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ORIGINAL PAPER

Molecular cloning and expression analysis of a cytosolic Hsp70 gene from Antarctic ice algae *Chlamydomonas* sp. ICE-L

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Abstract A cDNA encoding heat shock protein 70 of Antarctic ice algae *Chlamydomonas* sp. ICE-L (designated as CiHsp70) was identified by RT-PCR and rapid amplification of cDNA ends approaches. The full-length cDNA of CiHsp70 was 2,232 bp, consisting of a 5′-terminal untranslated region (UTR) of 76 bp, a 3′-terminal UTR of 203 bp with a poly (A) tail, and an open reading frame of 1,953 bp. The CiHsp70 cDNA encoded a polypeptide of 651 amino acids with an ATPase domain of 388 amino acids, the substrate peptide binding domain of 246 amino acids and a C-terminus domain of 17 amino acids. The inducible CiHsp70 cDNA was highly homologous to other plant cytosolic Hsp70 genes and clustered together with green algae and higher plant rather than brown algae, diatom and Cryptophyta. Antarctic ice algae were treated with different stress conditions and messenger RNA (mRNA) expression levels of CiHsp70 were quantified by quantitative RT-PCR. The results showed that both cold and heat shock treatments could stimulate CiHsp70 mRNA expression. Meanwhile, CiHsp70 mRNA expression level increased 2.9-fold in response to UV-B radiation for 6 h, while the expression levels of CiHsp70 were remarkably increased after removing the UV-B radiation and immediately providing additional 6 h visible light. Furthermore, treating with 62 or 93‰ NaCl for 2 h, CiHsp70 mRNA expression level increased 3.0- and 2.1-fold, respectively. Together, our observations revealed that CiHsp70 as a molecular chaperone might play an important role in Antarctic ice algae *Chlamydomonas* sp. ICE-L acclimatizing to polar environment.

Keywords Antarctic ice algae · *Chlamydomonas* sp. ICE-L · Heat shock protein · Hsp70 · Psychrophile · Stress

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Introduction

Antarctic terrestrial ecosystems are cold, dry, low nutrient environments, with drastic temperature fluctuations and relatively low levels of water availability (Pearce 2008). Such persistently cold environments constitute one of the largest ecosystems on earth, and photosynthetic microorganisms dominate the biomass and metabolic activity in these extreme environments (Morgan-Kiss et al. 2006). To successfully colonize Antarctic low-temperature environments, psychrophilic photoautotrophs have evolved a number of strategies that range from molecular to whole cell or even to ecosystem levels to achieve their survival and propagation (Thomas and Dieckmann 2002). Such acclimatization responses to transient ecophysical changes in the surrounding environment are dependent upon rapid signal transduction pathways, usually involving changes in the expression of specific genes (Zhu 2001; Hwang et al. 2008). Therefore, these plants offer exceptional opportunities for gaining novel insights into the

mechanisms of stress resistance under extreme conditions, plant genetic evolution, and the response to increased UV irradiation in Antarctic continent correlated to the global climate change.

The protein synthesizing system plays a crucial role in plant acclimation processes (Yordanov 1995; Chinnusamy et al. 2007). Accumulating evidences indicate that through stabilizing proteins in a particular state, heat shock proteins facilitate diversified intracellular processes including protein folding, transport of proteins across membranes and their correct conformation, the assembly of oligomeric proteins and modulation of receptor activities, DNA replication and mRNA turnover (Wang et al. 2004; Mayer and Bukau 2005; Swindell et al. 2007; Madden et al. 2008; Sharma and Masison 2009; Kocabiyik 2009). The heat shock 70 proteins (Hsp70s) are chaperones and crucial housekeeping proteins whose functional roles have become the focus for studying the mechanism of stress acclimation (Brocchieri et al. 2008; Reddy et al. 2010; Montero-Barrientos et al. 2010). All Hsp70s possess 3 distinct domains: an N-terminal adenosine triphosphatase (ATPase) domain of approximately 400 amino acids, a substrate-binding domain of approximately 200 amino acids, and a highly variable C-terminal domain. Photosynthetic eukaryotes possess four types of Hsp70s, each of which localizes to different subcellular compartments: cytoplasm, mitochondrion (MT), endoplasmic reticulum (ER), and chloroplast (CP) (Renner and Waters 2007).

The Antarctic *Chlamydomonas* sp. ICE-L is a unicellular photosynthetic eukaryote isolated from the floating ice of Antarctica and kept in a clonal culture in our laboratory (Liu et al. 2006). One basic prerequisite for thriving in permanent cold environments was found that *Chlamydomonas* sp. ICE-L can proliferate and propagate under the temperature of 0–10°C, in contrast it will not survive when the ambient temperature exceeds 15°C. Furthermore, this ice green alga is less sensitive or, in other words, more resistant to UV-B radiation compared to mesophilic green algae (Miao et al. 2002). These previous investigations demonstrate that Antarctic *Chlamydomonas* sp. ICE-L is a psychrophilic alga and has some unique physiological properties that correlate with Antarctic ice habitats.

