | 92 | 95.8 |
| *prx* | 2-Cysteine peroxiredoxin | CTTCGCCATCTCTACCGACT | CGTAATCGCGTGCAATCTTCA | 134 | 95.5 |
| *gst* | Glutathione S-transferase Gst2 | ACGCATTCCTTGCCTTGTTGAT | CTGGTTCGTCTGAGCCTTCT | 145 | 107.3 |
| *grxA* | Glutaredoxin | CCACGTCGTTGTGTTCTCCAA | GCCCTCATCCATCTGGTCTA | 115 | 94.2 |
| *grxB* | Monothiol glutaredoxin-5 | CGTGAGGGCATCAAGGAGT | TCCTCTAGCTCGCCCGATT | 118 | 93.7 |
| *cat* | Catalase | CTTCGAAGTCACGCACGATAT | CCTCCAACGGTAGAGAAACG | 102 | 96.9 |
| *MnSOD* | Manganese superoxide dismutase | CAGCACATCACGAGAACATCA | CGACTACGTGAACGTCAAGC | 147 | 98.4 |

[a] Candidate of reference genes

### qPCR data analysis

The correlation coefficient, $R^2$, and the primer efficiencies were determined using the iCycler software based on the standard curve. The standard curve was generated using five dilution series. The stability of five reference genes, *act3, act4, IdhD, tub2,* and *gdh* was analyzed using the geNorm (version 3.4) (Vandesompele et al. 2002) and NormFinder (Andersen et al. 2004) analysis tools to determine the most suitable reference genes for this work. The fold changes in expression were calculated using the gene expression macro software version 1.1 (Bio-Rad Laboratories). It was normalized against the *act4* and *IdhD* reference genes, and 12 °C was used as calibrator condition. The macro was derived based on the algorithms developed by Vandesompele et al. (2002).

A one-way analysis of variance (ANOVA) was performed to test whether there was a significant evolution in gene expression levels under all the thermal stress conditions. When one-way ANOVA was significant, the tukey HSD method ($n = 3$, $P < 0.05$) was used to locate significant differences. SigmaStat® Version 3.0.1 software (SPSS Inc.) was used to perform this statistical analysis. Genes were recorded as significantly down- or up-regulated if the fold change was at least 1.5-fold lower or higher than the calibrator condition at 12 °C, and the *P* value should be less than 0.05.

### Nucleotide sequence accession numbers

The EST sequences were deposited in NCBI GenBank and were assigned the following accession numbers: JF412496 (*tub2*), JF412497 (*act3*), JF412498 (*act4*), JF412499 (*gdh*), JF412500 (*IdhD*), JF412501 (*nth1*), JF412502 (*afp4*), JF412503 (*tps1*), JF412504 (*tps2*), JF412505 (*hsp70*), JF412506 (*hsp90*), JF412507 (*hsp100*), JF412508 (*prx*), JF412509 (*gst*), JF412511 (*grxA*), JF412512 (*grxB*), JF412513 (*cat*), JF412514 (*MnSOD*).

## Results

### Determination of optimal growth temperature

The growth curve revealed that the optimal growth temperature of *G. antarctica* PI12 was 12 °C since *G. antarctica* PI12 had the highest growth rate (0.6 $day^{-1}$) at this temperature (Fig. 1). The growth rate of *G. antarctica* PI12 decreased when it was grown at 1 °C lower or higher than 12 °C and drastically decreased at higher temperatures.

### Gene expression analysis

An exposure time of 1 h to 22 °C was found to be sufficient for the induction. An exposure of more than 1 h dramatically decreased the amount of RNA recovered (Fig. 2). In addition, *G. antarctica* PI12 grown to an $OD_{600nm}$ of not more than 1.5 because beyond this, there was a significant reduction and degradation of RNA (data not shown). The *act4* and *IdhD* genes were used as the reference genes in this analysis because they had the lowest expression stability values *(M).* This means that their expressions are stable when compared to the other three reference gene candidate across all the thermal conditions tested (Table 2).

