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Protective function of the recombinant Oshsp18.0-CII protein, a class II small heat shock protein of rice, in *Escherichia coli*

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ABSTRACT. All plants synthesize multiple families of small heat shock proteins (sHSP). A cDNA clone, *Oshsp18.0-CII*, encoding an 18.0 kDa class II sHSP was isolated from rice previously. The function of the rice class II sHSP was studied by overproduction of the Oshsp18.0-CII fusion protein in transformed *Escherichia coli* cells. The results suggest that heterologous expression of the Oshsp18.0-CII fusion protein increases thermotolerance of *E. coli* cells *in vivo* and provided thermoprotection to *E. coli* soluble proteins *in vitro*. The survival rate of Oshsp 18.0-CII fusion protein-accumulating cells treated at 50°C for 1 h was almost 1000-fold higher than that of the control cells transformed with *pET32a* expression vector. Overproduction of the Oshsp18.0-CII fusion protein in *E. coli* also confers tolerance of *E. coli* cells to ultraviolet (UV) irradiation. The post-UV survival of the Oshsp18.0-CII fusion protein-accumulating cells is about 7.29-fold over that of the control cells transformed with *pET32a* expression vector when exposed to 1700 μJ of UV. There is almost no post-UV survival (≦0.4%) in the untransformed cells after exposing to 900 μJ UV light.

Keywords: Heat shock; *Oryza sativa* L.; Thermotolerance; Thermoprotection; Ultraviolet.

Abbreviations: ER, endoplasmic reticulum; **HSP,** heat shock protein (s); **HRP,** horse radish peroxidase; **IPTG,** isopropyl β-D-thiogalactopyranoside; **LA,** Luria-Bertani broth containing 100 μg mL^{-1} of ampicillin; **LB,** Luria-Bertani broth; **PBS,** Phosphate-buffered Saline; **PCR,** polymerase chain reaction; **sHSP,** small heat shock protein (s); **SDS-PAGE,** sodium dodecyl sulfate polyacrylamide gel electrophoresis; **UV,** ultraviolet.

INTRODUCTION

Plant cells have evolved to cope with the heat stress in their environments. Under heat stress, plant may become tolerant to non-permissive high temperatures, as a result of the induction of heat shock proteins (HSP; Vierling, 1991). The roles of individual HSP in heat stress tolerance are reportedly different among different organisms; they rely on different sets of HSP to establish tolerance to similar levels of heat stress (Parsell and Lindquist, 1993; Krishna, 2003).

The HSP families in plants are more complex than those in other organisms. Based on the molecular mass, HSP are grouped into five major families (Wang et al., 2004; Kotak et al., 2007) including the families of HSP100/Clp (Schirmer et al., 1996; Nieto-Sotelo et al., 1999), HSP90 (Mogelsvang and Simpson, 1998), HSP70/DnaK (Miernyk, 1997), chaperonins (GroEL and HSP60) (Sigler et al., 1998; Krishna, 2003; Wang et al., 2004), and small molecular mass proteins (sHSP) ranging in size of approximate 16-42 kDa (Scharf et al., 2001; Siddique et al., 2008).

All plants synthesize multiple families of sHSP (Vierling, 1991). Under heat stress condition, the accumulation level of the sHSP in soybean could reach more than 1% of the total cellular proteins (Hsieh et al., 1992), and this accumulation is correlated with the acquisition of thermotolerance (Lin et al., 1997; Chang and Lin, 2000; Sun et al., 2002; Wang et al., 2003; 2004). Recently, the complexity of the heat stress responses in plants has been addressed (Kotak et al., 2007). Plant sHSP have been grouped further based on sequence homology, immunological cross-reactivity, and localization to different cellular compartments (Waters et al., 1996). There are three classes of cytosolic sHSP (class I, II, and III), as well as four distinct types of sHSP localized in the various organelles including endoplasmic reticulum (ER), mitochondria, chloroplasts,

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and peroxisomes (Siddique et al., 2008). Other proteins containing α-crystallin domain (ACD proteins) were also identified (Scharf et al., 2001). Plant sHSP have been shown to be induced by several abiotic stresses, photoperiod, and certain developmental programs (Krishna et al., 1992; Sun and MacRae, 2005; Waters et al., 2008; Lin and Chang, 2009).

In addition to heat tolerance, other functions of sHSP that protected plants under adverse environments were reported. Sun et al. (2001) reported that a class II sHSP, At-HSP17.6A, could increase salt and drought tolerance in *Arabidopsis*. Plant sHSP could increase tolerance to heavy metal (Heckathorn et al., 2004), oxidative stress (Banzet et al., 1998), and osmotic stress (Sun et al., 2001). Plant HSP (including sHSP) have been shown to posses chaperone function (Wang et al., 2004; Sun and MacRae, 2005), and could protect other cellular proteins to maintain correct conformation or translocation to other organelles under stresses. The sHSP may be activated and involved in plant growth and development, such as pollen differentiation, embryogenesis, seed germination, and fruit maturation (Sun et al., 2002).