In the present study, to further understand the intrinsic mechanism of Antarctic sea ice algae responding to the ecological and environmental stimulations and to speculate the role of Hsp70 in anti-stress activities, a cytosolic Hsp70 cDNA was isolated by RT-PCR and RACE technique from ice algae *Chlamydomonas* sp. ICE-L. Moreover, mRNA expression levels of Hsp70 under different stress conditions were investigated using real-time quantitative RT-PCR (qRT-PCR) approaches.

Materials and methods

Plant materials and stress treatments

The water samples containing Antarctic ice algae were provided by the Polar Research Institute of Shanghai, China. They were collected from the floating ice near the Zhongshan Research Station of Antarctica (69.8°S, 77.8°E) during the 18th Antarctic expedition of China. The unicellular Antarctic ice alga was identified as *Chlamydomonas* sp. ICE-L (Liu et al. 2006) and cultured at light density of 40 μmol photons m^{-2} s^{-1}, 12L:12D cycle, temperature of 6°C, in the Provasoli seawater medium (Provasoli 1968). Seawater used for ice algae culture was taken from the coast of Qingdao (Shandong, China) and the salinity was 31‰.

For isolating full-length cDNA of Hsp70 gene, *Chlamydomonas* sp. ICE-L were kept in 12°C for 6 h, and then total RNA or poly A^+ RNA were extracted. In temperature treatment, algae were kept in different temperatures (−20°C to 0°C freeze and thaw 3 times; 0°C for 6, 12 and 24 h; 12°C for 6, 12 and 24 h) to investigate the effects of temperatures on mRNA expression of CiHsp70. In salt stress treatments, additional NaCl was supplemented to Provasoli seawater medium to get the final salinity of 62 or 93‰. Algae were then kept in the culture medium containing 62 or 93‰ NaCl for 2, 6, 12, 24 and 36 h to investigate the effects of salt stress on mRNA expression of CiHsp70. In UV-B radiation treatment, algae were placed under 8 W UV-B lamp (302 nm, NatureGene, USA) reaching 70 μW cm^{-2} irradiance for 2, 6 and 12 h or remove radiation then immediately give additional 6 h visible light to investigate mRNA expression of CiHsp70.

Total RNA preparation

Total RNA was extracted from liquid nitrogen ground algae powder using cetyltrimethylammonium bromide (CTAB) extraction buffer composed of 2% CTAB, 1% polyvinylpyrrolidone (PVP) K-30 (soluble), 100 mM Tris HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, 0.5 g L^{-1} spermidine (free acid) and 2% β-mercaptoethanol (added just before use) (Liu et al. 2009). After adding 0.5 mL CTAB buffer (2% β-ME) to the ground powder, the samples were incubated at 60°C for 10 min, mixed well by vortexing. The samples were further extracted twice with an equal volume of chloroform:isoamyl alcohol (24:1) centrifuged at 13,000 × g for 10 min at 4°C and the aqueous phase was transferred to a new tube. The supernatant was precipitated with 1/4 volume of 10.0 M LiCl to each tube and mixed well by inverting. The RNA was precipitated overnight at 4°C and harvested by centrifugation at 13,000 × g for 30 min at 4°C. Pellet was washed with 75% ethanol twice and air-dried for 5 min. RNA was

dissolved in 100 μL DEPC-treated water and RNA from the same samples were pooled together into new 1.5-mL RNAse-free microfuge tubes. The integrity of total RNA was verified by running samples on 1.2% formaldehyde denaturing agarose gels.

Cloning of CiHsp70 gene fragment

The first-strand cDNA of CiHsp70 gene was amplified using a PrimeScript™ Reverse Transcriptase (TaKaRa, Tokyo, Japan). The degenerate primers were designed from the conserved regions of Hsp70 from *Arabidopsis thaliana*, *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Oryza sativa*, *Physcomitrella patens*. The forward primer 5′-TG CGCATCATCAACGAGCC(G/C)AC-3′ was designed from the conserved peptide sequence of LRIINEPT. The reverse primer 5′-ACTTCTCCGCCTCCTG(G/C)AC CAT-3′ was designed from the conserved peptide sequence of MVQEAEK. CiHsp70 gene related cDNA fragment was amplified by RT-PCR programmed for 35 cycles (94°C for 30 s; 60°C for 30 s; 72°C for 1.5 min). The fragment of interest of the PCR product was excised, purified by agarose gel DNA fragment recovery kit (Tiangen, Beijing, China), subcloned into pMD-18T vector (TaKaRa, Tokyo, Japan), and sequenced (Invitrogen, Shanghai, China).