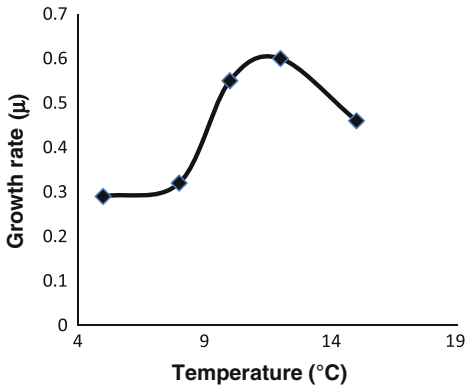


**Fig. 1** Growth rate curve of *G. antarctica* PI12 at five different growth temperatures (5, 8, 10, 12, and 15 °C) in YPD medium. $OD_{600}$ was used for growth rate calculation

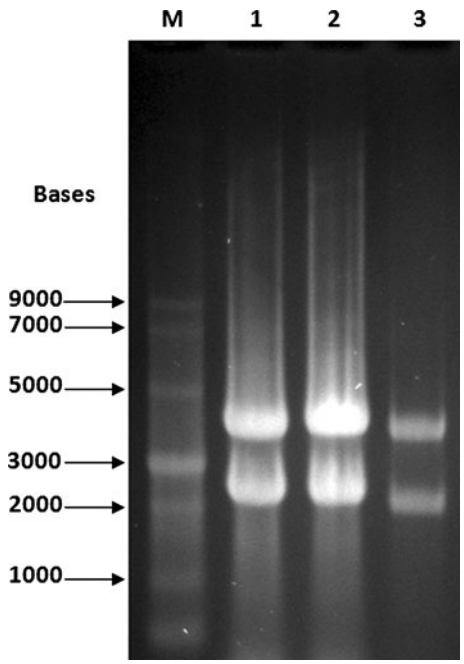


**Fig. 2** Total RNA isolated from *G. antarctica* PI12 after exposed to 22 °C for different time ranges. *M* ssRNA ladder (NEB), 1–30, 2–60, 3–90 min

**Table 2** Expression stability values (M) of five candidate reference genes as calculated by NormFinder and geNorm

| Rank | NormFinder | | geNorm | |
|---|---|---|---|---|
| | Gene | Stability | Gene | Stability |
| 1 | *act4* | 0.017 | *act4* | 0.194 |
| 2 | *IdhD* | 0.017 | *IdhD* | 0.194 |
| 3 | *tub2* | 0.156 | *tub2* | 0.282 |
| 4 | *gdh* | 0.167 | *gdh* | 0.302 |
| 5 | *act3* | 0.270 | *act3* | 0.41 |

The expression level of the 14 genes of *G. antarctica* PI12 exposed to 0, 5, and 22 °C was compared to the expression level at 12 °C (optimal growth temperature) in order to identify genes that were up- or down-regulated. Statistical analysis showed that the gene expression levels of three genes (*hsp100*, *gst,* and *tps1*) did not significantly change after exposure to all three thermal stress conditions. As stated above, only the genes with fold change ≥1.5-fold and $p$ value <0.05 are considered as significantly expressed.

The expression of *afp4* increased 15.3-fold and 2.9-fold at 0 and 5 °C, respectively. However, it was not induced under heat shock condition at 22 °C. The transcript level of the *fad* gene had increased by 1.8-fold when the cells were exposed to cold shock. The expression of *hsp70* significantly increased 3.2-fold when the cells were exposed to heat shock at 22 °C. Meanwhile, the expression of the *hsp90* and *hsp100* genes were maintained at constant levels with the fold change equal to one when the cells were exposed to 22 °C (Fig. 3). There was no significant induction of the three genes, *tps1*, *tps2,* and *nth1* required for the biosynthesis and degradation of trehalose under all the three thermal stress conditions.

The expression profiles of the oxidative stress-related genes varied. Under cold shock condition, up-regulation was observed for the *MnSOD* gene (1.9-fold), whereas down-regulation was observed for the *grxA* gene (−2.0-fold). The expression of the *prx* gene had increased by 1.8-fold when the cells were exposed to heat shock at 22 °C. On the other hand, the *cat* gene was down-regulated 1.8-fold under the same condition (Fig. 3). The *grxB* gene was down-regulated 3.2-fold under the mild cold shock condition at 5 °C when compared to 12 °C (Fig. 3).

## Discussion

Turkiewicz et al. (2005) have reported that the optimal growth temperature of *G. antarctica* strain 171 was 15 °C. However, *G. antarctica* strain PI12 used in this study grew best at 12 °C in YPD medium and its growth rate declined at 11 or 13 °C indicating that it is very sensitive to small temperature change. The slight differences are possibly due to strains variation among *G. antarctica* isolates originated from different geographic locations. Howarth and Ougham (1993), Cairns et al. (1995) reported that a 10 °C increase above the growth temperature of an organism is required to elicit heat shock response. Hence, *G. antarctica* PI12 was