Members of the sHSP family were shown to prevent aggregation of thermally inactivated reporter proteins *in vitro* (Horwitz, 1992; Jakob et al., 1993; Lee et al., 1995; 1997; Ehrnsperger et al., 1997; Krishna, 2003; Sun and MacRae, 2005). Direct evidence showing a molecular chaperone function of plant sHSP *in vivo* has also been reported. A dodecameric class II sHSP from pea, HSP17.7, prevented the aggregation of citrate synthase when heated to 45°C (Lee et al., 1995). Helm et al. (1997) found simultaneously expressing class I and class II sHSP could form distinct complexes *in vivo*, suggesting that they have subtly different functions. Basha et al. (2004) showed that the class II protein of wheat (TaHsp17.8C-II) comprises a smaller oligomer than the dodecameric TaHsp16.9C-I. This indicated that class II proteins may have a distinct mode of oligomer assembly as compared to the class I proteins. These authors also showed that heat-denaturing firefly luciferase was fully protected by TaHsp17.8C-II, but it did not interact significantly with TaHsp16.9C-I (Basha et al., 2004).

Before 2007, nine genes encoding class I sHSP have been characterized in rice (Guan et al., 2004) but only one class II sHSP gene, *Oshsp18.0-CII*, has been identified (Chang et al., 2007). The *Oshsp18.0-CII* gene is transcriptionally regulated by heat stress, but not by other environmental stresses such as cold, salt, and drought (Chang et al., 2007). Comparing to class I sHSP, fewer reports were focused on the function of class II sHSP in other plants, especially very few reports were for the class II sHSP of rice. The class II sHSP of pea (Lee et al., 1995; Basha et al., 2010), tomato (Löw et al., 2000), wheat (Basha et al., 2004; 2010), and Arabidopsis (Basha et al., 2010) were shown to act as molecular chaperones. Overexpression of an Arabidopsis class II sHSP enhanced osmotolerance (Sun et al., 2001). Hsp17.4-CII of tomato acts as corepressor and cytoplasmic retention factor of heat shock transcription factor HsfA2 (Port et al., 2004). Recently, one more class II sHSP gene, *Oshsp19.0-CII*, was identified by Sarkar et al. (2009) according to the phylogenetic and *in silico* localization analysis of the 40 α-crystallin domain containing genes in rice genome, which were identified using the database search of rice genome for sequences with the presence of α-crystallin domain at the C-terminus. Based on the expressed sequence tags (EST), reverse transcription-polymerase chain reaction (PCR), and microarray results, expression profiles of *Oshsp18.0-CII* and *Oshsp19.0-CII* were different under various stress treatments (heat, salt, UV, and anoxia), and developmental programs such as pollen and anther development, embryogenesis, and seed maturation (Sarkar et al., 2009). It appears that, except for anoxia, *Oshsp19.0-CII* was not abiotic stress induced based on the stresses examined including heat and UV-C treatments (Sarkar et al., 2009).

To understand the possible structure-function relationship of the rice class II sHSP, we introduced the *Oshsp18.0-CII* cDNA into *E. coli* and studied its thermotolerance in the transformed *E. coli* cells. In this study, heterologous expression of a rice 18.0-kDa sHSP resulted in thermotolerance of *E. coli* cells to a lethal heat treatment *in vivo* and provided thermoprotection to functional proteins of *E. coli in vitro*. The effects of rice Oshsp18.0-CII protein in resistance to ultraviolet (UV) irradiation were also observed in *E. coli* cells.

MATERIALS AND METHODS

Preparation of the *Oshsp18.0-CII* gene expression construct

The open reading frame of the *Oshsp18.0-CII* gene was amplified by PCR with primers covering both termini of the coding region of the *Oshsp18.0-CII* cDNA which encodes the full-length polypeptide of 210 amino acids. For gene construction, a *Eco*RI site (single underlined) was introduced to the 5' primer (5'-CATGAATTCATGGAGAGCGC-3') and a *Xho*I site (double underlined) to the 3' primer (5'-CATCTCGAGGATAAGCAGCAG-3'), respectively. Five ng of the *Oshsp18.0-CII* gene cloned in the pGEM®-T Easy Vector (Promega Co., Madison, WI, USA) was used as template for PCR reaction containing 10× PCR Reaction Buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl; 25 mM $MgCl_2$; Roche Applied Science, Mannheim, Germany), 0.3 mM each of four dNTPs, 0.4 mM each of two primers, 2 units FastStart Taq DNA Polymerase (Roche Applied Science, Mannheim, Germany). The PCR program was 1 min at 94°C, 1 min at 38°C, 40 s at 72°C for 35 cycles followed by 10 min at 72°C. The PCR product was digested with *Eco*RI and *Xho*I restriction enzymes (New England Biolabs, Beverly, MA, USA), and ligated into the *pET32a* expression vector (Novagen, Madison, WI, USA) at the *Eco*RI and *Xho*I sites to produce the recombinant plasmid, *pETHSP*. The nucleotide sequence of the cloned *Oshsp18.0-CII* gene was confirmed by sequencing. The *E. coli* strain BL21 (DE3) was transformed with