Rapid amplification of cDNA ends

Total RNA was extracted using CTAB extraction buffer, and was further purified by Oligotex mRNA Mini Kit (Qiagen, CA, USA). 5′- and 3′-RACE cDNA synthesized with primers designed from the amplified middle cDNA sequence of CiHsp70 (Table 1). The reverse transcription reaction was performed using 5′-RACE CDS Primer A and 3′-RACE CDS Primer A and PrimeScript™ Reverse Transcriptase (TaKaRa, Tokyo, Japan). The RACE reactions were performed using mRNA, primers GSP1 and GSP2 and SMART RACE cDNA Amplification Kit (Clontech, CA, USA) according to the manufacturer's instructions. The 5′- and 3′-RACE products were purified from an agarose gel using a Tiangen gel purification kit (Tiangen, Beijing, China) and were ligated into the T/A cloning vector pMD18-T (TaKaRa, Tokyo, Japan) and transformed into competent *Escherichia coli* Top 10 (Tiangen, Beijing, China). Five randomly selected clones were identified as positive clones using restriction endonuclease XbaI and PstI (TaKaRa, Tokyo, Japan) and were sequenced at least twice (Invitrogen, Shanghai, China).

Table 1 Primers

Genes	Primers	Oligonucleotide sequence (5′–3′)
Hsp70	Forward primer	TGCGCATCATCAACGAGCC(G/C)AC
Hsp70	Reverse primer	ACTTCTCCgCCTCCTg(G/C)ACCAT
5′Race	GSP1-Hsp70	CCTTGGACAGGCGTCCCTTGTCGTT
3′Race	GSP2-Hsp70	CGCCCGCACCAAGGACAACAACTC
Hsp70	HSP70 qPCR1	CGACCAATCATCCACACCTCTAC
Hsp70	Hsp70 qPCR2	GAGAAGCTGAATGCCAGACTTCCT
rbcl	rbcl qPCR1	ACATTCTCTGGTCCTCCTCACG
rbcl	rbcl qPCR2	AAGGAAACGGTCTCTCCAACGC

Bioinformatics analysis

The full-length cDNA sequence of CiHsp70 was spliced according to the RACE-PCR results by DNAStar 7.1 (DNASTAR Inc., USA). The theoretical molecular weight (Mw) and isoelectronic point (PI) of CiHsp70 protein were computed by ExPASy Compute pI/Mw tool (http://au.expasy.org/tools/pi_tool.html). Multiple alignments of Hsp70s were performed with ClustalW multiple alignment program which accessorily integrated in DNAStar 7.1 (DNASTAR Inc., USA) and BioEdit Sequence Alignment Editor program v7.0.5 (Hall 1999). A phylogenetic tree was constructed using the program Mega 4.0 (Kumar et al. 2008) and the reliability of the tree was analyzed using bootstrap probabilities.

Analysis of CiHsp70 mRNA expression by quantitative real-time PCR analysis

SYBR® PrimeScript™ RT-PCR Kit (TaKaRa, Tokyo, Japan) was used for qRT-PCR analysis. The cDNA for qRT-PCR was prepared using 500 ng of total RNA with Random 6 primers. The primers targeted against sequences of the CiHsp70 gene and ice algae rbcl gene were listed in Table 1. qRT-PCR was performed on Stratagene Mx3000P® qPCR System for 40 cycles (95°C for 10 s; 60°C for 10 s; 72°C for 20 s). All reactions were carried out at least in three duplicates. Quantification of mRNA level was based on Ct (threshold cycle) values. The Ct values of CiHsp70 were normalized using the Ct value corresponding to ice algae rbcl gene. The efficiency of each qRT-PCR was also calculated. Data analysis was executed using comparative Ct ($2^{-\Delta\Delta Ct}$) method (Livak and Schmittgen 2001).

Results

RNA extraction, cDNA cloning, and sequencing of CiHsp70 gene

High-quality total RNA was obtained by using modified CTAB extraction buffer. The formaldehyde agarose gel electrophoresis demonstrated that the extraction protocol

described here was efficient in yielding a high quality of total RNA from Antarctic unicellular algae (Fig. 1a).

CiHsp70 cDNA fragment was amplified from the total RNA and showed a 1,071 bp band by agarose gel electrophoresis. Taking CiHsp70 middle sequence as a template, two cDNA fragments of 1,625 and 891 bp were obtained through 5′RACE and 3′RACE approaches, respectively (Fig. 1b). The full length of cDNA contained an open reading frame (ORF) of 1,953 bp with 5′ UTR of 76 bp and 3′ UTR of 203 bp encoding a heat shock protein of 651 amino acids with a deduced molecular weight of 71.45 kDa and pI of 5.08. These results indicated that this new CiHsp70 gene belongs to the subfamily of Hsp70s gene. The sequence was submitted to GenBank with an accession number GQ888687.