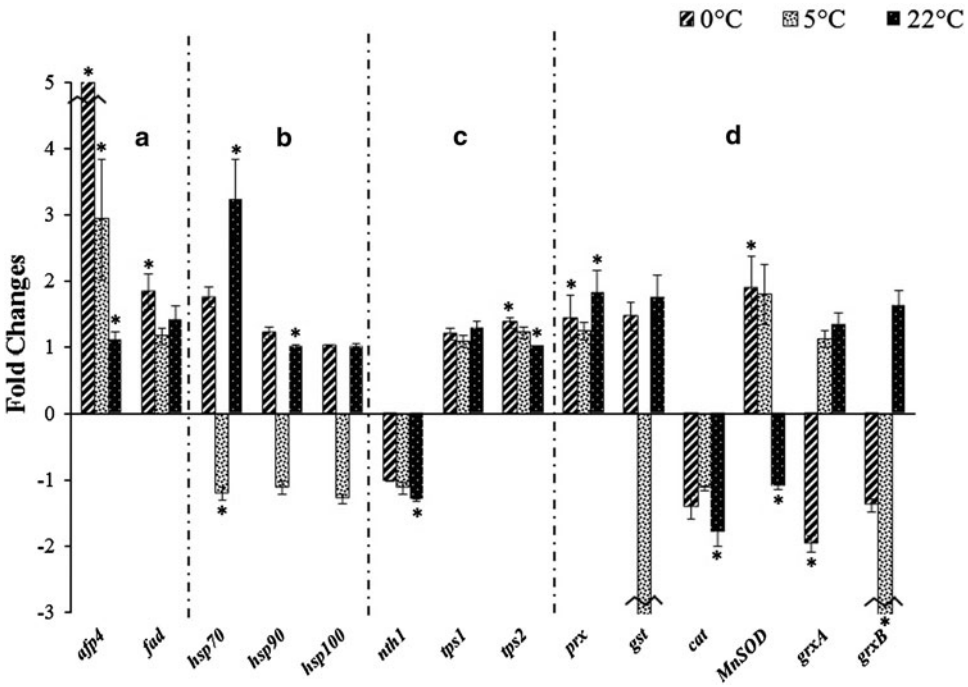


**Fig. 3** Fold changes for 14 thermal stress-related genes after exposed to cold shock (0 °C), mild cold shock (5 °C), and heat shock (22 °C) conditions. *a* AFP and fatty acid desaturase genes, *b* HSPs genes, *c* trehalose biosynthesis and degradation genes, *d* oxidative stress-related genes. Gene expression was quantified using the gene expression macro software (Bio-rad). The growth condition at 12 °C was used as the calibrator condition, and *act4* and *IdhD* gene were used as internal control. *Asterisk* indicates significant difference ($P < 0.05$)

exposed to 22, 10 °C above the optimum growth temperature to induce heat shock response.

The 14 genes assayed in this work were previously found to respond to thermal stress (Fulco and Fuji 1980; Deegenaars and Watson 1998; Porankiewiz et al. 1998; Methé et al. 2005; Hu et al. 2008; Gocheva et al. 2009) in mesophilic or psychrophilic organisms. They were the (1) *afp4* gene encoding antifreeze protein (AFP) which is important to provide protection to cells in freezing temperatures (Davies et al. 2002), *fad* gene encoding the fatty acid desaturase enzyme which is involved in the maintenance of membrane fluidity (Fulco and Fuji 1980); (2) *hsp70*, *hsp90,* and *hsp100* genes encoding heat shock proteins (HSPs) that act as molecular chaperones for protein folding (Gething and Sambrook 1992); (3) *nth1* gene encoding neutral trehalase act as trehalose degradation enzyme, and *tps1* and *tps2* genes that are involved in the biosynthesis of trehalose (Kopp et al. 1993; Murata et al. 2006); and (4) *prx*, *gst*, *grxA*, *grxB*, *cat,* and *MnSOD* genes encoding anti-oxidant enzymes that are important for reduction in oxidative stress caused by accumulation of ROS (Berlett and Stadtman 1997; Toledano et al. 2003).

The expression profiles of this Antarctic yeast *G. antarctica* PI12 exposed to cold shock (0 °C), mild cold shock (5 °C), and heat shock (22 °C) were compared to the thermal stress responses of other Antarctic organisms to assess the similarity and differences between them. Firstly, our study showed that AFP and fatty acid desaturase played an important role for cold adaptation in *G. antarctica* PI12. AFPs have been discovered in a wide range of Antarctic organisms such as algae (Hu et al. 2008; Gwak et al. 2009), fish (Hofmann et al. 2000), plant (Bravo and Griffith 2005), and bacteria (Gilbert et al. 2004). AFPs prevent the formation of ice crystals and lower the freezing temperature of the water without altering its melting point (Davies et al. 2002). Exposure of *G. antarctica* PI12 to 0 and 5 °C caused significant induction of *afp4*. Similar results have been noted for two Antarctic algae, *Chlorella vulgaris* NJ-7 and *Chaetoceros neogracile* (Hu et al. 2008; Gwak et al. 2009). Although the amino acid sequences of AFP or IBP (ice binding protein) were very diverse among different organisms but the amino acid sequence of AFP4 of *G. antarctica* PI12 was 93 % similar with IBP of Arctic yeast, *Leucosporidum* sp. AY30 (Lee et al. 2010; Park et al. 2011). Turchetti et al. (2011) have stated that this Arctic yeast probably belonged to *G. watsonii* rather than *G. antarctica* based on the few characteristics described by Lee et al. (2010). Nevertheless, the AFPs produced by *G. antarctica* PI12 and *G. watsonii* AY30 which under the same genus of Glaciozyma probably share some similarities in their functional sites and protein domains.