either the *pET32a* expression vector alone to produce the ET32A cells or with the recombinant plasmid (*pETHSP*) to produce the ETHSP cells.

Expression of the Oshsp18.0-CII fusion protein in transformed *E. coli*

The ET32A and ETHSP cells were grown in Luria-Bertani (LB) broth (Tryptone 10 g, Yeast extract 5 g, NaCl 10 g per liter) containing 100 μg mL^{-1} of ampicillin (LB-Amp) at 37°C overnight. The overnight cultures were diluted 50-fold using fresh LB broth and incubated at 37°C until OD_{600} reached 0.5 to 0.8 (mid-log phase), then recombinant protein expression was induced by the addition of isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM, followed by incubation at 37°C for 4 h. The bacterial cells were harvested by centrifugation and disrupted by sonication at 7.5Hz for 15s each time for ten times and centrifuged at 24,000 ×*g* for 10 min at 4°C. The supernatant containing the crude protein extracts were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Protein samples were dissolved in sample buffer (50 mM Tris-HCl, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol), separated in a 12% polyacrylamide gel (acrylamide:Bis = 29:1) containing 0.1% SDS and electro-blotted onto a PROTRAN® nitrocellulose transfer membrane (Schleicher & Schuell, Einbeck, Germany) using a Bio-Rad transblot apparatus (Trans-Blot Transfer Medium, Bio-Rad, Hercules, CA, USA). The membrane was submerged in 3% non-fat powdered milk with gentle shaking for 1 h, and then incubated with either 1:2000 diluted monoclonal antibodies against the N-terminal histidine residues of the expression protein (Amersham Pharmacia Biotech, Buckinghamshire, England) or with 1:2000 diluted polyclonal antibodies against the pea 17.7 kDa class II HSP (Pshsp17.7) (provided by Dr. Elizabeth Vierling of the Department of Biochemistry, University of Arizona) in TTBS buffer (20 mM Tris-HCl, pH 7.5; 500 mM NaCl; and 0.05% Tween-20) containing 1% non-fat powdered milk for 2 h. After 3 rinses with TTBS buffer for 10 min each, the membranes were incubated with 1:3000 dilution of either donkey anti-mouse or donkey anti-rabbit antibodies conjugated with horseradish peroxidase (HRP) (Amersham Pharmacia Biotech, Buckinghamshire, England) for 1 h. The blot was then washed twice with TTBS buffer for 10 min each, and once with TBS (20 mM Tris-HCl, pH 7.5; and 500 mM NaCl) buffer for 10 min. Signal detection was initiated by submerging the membranes in HRP color development solution (15 mg 4-chloro-1-naphtol in 5 mL cold methanol; 15 μL cold 30% H_2O_2 in 20 mL TBS) for 45 min. The membrane was rinsed with distilled water to stop the reaction.

Thermotolerance of transformed *E. coli* cells

The ET32A and ETHSP cells were used for the thermotolerance assay. Cell cultures were grown in LB-Amp broth as mentioned above. After IPTG induction, 5 mL of the cell culture was transferred to 50°C. The cultures were taken at 0.5, 1, 1.5, 2, and 4 h after 50°C treatment, and serial dilutions of the cultures were plated in triplicate on LB-Amp agar medium. The plates were incubated overnight at 37°C prior to scoring numbers of colony formation to determine the percentage of viable cells.

UV sensitivity of transformed *E. coli* cells

After IPTG induction, the BL21 (DE3), ET32A, and ETHSP cultures were first adjusted to the same concentrations (OD_{600} = 1.0 to 1.5) and diluted 10-fold at each step. Diluted samples of 100 μl were spotted in triplicate onto the LB plates. The plates were subjected to 500, 900, 1300, and 1700 μJ UV (UV-C, 254 nm) treatments (UV Stratalinker 1800, Stratragene, La Jolla, CA, USA). The plates were then incubated overnight at 37°C prior to scoring colony formations to determine the percentage of viable cells.