Bioinformatic analysis of CiHsp70

The deduced amino acid sequence from Antarctic CiHsp70 cDNA was compared with those from other higher plants and algae by DNAStar 7.1 software. Our results showed that CiHsp70 was homologous to Hsp70s of other species such as *Arabidopsis thaliana* (GenBank accession No. NP_195870), *Oryza sativa* (japonica cultivar-group) (GenBank accession No. NP_001068540), *Chlamydomonas reinhardtii* (GenBank accession No. XP_001701326) and *Dunaliella salina* (GenBank accession No. AAL79999). The amino acid sequence deduced from CiHsp70 was 87.1 and 85.2% identity with Hsp70s of *C. reinhardtii* and *D. salina*, respectively. However, the identity was both about 80% when compared with Hsp70 of *A. thaliana*, *O. sativa* (Fig. 2).

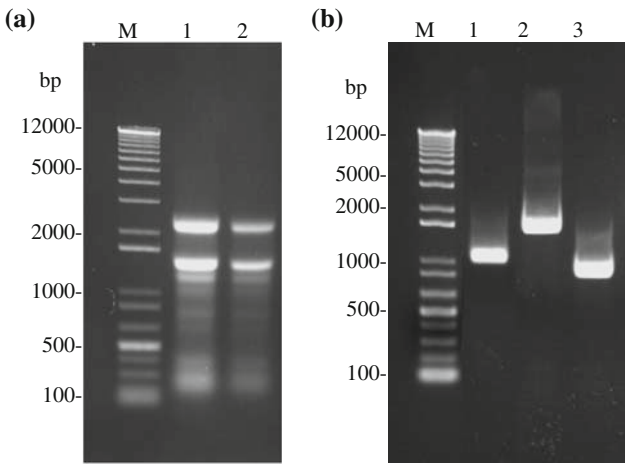

Fig. 1 **a** Total RNA isolated using CTAB extraction protocol was separated on a 1.2% denaturing formaldehyde agarose gel and stained with ethidium bromide. *M* 1 kb DNA ladder, *1* ice algae total RNA (~4 μg), *2* ice algae total RNA (~1 μg). **b** Agarose gel electrophoresis of double stranded cDNA amplified by long-distance PCR. *M* 1 kb DNA ladder, *1* middle sequence cDNA fragments, *2* 5′RACE cDNA fragments, *3* 3′RACE cDNA fragments

Multiple sequence alignment revealed that the deduced amino acid sequence of the CiHsp70 was highly conserved and shared homology with amino acid sequences of other known cytosolic Hsp70s (Fig. 2). In detail, the deduced amino acid sequence of CiHsp70 cDNA also included three typical motifs of the Hsp70 family at residues 9-16 (IDLGTTYS), 201-214 (IFDLGGGTFDVSLL), and 338-351 (VVLVGGSTRIPKVQ). The CiHsp70 was composed of an ATPase domain from residues 1 of ATG to 388 of AILTGEG motif, a substrate peptide binding domain from residues 389 to 634, and a C-terminus domain from residues 635 to 651. CiHsp70 contains the conserved cytosolic C-terminal sequence of GPKIEEVD and lacks any N-terminal signal or transit sequence (Fig. 2). However, in addition to the C-terminal sequence of GPKIEEVD, CiHsp70 terminated with the sequence of QSSTPLPDF, which is different from other cytosolic Hsp70s.

According to the method used in Renner and Waters (2007) and Fu et al. (2009), representative cytosolic Hsp70s of land plants and algae were selected to construct phylogenetic tree using the programs of DNAStar 7.1, BioEdit 7.0 and Mega 4.0. Meanwhile, some Hsp70s belonging to other groups (CP, MT, and ER groups) were also selected and used to construct phylogenetic tree to study phylogenetic relationship among different Hsp70 groups. In Hsp70s phylogenetic tree, *Chlamydomonas* sp. ICE-L (GQ888687) clustered together with *D. salina* (AAL79999), *C. reinhardtii* (EDO97621 and AAB00730), *Volvox carteri* f. *nagariensis* (AAZ04921 and AAZ04922), which all had the conserved cytosolic C-terminal sequence of GPKIEEVD and formed a sister group (Fig. 3). Furthermore, CiHsp70 kept relatively closer with cytosolic Hsp70s of green algae and higher plants, but was relatively distant from Cryptophyta, red algae and diatom. In general, the relationships displayed in the phylogenic tree were consistent with traditional taxonomy.