Apart from the AFP, fatty acid desaturase (FAD) is important to maintain the membrane fluidity in *G. antarctica* PI12 under cold shock conditions by converting pre-existing saturated fatty acids to the unsaturated forms (Vigh et al. 1998). Induction of desaturases by cold has been described in an Antarctic algae, *C. vulgaris* NJ-7. This alga possesses two fatty acid desaturase genes, CvFAD2 and CvFAD6, induced at different temperatures. CvFAD2 mRNA increased when the alga was transferred from 25 to 15 °C or 4 °C, whereas CvFAD6 was only up-regulated under extreme low temperature at 4 °C. The fatty acid desaturase gene, *fad,* in *G. antarctica* PI12 was up-regulated at 0 °C but not under mild cold shock condition at 5 °C which has the similar expression pattern to CvFAD6. These results suggested that the fatty acid desaturase genes in some Antarctic organisms have evolved in such a way that they are induced only under extreme low temperature (≤4 °C).

The classical heat shock response involving a strong up-regulation of HSP70 production has been demonstrated in most of the Antarctic organisms examined to date with exception in some Antarctic marine organisms such as fish (Hofmann et al. 2000; Place and Hofmann 2005; Clark et al. 2008a), ciliate (LaTerza et al. 2001, LaTerza et al. 2004), sea star, and gammarid (Clark et al. 2008b). They have possibly lost their ability to respond to heat shock due to evolution after living in the cold environment for a long period of time. *G. antarctica* PI12 was found to harbor a gene encoding the HSP70 family protein too. The amino acid sequence of *hsp70* of *G. antarctica* PI12 consisted three heat shock protein 70 family signatures, signature 1 (IDGTTYS), signature 2 (IFDLGGGTFD), and signature 3 (IVIVGGSTRIP) indicating that that it belongs to the HSP70 family. Piano et al. (2005) have pointed out that the GGMP repeats are present in the bivalve heat shock cognate 70 (HSC70) but absent in the heat shock protein 70 (HSP70), so the presence of GGMP tetrapeptide repeats in the amino acid sequence probably can be used to differentiate the HSC70 and HSP70. However, the *hsp70* of *G. antarctica* PI12 carries only a single copy of the GGMP amino acid sequence. Hence, it is not known whether *hsp70* of *G. antarctica* PI12 is a cognate or inducible gene based solely on the amino acid alignment analysis. Nevertheless, based on the qPCR results of this study, it was found that the *hsp70* of *G. antarctica* PI12 was heat-inducible at 22 °C and this indicates that *hsp70* is an inducible HSP70.

HSP genes were probably not important for cold adaptation since none of them were induced at 0 and 5 °C. Incidentally, low temperature is well known to reduce the probability of protein misfolding and aggregation (King et al. 1996), therefore possibly reducing the need for HSP chaperones. In fact, it has been shown that the induced expression of HSPs in *Eschericia coli* is harmful to cells at 4 °C to the extent that it reduces cell viability at this

temperature (Kandror and Goldberg 1997). Additionally, it was reported that cold repression of HSPs is beneficial to the Antarctic pscyhrophilic bacteria *Pseudoalteromonas haloplanktis* and *Exiguobacterium sibiricum* (Piette et al. 2011).

The over-expression of antioxidant genes, *MnSOD* and *prx,* in this work suggests that the oxidative stress was induced in *G. antarctica* PI12 under cold shock and heat shock conditions. However, the antioxidant genes induced under heat shock and cold shock conditions are different. This probably has something to do with the thermal stability of enzymes under different thermal conditions. Our results showed that *MnSOD* is the only gene up-regulated at 0 °C but not at 5 °C. Similar data were reported that cold exposure significantly increased activities of superoxide dismutase (SOD) in Antarctic plants (Arora et al. 2002), algae (Collén and Davison 2001), and animals (Ansaldo et al. 2000; Abele et al. 2001; Heise et al. 2006). Meanwhile, one of the glutaredoxin gene, *grxA* in *G. antarctica* PI12, was down-regulated under cold shock condition. In contrast, Hwang et al. (2008) reported that the glutaredoxin gene was up-regulated under cold shock condition at 4 and 10 °C in *C. neogracile*. Meanwhile, Gidekel et al. (2003) demonstrated that the glutaredoxin gene was also up-regulated at 4 °C in the Antarctic monocot plant, *Deschampsia antarctica*.