Thermo-stability of proteins in the *E. coli* cell lysate

Following IPTG induction, the transformed *E. coli* cells were centrifuged at 3,800 ×*g* (Beckman, Harbor Boulevard, CA, USA) for 10 min at 4°C, and resuspended in Phosphate-buffered Saline (PBS) buffer (pH 7.4; 2.7 mM KCl, 2 mM KH_2PO_4, 10 mM NaH_2PO_4, 150 mM NaCl). Cells were disrupted by passing through a French Pressure (Sim-AMINCO, Silver Spring, MD, USA) three times at 1,200 psi. The disrupted cells were spun down at 16,000 ×*g* (Labnet International, Edison, NJ, USA) for 3 min at 28°C, and soluble proteins were quantified by Bio-Rad Protein Assays Kit (Bio-Rad, Hercules, CA, USA). The soluble protein concentration was adjusted to 1 mg/500 μL PBS buffer. The adjusted protein samples were heat treated individually at 50, 60, 70, 80, 90, and 100°C for 15 min. After heating, supernatants and precipitated pellets were separated by centrifugation at 16,000 ×*g* for 10 min at 28°C, and their protein concentrations were determined as described above.

RESULTS

Induction of fusion protein in transformed *E. coli* cells

The rice *Oshsp18.0-CII* gene, encoding a class II sHSP, was introduced into *E. coli* BL21 (DE3) cells for its expression and function analysis in bacterial cells. The *pET32a* expression vector would produce a ~20 kDa protein in bacteria. With the recombinant plasmid *pETHSP,* the bacterial cells would produce a ~38 kDa fused protein, which contained the Oshsp18.0-CII polypeptide sequence. It appeared that the proteins in these two vectors could be induced after one hour (h) incubation with 1 mM IPTG (Figure 1A). It also showed that the proteins could be induced by as low as 0.5 mM IPTG in bacterial cells (Figure 1B). After 4 h incubation, the accumulation of Oshsp18.0-CII fusion protein in the ETHSP cells was a little bit higher

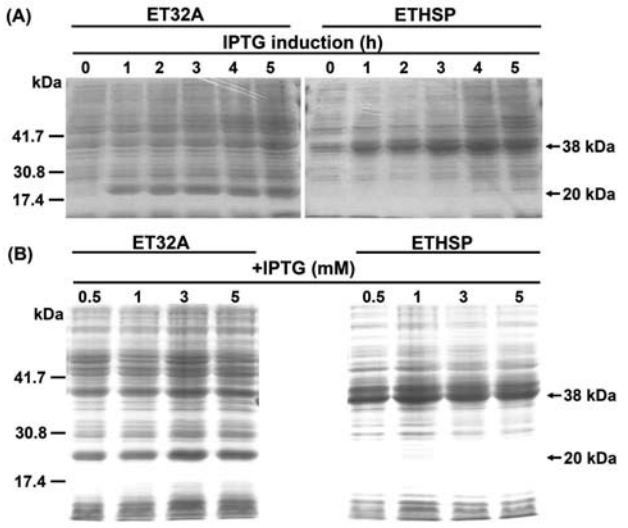

Figure 1. SDS-PAGE analysis of total proteins extracted from ET32A cells containing the *pET32a* expression vector and the ETHSP cells with the recombinant plasmid *pETHSP* overexpressing recombinant Oshsp18.0-CII. A: Total proteins extracted from ET32A and ETHSP cells after 0, 1, 2, 3, 4, and 5 hour (h) 1 mM IPTG induction; B: Total proteins extracted from ET32A and ETHSP cells after 0.5, 1, 3, and 5 mM IPTG induction for 4 h. Ten μL of protein samples were subjected to SDS-PAGE analysis. The recombinant protein expressed in ET32A and ETHSP cells was ~20 kDa and ~38 kDa in size, respectively.

by 1 mM IPTG induction (Figure 1B). The western blotting analysis showed that the IPTG induced Oshsp18.0-CII fusion protein in the transformed ETHSP cells was histidine-tagged and recognized by the polyclonal antibodies against Pshsp17.7, the 17.7 kDa class II sHSP of pea (Figure 2).

Growth rates of the transformed *E. coli* cells

The ET32A and ETHSP cells had similar growth rates at 37°C when compared with the non-transformed *E. coli* BL21 (DE3) cells (Figure 3, open symbols), although slightly higher OD_{600} values were obtained in ETHSP cells after growth at 37°C for 480 min. After 1 mM IPTG induction, similar growth rates of the non-transformed BL21 (DE3), ET32A, and ETHSP cells were found (Figure 3, closed symbols). The growth rates of the above three culture cells, however, were lower when subjected to 1 mM IPTG treatment than without IPTG. Overall, we observed no difference in the growth of these three *E. coli* cells at 37°C.