Expression levels of CiHsp70 in response to different temperature treatment

To investigate the effects of temperatures on expression level of CiHsp70 mRNA, ice algae *Chlamydomonas* sp. ICE-L in exponential growth status were treated with different temperatures and times (6°C for control; −20°C to 0°C freeze and thaw 3 times; 0°C for 6, 12 and 24 h; 12°C for 6, 12 and 24 h). After treatment, ice algae cells were quickly centrifuged at 4°C and stored at −80°C. qRT-PCR analysis showed that freeze and thaw the medium together with algae can increase the mRNA expression levels of CiHsp70 when compared to control. Meanwhile, chilling the ice algae cells at 0°C can also increase the mRNA expression of CiHsp70 with a maximum value of 4.4-fold after 12 h treatment. Furthermore, mRNA expression

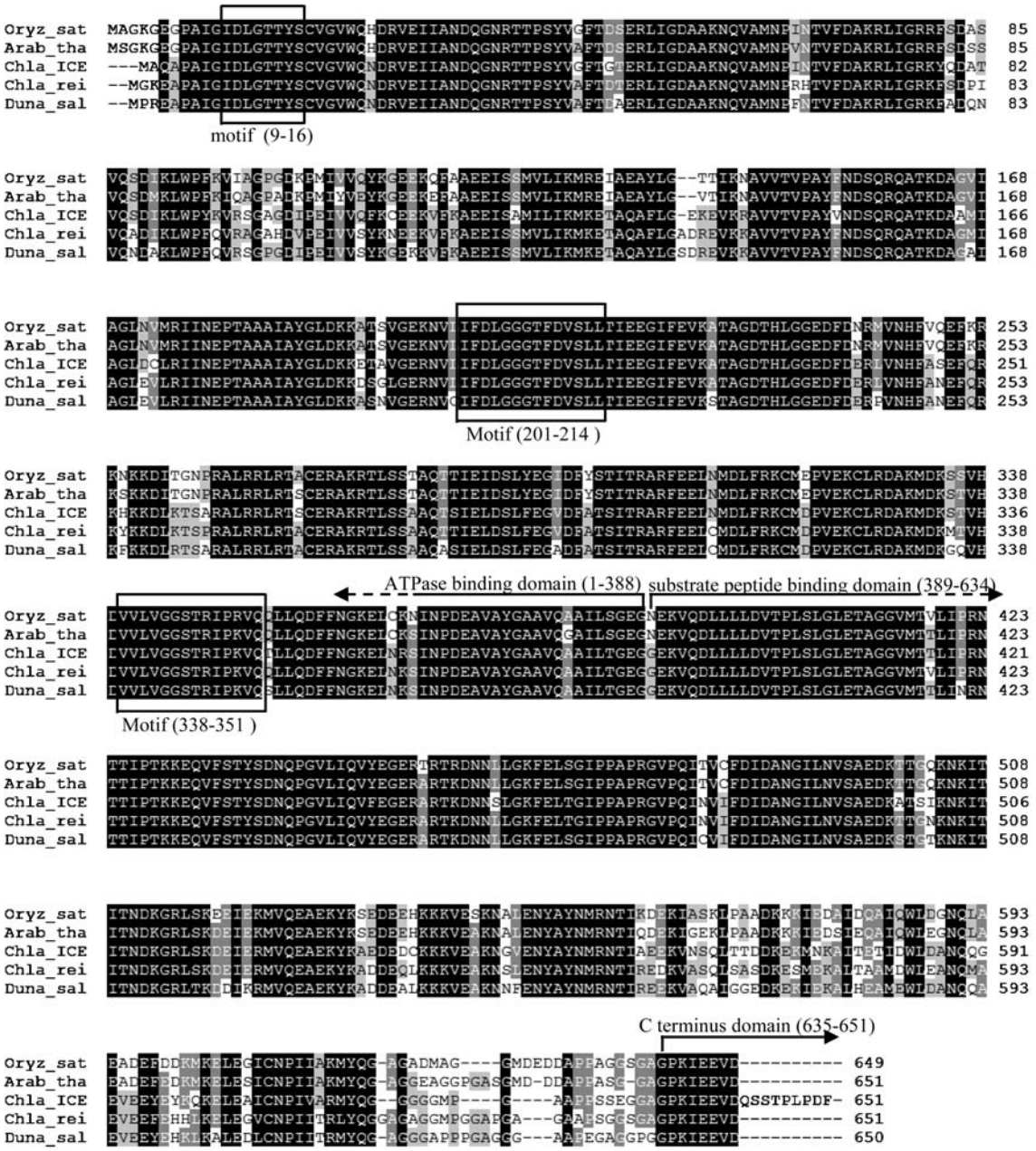

Fig. 2 Alignment and comparison of the deduced amino acid sequences of CiHsp70 with different species. Oryz_sat [*Oryza sativa* (japonica cultivar-group), NP_001068540], Arab_tha (*Arabidopsis thaliana*, NP_195870), Chla_ICE (*Chlamydomonas* sp. ICE-L, GQ888687) and Chla_rei (*Chlamydomonas reinhardtii*, XP_001701326), Duna_sal (*Dunaliella salina*, AAL79999). *Black bars* show the identical amino acid residues. Deletions are indicated by *dashes*

levels of CiHsp70 increased 3.0-fold after 6 h heat shock treatment at 12°C (Fig. 4).