Similar with cold shock conditions, there was only one antioxidant gene, *prx* that was significantly up-regulated under heat shock conditions. A similar observation was reported for the Antarctic bivalve *Laternula elliptica* whereby two of its peroxiredoxins were up-regulated significantly when exposed to 10 °C (Park et al. 2008). Kim et al. (2009) reported that the pi-class glutathione S-transferase of *L. elliptica* was also up-regulated upon heat treatment. However, the glutathione S-transferase gene, *gst* in *G. antarctica* PI12, was not induced at 22 °C. This indicated that the expression of *gst* in *G. antarctica* PI12 is possibly triggered by temperatures higher than 22 °C.

Besides, the results in this work revealed that the trehalose accumulation under thermal stress conditions in *G. antarctica* PI12 was possibly caused by the repression of *nth1* but not due to over-expression of the *tps1* and *tps2* genes. *tps1* and *tps2* which are involved in the biosynthesis of trehalose are normally up-regulated when cells are exposed to cold shock (Inouye and Phadtare 2004; Kandror et al. 2004; Murata et al. 2006) or heat shock (Attfield 1987; Bell et al. 1992 and Vuorio et al. 1993). Deegenaars and Watson (1998) reported that *G. antarctica* has the greatest intrinsic level of trehalose when exposed to heat shock when compared to three other psychrophilic yeasts. However, our study showed that there is no significant induction of *tps1* and *tps2* under heat shock and cold shock conditions in *G. antarctica* PI12. Hence, we have speculated that the accumulation of trehalose in *G. antarctica* PI12 under stress conditions is not caused by the over-expression of *tps1* and *tps2* but is due to other factors. Neutral trehalase is an enzyme involved in the hydrolysis of trehalose and which degrades excess amounts of the disaccharide in the cell (Kopp et al. 1993). When cells are exposed to heat shock or cold shock, trehalose is allowed to accumulate as a way to protect the *G. antarctica* PI12 cells by down-regulating the *nth1* gene as found in this work. Similar findings were reported for fungi (Ocón et al. 2007) and yeast (Kim et al. 2011) exposed to stresses. These organisms were found to accumulate trehalose through down-regulation of trehalase gene, *nth1*.

The results of this work lead us to conclude *G. antarctica* PI12 possibly avoids ice crystallization and maintains the membrane fluidity in the cells by over-expressing the AFP and fatty acid desaturase genes under cold stress (Lee et al. 2010; Park et al. 2011). At the same time, the antioxidant enzyme, *MnSOD,* is highly up-regulated to neutralize the oxidative stress. When the condition is in the reverse, *G. antarctica* PI12 produces large amounts of *HSP70* as chaperone for protein folding. Antioxidant gene *prx* is over-expressed under heat shock condition. These findings are interesting and warrant a more extensive analysis to provide a better understanding of what is happening in the cells when they are exposed to heat or cold shocks. As sequencing using the next generation sequencing is becoming more affordable, it will be ideal to conduct RNA sequencing to look at the transcriptome profiles of *G. antarctica* PI12 under these thermal stress conditions.

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## References

Abele D, Tesch C, Wencke P, Pörtner HO (2001) How do oxidative stress parameters relate to thermal tolerance in the Antarctic bivalve *Yoldia eightsi*? Antarct Sci 13:111–118

Andersen CL, Jensen JL, Orntoft TF (2004) Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res 64:5245

Ansaldo M, Polo J, Evelson P, Luqet C, Llesuy S (2000) Antioxidant levels from different Antarctic fish caught around South Georgia Island and Shag Rocks. Polar Biol 23:160–165

Arora A, Sairam RK, Srivastava GC (2002) Oxidative stress and antioxidative system in plants. Curr Sci India 82:1227–1238

Attfield PV (1987) Trehalose accumulates in *Saccharomyces cerevisiae* during exposure to agents that induce heat shock response. FEBS Lett 225:259–263

Bell W, Klaassen P, Ohnacker M, Boller T, Herweijer M, Schoppink P, Van der Zee P, Wiemken A (1992) Characterization of the 56-kDa subunit of yeast trehalose-6-phosphate synthase and cloning of its gene reveal its identity with the product of CIF1, a regulator of carbon catabolite inactivation. Eur J Biochem 209:951–959