Thermotolerance of the *E. coli* cells containing the Oshsp18.0-CII fusion protein

To investigate the molecular chaperone function of Oshsp18.0-CII *in vivo*, the attenuation in lethality at 50°C was checked for the *E. coli* cells producing the recombinant Oshsp18.0-CII protein. As shown in Figure 4, after exposing cells to 50°C for 0.5 h, the survival rate of ET32A cells dropped to 0.4%, whereas that of the ETHSP cells was 33%. The difference in survival rates at 50°C for 0.5 h was greater than 80 folds. Even after 1 h exposure to 50°C, the ETHSP cells still had a survival rate of about 10%, almost 1000-fold higher as compared with the ET32A cells, which had only 0.01% survival rate. Heating at 50°C for 1.5 h dropped the survival rate of the ETHSP cells to 1.8%, whereas no ET32A cells survived under the same condition. It was evident that 0.02% ETHSP cells could survive 2 h exposure to 50°C (Figure 4). It appeared that the transformed *E. coli* cells (ETHSP) producing the

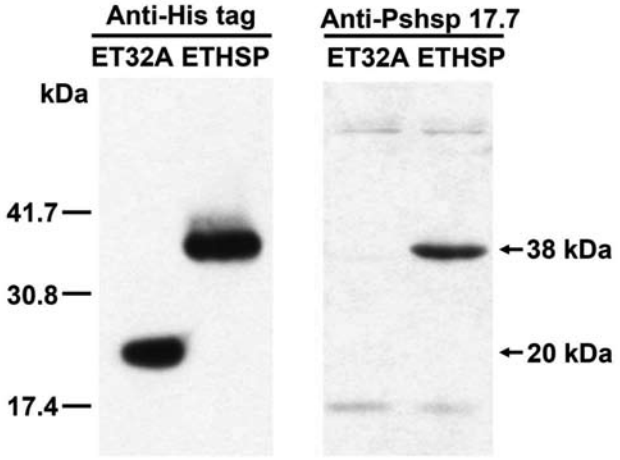

Figure 2. Immunological gel blots of the recombinant proteins produced in ET32A and ETHSP cells. Monoclonal antibodies against histidine tag (Anti-His tag) and polyclonal antibodies against the 17.7 kDa class II HSP of pea (Anti-Pshsp 17.7) were used for blotting. The His-tagged recombinant Trx·Tag™ thioredoxin protein of ~20 kDa in ET32A and ETHSP cells and the His-tagged Oshsp18.0-CII fusion protein of ~38 kDa in ETHSP cells were detected after incubation 4 h with 1 mM IPTG.

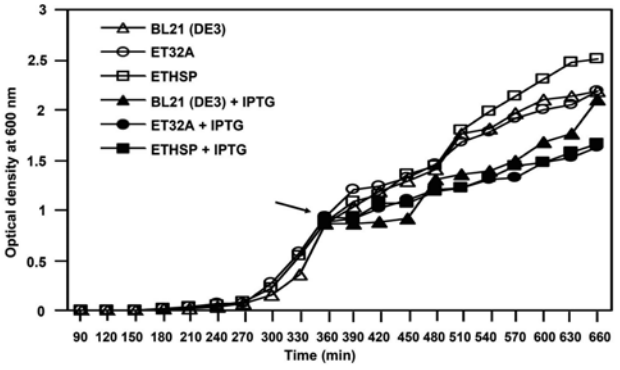

Figure 3. Growth of wild-type and transformed *E. coli* BL21 (DE3) cells at 37°C with or without IPTG. Cultures of *E. coli* BL21 (DE3) cells (△ and ▲), ET32A cells (○ and ●) carrying the *pET32a* vector, and ETHSP cells (□ and ■) carrying the recombinant *pETHSP* plasmid were grown at 37°C for 10 h. When OD_{600} reached to 0.8-1.0 (6 h after growth), half of the cultures were added with (closed symbols) or without (open symbols) IPTG to a final concentration of 1 mM, and subjected to further growth at 37°C for 4 h. Arrow indicates the addition of IPTG. Optical density at 600 nm was measured for cell growth.

Oshsp18.0-CII fusion protein was more thermotolerant than the cells harboring only the *pET32a* vector producing the recombinant Trx·Tag™ thioredoxin protein.

Ultraviolet irradiation protection by rice Oshsp18.0-CII fusion protein in *E. coli*

Induction of the Oshsp18.0-CII fusion protein in the UV-sensitive *E. coli* strain BL21 (DE3) cells increased the survival of *E. coli* after UV irradiation (post-UV survival) (Figure 5). The data indicated that overproduction of Oshsp18.0-CII fusion protein protects ETHSP cells against UV irradiation at the dosages of 500 μJ to 1700 μJ. It is surprising that after IPTG induction, the post-UV survival of the ET32A strain harboring the control plasmid *pET32a* was higher than that of the plasmid-free non-transformed BL21 (DE3) cells. The increase in the post-UV survival of the ETHSP in comparison with other cells was observed when cells were subjected to 500 μJ or higher UV irradiation. The significant increase in the post-UV survival of the ETHSP cells was from ~2.02-fold (at 500 μJ UV) to ~7.29-fold (at 1700 μJ) when compared with the ET32A cells. These results suggested that the Oshsp18.0-CII fusion protein may lead to UV-tolerance of *E. coli* and therefore increased the post-UV survival.