Expression levels of CiHsp70 in response to UV-B radiation

In UV-B radiation treatment, *Chlamydomonas* sp. ICE-L was placed under different times of UV-B radiation or visible light to investigate the effects of UV-B radiation on mRNA expression levels of CiHsp70. The results of qRT-PCR analysis showed that CiHsp70 mRNA expression levels increased 2.7-, 2.9- and 2.3-fold corresponding to 2, 6 and 12 h UV-B radiation, respectively. Furthermore, after removing the UV-B radiation at 2, 6 and 12 h and then offering additional 6 h visible light, CiHsp70 mRNA expression levels were remarkably increased and reached 3.4-, 7.1- and 7.9-fold when compared to control (Fig. 4).

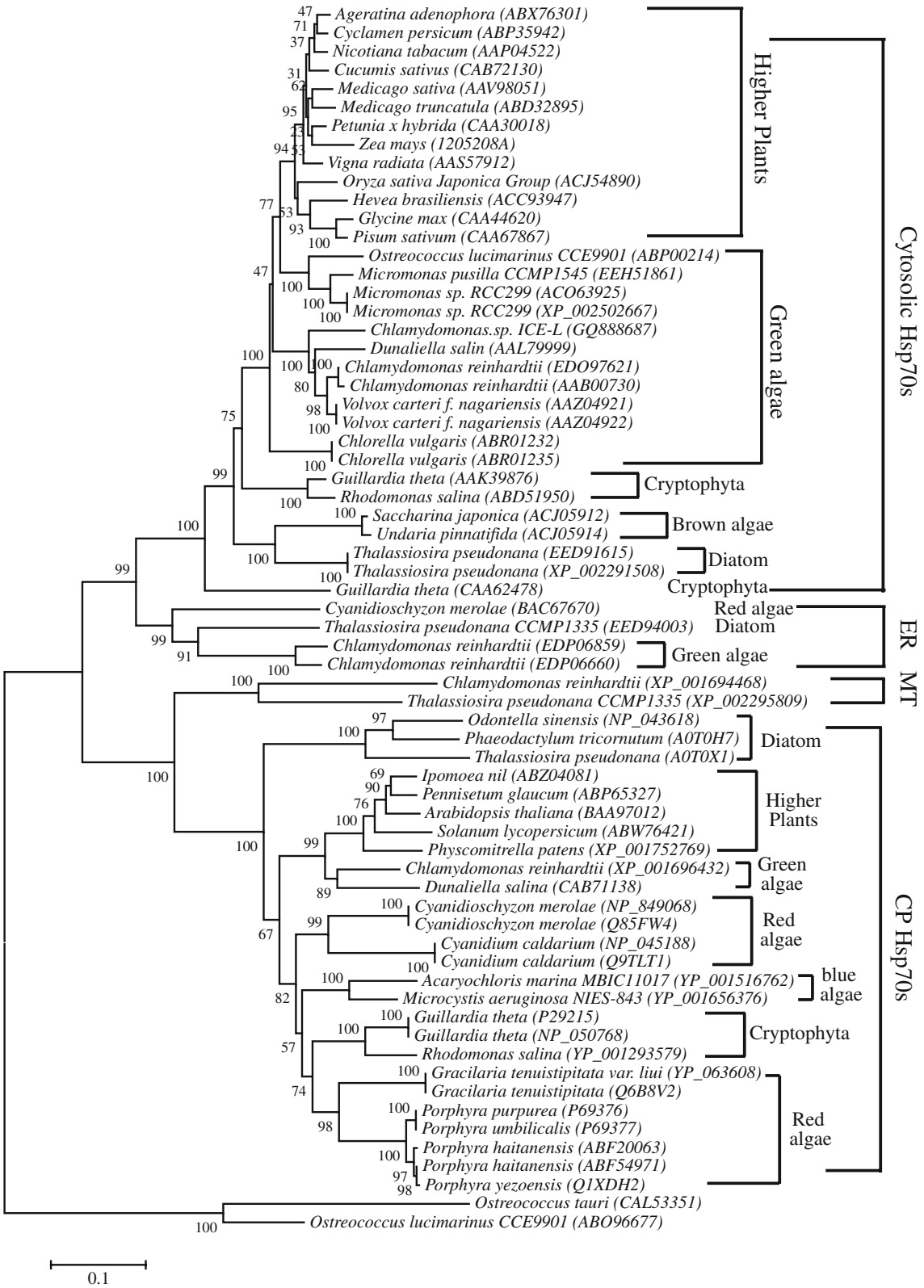

Fig. 3 Phylogenetic tree of Hsp70 family members constructed with the neighbor-joining method. Accession numbers are shown in *parentheses*