Berlett BS, Stadtman ER (1997) Protein oxidation in aging, disease, and oxidative stress. J Biol Chem 272:20313–20316

Borges N, Ramos A, Raven ND, Sharp RJ, Santos H (2002) Comparative study of the thermo stabilizing properties of mannosylglycerate and other compatible solutes on model enzymes. Extremophiles 6:209–216

Bravo LA, Griffith M (2005) Characterization of antifreeze activity in Antarctic plants. J Exp Bot 56:1189–1196

Cairns A, Howarth CJ, Pollock CJ (1995) Submerged batch culture of the psychrophile *Monographella nivalis* in a defined medium: growth, carbohydrate utilization and responses to temperature. New Phytol 129:299–308

Chen Z, Cheng CH, Zhang J, Cao L, Chen L, Zhou L, Jin Y, Ye H, Deng C, Dai Z, Xu Q, Hu P, Sun S, Shen Y, Chen L (2008) Transcriptomic and genomic evolution under constant cold in Antarctic notothenioid fish. Proc Natl Acad Sci USA 105:12944–12949

Chong GL, Chu WL, Othman RY, Phang SM (2010) Differential gene expression of an Antarctic chlorella in response to temperature stress. Polar Biol 34:637–645

Clark MS, Fraser KPP, Burns G, Peck LS (2008a) The HSP70 heat shock response in the Antarctic fish *Harpagifer antarcticus*. Polar Biol 31:171–180

Clark MS, Fraser KPP, Peck LS (2008b) Lack of an HSP70 heat shock response in two Antarctic marine invertebrates. Polar Biol 31:1059–1065

Clarke A, Crame JA (1992) The southern ocean benthic fauna and climate change: a historical perspective. Phil Trans R Soc B 338:299–309

Cocca E, Ratnayakelecamwasam M, Parker SK, Camardella L, Ciaramella M, Di Prisco G, Detrich HW (1997) Do the hemoglobinless icefishes have globin genes? Comp Biochem Physiol 118:1027–1030

Collén J, Davison IR (2001) Seasonality and thermal acclimation of reactive oxygen metabolism in *Fucus vesiculosus* (Phaeophyceae). J Phycol 37:474–481

Connell L, Redman R, Craig S, Scorzetti G, Iszard M, Rodriguez R (2008) Diversity of soil yeasts isolated from South Victoria Land, Antarctica. Microb Ecol 56:448–459

Davies PL, Baardsnes J, Kuiper MJ, Walker VK (2002) Structure and function of antifreeze proteins. Phil Trans R Soc B 357:927–935

Deegenaars ML, Watson K (1997) Stress proteins and stress tolerance in an Antarctic psychrophilic yeast, *Candida psychrophila*. FEMS Microbiol Lett 151:191–196

Deegenaars ML, Watson K (1998) Heat shock response in psychrophilic and psychrotrophic yeast from Antarctica. Extremophiles 2:41–49

Deming JW (2002) Psychrophiles and polar regions. Curr Opin Microbiol 5:301–309

Donachie SP (1995) Ecophysiological description of marine bacteria from Admiralty Bay (Antarctica), and the digestive tracts of selected *Euphausiidae*. Ph. D. Thesis. Department of Antarctic Biology, Polish Academy of Sciences, Warsaw

Ermolenko DN, Makhatadze GI (2002) Bacterial cold-shock proteins. Cell Mol Life Sci 59:1902–1913

Fell JW, Statzell AC, Hunter IL, Phaff HJ (1969) *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. Antonie Van Leeuwenhoek 35:433–462

Fulco AJ, Fuji DK (1980) Adaptive regulation of membrane lipid biosynthesis in bacilli by environmental temperature. In: Kates M, Kuksis A (eds) Membrane fluidity, biophysical techniques and cellular regulation. Humana Press, Totowa, New Jersey, pp 77–98

Gething MJ, Sambrook J (1992) Protein folding in the cell. Nature 355:33–45

Gidekel M, Destefano-Beltrán L, García P, Mujica L, Leal P, Cuba M, Fuentes L, Bravo LA, Corcuera LJ, Alberdi M, Concha I, Gutiérrez A (2003) Identification and characterization of three novel cold acclimation-responsive genes from the extremophile hair grass *Deschampsia antarctica* Desv. Extremophiles 7:459–469

Gilbert JA, Hill PJ, Dodd CE, Laybourn-Parry J (2004) Demonstration of antifreeze protein activity in Antarctic lake bacteria. Microbiology 150:171–180