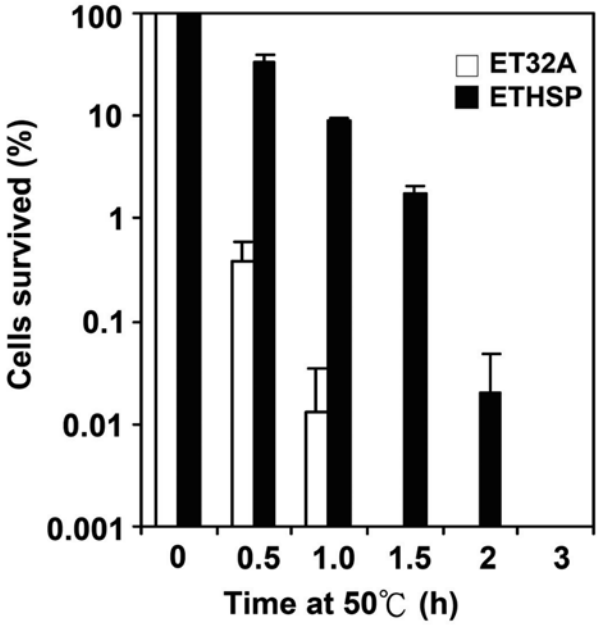

Figure 4. Survival rates of the transformed *E. coli* ETHSP cells after heat treatments. After 4 h of 1 mM IPTG induction, the *E. coli* cells carrying *pET32a* expression vector (ET32A, open columns) and cells producing the Oshsp18.0-CII fusion protein (ETHSP, filled columns) were heat-treated at 50°C for 0, 0.5, 1, 1.5, 2, and 3 h. Percentages of cells survived after 50°C treatment were measured using the cell numbers obtained at treatment of 0 h as 100%. The percentage of the ET32A cells survived after 0, 0.5, 1, 1.5, 2, and 3 h heat treatment was 100%, 0.4%, 0.01%, 0%, 0%, and 0%, respectively, while that of the ETHSP cells was 100%, 33%, 9.2%, 1.8%, 0.02%, and 0%, respectively. Values are means ± SE (n = 5).

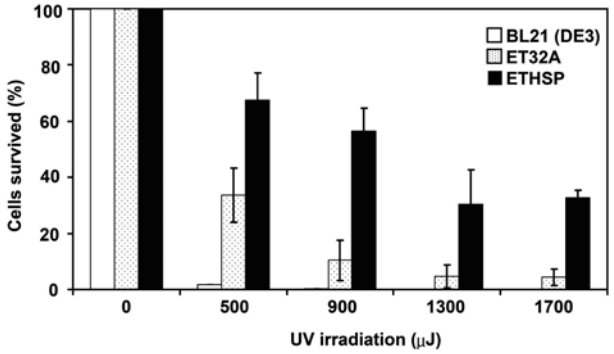

Figure 5. Influence of Oshsp18.0-CII on cell viability of *E. coli* under UV irradiation. After 3 h of 1 mM IPTG induction, *E. coli* cells carrying *pET32a* vector (ET32A, spotted column), cells overproducing the Oshsp18.0-CII fusion protein (ETHSP, filled column), and non-transformed BL21 (DE3) cells (BL21 (DE3), open column) were diluted, plated on LA, and subjected to 0, 500, 900, 1300, and 1700 μJ UV irradiation. Percentage of cells survived after UV irradiation was measured using the cell number obtained at treatment of 0 μJ as 100%. The percentage of the ET32A cells survived after 0, 500, 900, 1300, and 1700 μJ UV irradiation was 100%, 33.5%, 10.4%, 4.6%, and 4.5%, respectively; that for BL21 (DE3) cells was 100%, 1.8%, 0.4%, 0.1%, and 0.1%, respectively; whereas that for ETHSP cells was 100%, 67.7%, 56.3%, 30.3%, and 32.8%, respectively. Values represent means ± SE (n = 3).