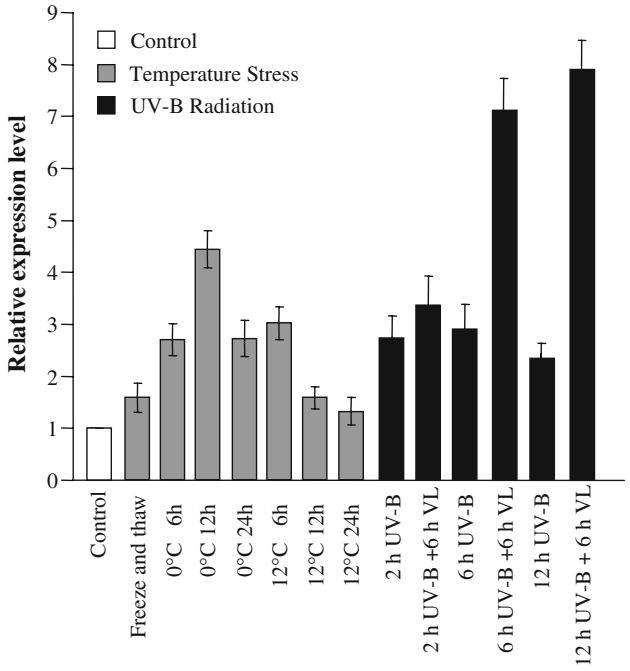

Fig. 4 CiHsp70 mRNA expression levels relative to rbcl mRNA levels under different temperatures and UV-B radiation analyzed by real-time quantitative RT-PCR. *VL* represents visible light irradiation. Standard error bars are shown

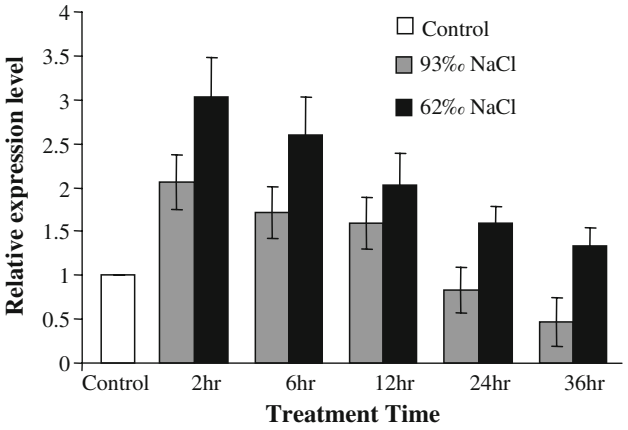

Fig. 5 CiHsp70 mRNA expression levels relative to rbcl mRNA levels under 62 or 93‰ salt concentrations analyzed by real-time quantitative RT-PCR. Standard error bars are shown

Expression levels of CiHsp70 in response to salt stress

In salt stress treatments, *Chlamydomonas* sp. ICE-L were kept in culture medium containing 62 or 93 ‰ NaCl, respectively, for 2, 6, 12, 24 and 36 h to investigate the expression levels of CiHsp70 mRNA. qRT-PCR analysis showed that after 2 h treatment, CiHsp70 mRNA expression levels increased 2.1-fold in 93‰ NaCl culture medium, while CiHsp70 mRNA expression levels increased threefold in 62‰ NaCl culture medium. However, CiHsp70 mRNA expression levels were gradually decreased with extending treatment time both in 93 and 62‰ NaCl culture medium (Fig. 5).

Discussion

The 70 kDa heat shock protein family is considerably and highly conserved across prokaryotes and eukaryotes (Renner and Waters 2007; Wang et al. 2009; Fu et al. 2009; Reddy et al. 2010). In the present study, a CiHsp70 gene encoding a mature protein of 651 amino acids was isolated from Antarctic ice algae by RT-PCR and RACE techniques (Figs. 1, 2). CiHsp70 containing the conserved cytosolic C-terminal sequence of GPKIEEVD and lacks any N-terminal signal or transit sequence suggests that CiHsp70 is a member of the cytoplasmic Hsp70 family or lineage (Fig. 2). Phylogenetic tree analysis showed that CiHsp70 clustered together with other cytoplasmic Hsp70s, which is consistent with the result of multiple sequence alignment (Fig. 3).