Gocheva YG, Tosi S, Krumova ET, Slokoska LS, Miteva JG, Vassilev SV, Angelova MB (2009) Temperature downshift induces antioxidant response in fungi isolated from Antarctica. Extremophiles 13:273–281

Gwak IG, Jung W, Kim HJ, Kang SH, Jin E (2009) Antifreeze Protein in Antarctic Marine Diatom, *Chaetoceros neogracile*. Mar Biotechnol 12:630–639

Heise K, Puntarulo S, Nikinmaa M, Lucassen M, Pörtner HO, Abele D (2006) Oxidative stress and HIF-1 DNA binding during stressful cold exposure and recovery in the north sea eelpout (*Zoarces viviparous*). Comp Biochem Physiol 143:494–503

Hofmann GE, Buckley BA, Airaksinen S, Keen JE, Somero GN (2000) Heat-shock protein expression is absent in the Antarctic fish *Trematomus bernacchii* (family *Nototheniidae*). J Exp Biol 203:2331–2339

Hottiger T, Boller T, Wiemken A (1989) Correlation of trehalose content and heat resistance in yeast mutants altered in the RAS/adenylate cyclase pathway: is trehalose a thermoprotectant? FEBS Lett 255:431–434

Howarth CJ, Ougham HJ (1993) Gene expression under temperature stress. New Phytol 125:1–26

Hu H, Li H, Xu X (2008) Alternative cold response modes in *Chlorella* (Chlorophyta, Trebouxiophyceae) from the Antarctic. Phycologia 47:28–34

Hwang Y, Jung G, Jin E (2008) Transcriptome analysis of acclimatory responses to thermal stress in Antarctic algae. Biochem Biophys Res Comm 367:635–641

Inouye M, Phadtare S (2004) Cold shock response and adaptation at near-freezing temperature in microorganisms. Sci STKE 237:26

Iwahashi H, Obuchi K, Fujii S, Komatsu Y (1995) The correlative evidence suggesting that trehalose stabilizes membrane structures in the yeast *Saccharomyces cerevisiae*. Cell Mol Biol 41:763–769

Kandror O, Goldberg AL (1997) Trigger factor is induced upon cold shock and enhances viability of *Escherichia coli* at low temperatures. Proc Natl Acad Sci USA 94:4978–4981

Kandror O, Bretschneider N, Kreydin E, Cavalieri D, Goldberg AL (2004) Yeast adapt to near-freezing temperatures by STRE/Msn2,4-dependent induction of trehalose synthesis and certain molecular chaperones. Mol Cell 13:771–781

Kim M, Ahn IY, Cheonand Hyun J (2009) Molecular cloning and thermal stress-induced expression of a pi-class glutathione S-transferase (GST) in the Antarctic bivalve *Laternula elliptica*. Comp Biochem Phys A 152:207–213

Kim IS, Sohn HY, Jin I (2011) Adaptive stress response to menadione-induced oxidative stress in *Saccharomyces cerevisiae* KNU5377. J Microbiol 49:816–823

King J, Haase-Pettingell C, Robinson AS, Speed M, Mitraki A (1996) Thermolabile folding intermediates: inclusion body precursors and chaperonin substrates. FASEB J 10:57–66

Kopp M, Muller H, Holzer H (1993) Molecular analysis of the neutral trehalase gene from *Saccharomyces cerevisiae*. J Biol Chem 268:4766–4774

LaTerza AL, Miceli C, Luporini P (2001) Divergence between two Antarctic species of the ciliate *Euplotes, E. focardii* and *E. nobilii*, in the expression of heat-shock protein 70 genes. Mol Ecol 10:1061–1067

LaTerza AL, Miceli C, Luporini P (2004) The gene for the heat-shock protein 70 of *Euplotes focardii*, an Antarctic psychrophilic ciliate. Antarct Sci 16:23–28

Lee JK, Park KS, Park S, Park H, Song YH, Kang SH, Kim HJ (2010) An extracellular ice-binding glycoprotein from an Arctic psychrophilic yeast. Cryobiology 60:222–228

Lindquist S, Craig EA (1988) The heat shock proteins. Annu Rev Genet 55:1151–1191

Médigue C, Krin E, Pascal G, Barbe V, Bernsel A, Bertin PN, Cheung F et al (2005) Coping with cold: the genome of the versatile marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125. Genome Res 15:1325–1335

Methé BA, Nelson KE, Deming JW, Momen B, Melamud E, Zhang Z, Moult J, Madupu R, Nelson WC, Dodson RJ et al (2005) The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. Proc Natl Acad Sci USA 102:10913–10918