Thermo-stability of proteins prepared from the *E. coli* cells producing the Oshsp18.0-CII fusion protein

To understand if rice Oshsp18.0-CII could protect *E. coli* proteins from denaturation during heat stress, the protein precipitation in the ET32A and ETHSP cell lysates were analyzed after heat treatments at 50, 60, 70, and 80°C for 15 min (Figure 6). The denatured proteins were precipitated by centrifugation after heat treatments. As the temperature was increased from 50°C to 80°C, an increase in the ratio of denatured proteins to soluble proteins in *E. coli* cell lysate was observed (Figure 6). The change of thermo-denatured proteins over the soluble proteins in ET32A cells after 15 min of 50°C, 60°C, 70°C, and 80°C treatments was 0.48, 1.45, 3.38, and 3.88-fold, respectively, whereas that in ETHSP cells was 0.4, 1.1, 2.17, and 2.73-fold, respectively. Nevertheless, in the lysate of ETHSP cells, which produced Oshsp18.0-CII fusion protein, heat-denaturation of soluble proteins was less, especially at 70°C. At this temperature, some Oshsp18.0-CII fusion protein still remained in the soluble fraction without denaturation, although at 80°C or higher temperature such as 100°C, most of the soluble proteins including Oshsp18.0-CII fusion protein were denatured as monitored by Anti-Pshsp 17.7 (data not shown).

DISCUSSION

For studying the function of HSP *in vivo*, null mutants are usually used and transformed with a specific HSP gene

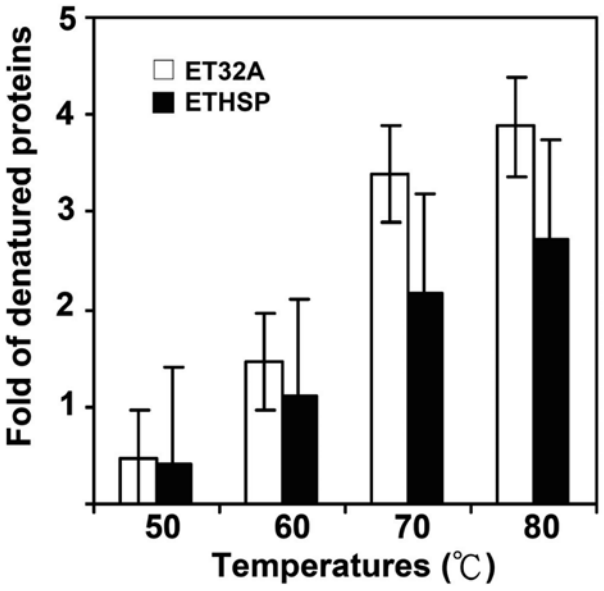

Figure 6. Thermo-denaturation of soluble proteins in cell lysate of transformed bacterial cells. The ET32A and ETHSP cells were subjected to 4 h of 1 mM IPTG induction for producing the corresponding recombinant proteins. The cell lysate prepared from the IPTG-induced *E. coli* cells were heated for 15 min at 50, 60, 70, and 80°C. The denatured protein in pellet and in its corresponding supernatant was compared. Values represent means ± SE (n = 5).

to confirm the function of the transferred gene (Lee et al., 1994; Schirmer et al., 1994; Yeh et al., 1995; 1997). In order to study the function of the plant class II sHSP *in vivo*, which has not been studied before, a rice sHSP gene was transferred into *E. coli* that does not indigenously produce an equivalent HSP during heat stress, even though *E. coli* produces IbpA and IbpB (Vladimir et al., 1995) which are not homologous to rice sHSP. In this study, the *Oshsp18.0-CII* cDNA, encoding an 18.0 kDa class II sHSP of rice, was introduced into the *pET32a* vector with 167 extra amino acids at the N-terminus. These 167 extra amino acids do not affect the function of the Oshsp18.0-CII protein, although the growth rate of *E. coli* after IPTG (1 mM) addition was slightly slower than the cells without IPTG. This may be due to the result of "metabolic burden" by channeling resources into the synthesis of a single foreign protein in *E. coli* (Dong et al., 1995; Hoffmann and Rinas, 2004). The function of plant class II sHSP in *E. coli* may be involved in providing thermotolerance by acting as chaperones similar to the class II sHSP from pea (HSP17.7; Lee et al., 1995) and TaHsp17.8C-II (Basha et al., 2004) in protecting *E. coli* proteins from heat denaturation.

Evidence is accumulating for the function of plant sHSP in heat-resistance. The rice class I sHSP (Oshsp16.9A) can protect *E. coli* proteins from heat denaturation and also increase thermotolerance of *E. coli* (Yeh et al., 1995; 1997), and the class I sHSP are exchangeable among different species for this protective function (Jinn et al., 1995). Transgenic expression of the rice sHSP17.7 (a class I sHSP) gene enhanced bacterial viability under heat stress, and recombinant sHSP17.7 protein prevented thermal aggregation of catalase *in vitro* (Murakami et al., 2004). It was demonstrated that the overexpression of a chloroplast small heat shock protein (Hsp21) in *A. thaliana* could enhance the tolerance against heat under high light conditions (Härndahl et al., 1999). Accumulation of sHSP in abundance could protect or repair plant cells from damages caused by stresses (Krishna, 2003; Murakami et al., 2004). In this study, Oshsp18.0-CII was shown to provide thermotolerance and thermoprotection in *E. coli*. Whether Oshsp18.0-CII prevents thermal aggregation of other proteins or if it can form a protein complex with specific region important for binding to the substrate polypeptide warrant further investigation.