Recently, due to the highly conserved structure and important function of Hsp70s, the degree of difference in the structure might reflect the phylogenetic relationship across species of algae and land plants. The Hsp70 phylogenetic tree constructed here was largely consistent with the phylogenetic trees of Renner and Waters (2007) and Fu et al. (2009). In our previous study, the taxonomic characterization of Antarctic ice algae *Chlamydomonas* sp. ICE-L was analyzed on the basis of morphological and 18S rDNA and ITS-1 sequence traits (Liu et al. 2006). The results indicated that Antarctic ice algae *Chlamydomonas* sp. ICE-L belongs to *Chlamydomonas* (Chlorophyta) and maintains a very close relative of *Chlamydomonas* sp. Antarctic 2E9. The phylogenetic relationship analysis suggests that *Chlamydomonas* sp. ICE-L is clearly a new species that lies within the cluster of green algae, but the results of CiHsp70 phylogenetic tree indicate that it is more closely related to *Dunaliella salin* rather than *C. reinhardtii*.

In Arctic and Antarctic oceans, the semisolid ice matrix provides a range of habitats in which low temperature, salinity, ultraviolet radiation and dissolved gases and so on are the important factors that affect the physiology and metabolism of algae in polar environment (Thomas and Dieckmann 2002; Iwamoto and Shiraiwa 2005; Mock and Thomas 2005). Psychrophiles are able to thrive in these cold environments, and have developed a variety of strategies for successful colonization (Morgan-Kiss et al. 2006). It has been demonstrated that the Hsp70s can be activated rapidly for protecting the cell from a wide variety of physiological and environmental stress (Yordanov 1995;

Feder and Hofmann 1999). In ice algae *Chlamydomonas* sp. ICE-L, qRT-PCR analysis showed that CiHsp70 mRNA expression levels were increased not only in response to heat shock stress but also to chilling stress. In Antarctic alga *Plocamium cartilagineum* (L. Dix.), heat-shock response results suggested that alga incubations at higher temperatures or for longer periods reduced the amount of Hsp70 mRNA (Vayda and Yuan 1994). This observation was consistent with the heat-shock response of CiHsp70 after *Chlamydomonas* sp. ICE-L treatment at 12°C (Fig. 4). To imitate the later summer ice condition in Antarctic, ice algae *Chlamydomonas* sp. ICE-L were freezed and thawed for three times within culture medium. Result showed that mRNA expression levels of CiHsp70 were also increased in response to repeated freeze and thaw cycles.

Particularly, polar organisms suffer from strong seasonal changes in the radiation climate (Karentz and Bosch 2001). This is further intensified by the fact that the stratospheric ozone depletion, increasing the UV-B on the Earth's surface coincides with spring conditions (Karentz 1989; Hanelt et al. 2001). UV-B radiation exerts many potentially harmful effects on prokaryotic and eukaryotic cells including mutagenesis, chronic depression of key physiological processes, and acute physiological stress that may result in death (Vincent and Roy 1993), while heat shock proteins may help the cell to cope with UV-induced damage (Simon et al. 1995; Trautinger et al. 1996; Park et al. 2000). In the present study, CiHsp70 mRNA expression levels increased 2.9-fold in response to 6 h UV-B radiation, while the mRNA expression of CiHsp70 were remarkably increased after removing the UV-B radiation and then immediately offering additional 6 h visible light (Fig. 4). Therefore, these data indicate that CiHsp70 might provide an adaptive cellular response to UV radiation and involve in damage repairing after removing the UV-B radiation.

In natural environment, ice algae *Chlamydomonas* sp. ICE-L generally grows in seawater with salt concentration about 30‰, but the sea ice will freeze and thaw in different seasons which in turn affects the salt concentration of estuaries and offshore where ice algae inhabits. Several reports suggested that the Hsp70 mRNA expression was regulated by osmotic stress (Shim et al. 2002; Dong et al. 2008). In the present study, qRT-PCR analysis showed treating with 62‰ NaCl from 2 to 12 h, mRNA expression level of CiHsp70 were increased 3.0-fold when compared to the control (Fig. 5). Further increasing the medium salinity to 93‰ also increased CiHsp70 mRNA expression when compared to the control, but the increasing amplitudes were lower than that of 62‰ salinity. In *Laminaria japonica*, study showed that the expression levels of LJHsp70 were increased in low salt concentration rather that in higher salt concentration (Fu et al. 2009).

Although stress-induced expression of Hsp70 genes have been widely studied in photosynthetic eukaryotes (Renner and Waters 2007) and this study has only examined the accumulation of CiHsp70 in transcription levels, to our knowledge, this is the first characterized Hsp70 with the response to low temperature, high salinity and UV-B radiation all together. Further study are therefore recommended with regard to the translation and hetero-expression levels as well as other differentially expressed proteins in *Chlamydomonas* sp. ICE-L to clarify the mechanism of ice algae in acclimating Antarctic environment.

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