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol Rev 70:222–252

Murata Y, Homma T, Kitagawa E, Momose Y, Sato MS, Odani M, Shimizu H, Hasegawa-Mizusawa M, Matsumoto R, Mizukami S et al (2006) Genome-wide expression analysis of yeast response during exposure to 4°C. Extremophiles 10:117–128

Ocón A, Hampp R, Requena N (2007) Trehalose turnover during abiotic stress in arbuscular mycorrhizal fungi. New Phytol 174:879–891

Park H, Ahn IY, Kim HJ, Cheon J, Kim M (2008) Analysis of ESTs and expression of two peroxiredoxins in the thermally stressed Antarctic bivalve *Laternula elliptica*. Fish Shellfish Immun 25:550–559

Park AK, Park KS, Kim HJ, Park H, Ahn IY, Chi YM, Moon JH (2011) Crystallization and preliminary X-ray crystallographic studies of the ice-binding protein from the Antarctic yeast *Leucosporidium* sp. AY30. Acta Cryst 67:800–802

Piano A, Franzellitti S, Tinti F, Fabbri E (2005) Sequencing and expression pattern of inducible heat shock gene products in the European flat oyster, *Ostrea edulis*. Gene 361:119–126

Piette F, Struvay C, Feller G (2011) The protein folding challenge in psychrophiles: facts and current issues. Environ Microbiol 13:1924–1933

Place SP, Hofmann GE (2005) Constitutive expression of a stress-inducible heat shock protein gene, hsp70, in a phylogenetically distant Antarctic fish. Polar Biol 28:261–267

Porankiewiz J, Schelin J, Clarke AK (1998) The ATP-dependent Clp protease is essential for acclimation to UV-B and low temperature in the cyanobacterium *Synechococcus*. Mol Microbiol 29:275–283

Rinehart JP, Hayward SA, Elnitsky MA, Sandro LH, Lee RE, Denlinger DL (2006) Continuous up-regulation of heat shock proteins in larvae, but not adults, of a polar insect. Proc Natl Acad Sci USA 103:14223–14227

Sidell BD, Vayda ME, Small DJ, Moylan TJ, Londraville RL, Yuan ML, Rodnick KJ, Eppley ZA, Costello L (1997) Variable expression of myoglobin among the hemoglobinless Antarctic icefishes. Proc Natl Acad Sci USA 94:3420–3424

Somero GN, Fields PA, Hofmann GE, Weinstein RB, Kawall H (1998) Cold adaptation and stenothermy in Antarctic notothenioid fishes: what has been gained and what has been lost? In: DiPrisco G, Pisano E, Clarke A (eds) Fishes of Antarctica. A biological overview. Springer, Italia, pp 97–109

Toledano MB, Delaunay A, Biteau B, Spector D, Azevedo D (2003) Oxidative stress responses in yeast. In: Hohman S, Mager WH (eds) Yeast stress responses. Springer, Berlin, pp 241–303

Tosi S, Onofri S, Brusoni M, Zucconi L, Vishniac H (2005) Response of Antarctic soil fungal assemblages to experimental warming and reduction of UV radiation. Polar Biol 28:470–482

Turchetti B, Hall SRT, Connell LB, Branda E, Buzzini P, Theelen B, Müller WH, Boekhout T (2011) Psychrophilic yeasts from Antarctica and European glaciers: description of *Glaciozyma* gen. nov., *Glaciozyma martini* sp. nov. and *Glaciozyma watsonii* sp. nov. Extremophiles 15:573–586

Turkiewicz M, Pazgier M, Donachie SP, Kalinowska H (2005) Invertase and α-glucosidase production by the endemic Antarctic marine yeast *Leucosporidium antarcticum*. Pol Polar Res 26:125–136

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3:research0034–research 0034.11

Vigh L, Maresca B, Harwood JL (1998) Does the membrane's physical state control the expression of heat shock and other genes? Trends Biochem Sci 23:369–374

Vishniac HS (2006) Yeast biodiversity in the Antarctic. In: Péter G, Rosa C (eds) Biodiversity and ecophysiology of yeasts. The yeast handbook. Springer, Berlin, pp 419–440

Vuorio OE, Kalkkinen N, Londesborough J (1993) Cloning of two related genes encoding the 56-kDa and 123-kDa subunits of trehalose synthase from the yeast *Saccharomyces cerevisiae*. Eur J Biochem 216:849–861

Watson K (1990) Microbial stress proteins. Adv Microb Physiol 31:183–223

Zucconi L, Pagano S, Fenice M, Selbmann L, Onofri S (1996) Growth temperature preferences of fungal strains from Victoria Land, Antarctica. Polar Biol 16:53–61