Reports have indicated that UV itself may induce the expression of specific HSP. HSP may provide an adaptive cellular response to tolerate the exposure of UV (Trautinger et al., 1996). For two class II sHSP in rice, it was shown that Oshsp18.0-CII but not Oshsp19.0-CII was induced by UV (Sarkar et al., 2009). Therefore, the effect of Oshsp18.0-CII fusion protein on sensitivity to UV irradiation was observed in *E. coli*.

Over-expression of a class I sHSP gene isolated from rice seedling, *sHSP17.7*, conferred heat tolerance in *E. coli* and both heat tolerance and UV-B resistance in rice plants (Murakami et al., 2004). Abundant accumulation of sHSP17.7 mRNA and protein also resulted in a significant increase in thermotolerance and UV-B resistance (302 nm, 3000 mJ cm^{-1}) in transgenic rice plants (Murakami et al., 2004). Recently, Basha et al. (2010) described some mechanistic differences between class I and class II sHSP. Herein, the effect of a class II sHSP of rice, Oshsp18.0-CII, on decreasing sensitivity to UV irradiation in *E. coli* was demonstrated. It is also possible that the thioredoxin part of the fusion protein might play a role in the increase of tolerance against heat or UV. Thioredoxin is a small, ubiquitous protein which plays an important role in the antioxidant systems by repairing oxidative protein damage through the reversible oxidation of its active center dithiol to a disulfide (Seo and Lee, 2006). It was reported that thioredoxin is an essential protein in *Bacillus subtilis*. A variety of stresses, including heat, salt stress, and ethanol treatment, strongly enhanced the synthesis of thioredoxin in *B. subtilis* (Scharf et al., 1998). The increased levels of thioredoxin might help stressed *B. subtilis* cells to maintain the native and reduced state of cellular proteins (Scharf et al., 1998). Bone marrow cells from transgenic mice overexpressing human thioredoxin were more resistant to UV-C-induced cytocide compared with those from wild type C57BL/6 mice (Mitsui et al., 2002). In addition, LaVallie et al. (1993) reported that the thioredoxin tag in this fusion protein increased the intrinsic thermo-stability of the host cells. Therefore, by using another expression system without any polypeptide fused to the Oshsp18.0-CII may

provide further evidence for the role of Oshsp18.0-CII on UV tolerance and thermotolerance in *E. coli*. Nevertheless, in the thioredoxin overproduced ET32A (vector only) cells, the UV protection was not as high as the ETHSP cells which overproduced thioredoxin-Oshsp18.0-CII fusion protein. This suggested that Oshsp18.0-CII did have some function on UV protection in *E. coli*. Actually, when thioredoxin gene in *pETHSP* was deleted from the ETHSP cells, the cells still showed higher UV tolerance than the control cells carrying the *pET32a* with thioredoxin gene deleted (Chang, unpublished results). Besides, when measuring the growth of the tested cell lines under heat condition (50°C), the growth of ETHSP cells was much lower than that of the ET32A cells and the BL21 (DE3) control cells (Chang, unpublished results). In the future, transgenic plants overproducing the Oshsp18.0-CII may be needed for the *in vivo* study of its function. The correlation of sHSP and UV protection warrants further studies in both *E. coli* and plants.

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重組水稻第二族小分子量熱休克蛋白質 Oshsp18.0-CII 在大腸桿菌的保護功能

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所有植物都會合成多種小分子量熱休克蛋白質。本研究在大腸桿菌 (*Escherichia coli*) 中大量表現 Oshsp18.0-CII 這個水稻 18.0 kDa 第二族小分子量熱休克蛋白質的基因，以研究其功能。結果顯示除了可因此提高大腸桿菌的耐熱性、且對大腸桿菌的水溶性蛋白質具有熱保護性之外，更可因此提高大腸桿菌對紫外光的耐受性，其對 50°C 一小時處理的耐熱性可比只轉入 *pET32a* 表現載體的對照組細胞增高幾乎 1000 倍的存活率；而對 1700 μJ 的紫外光照射的耐性約為只轉入 *pET32a* 表現載體之對照組細胞的 7.29 倍。在 900 μJ 的紫外光照射之後，未轉型的細胞幾乎無法存活，其存活率不高於 0.4%。

關鍵詞：熱休克；水稻；耐熱性；熱保護性；紫外光。