Development of Transgenic *Escherichia coli* with Improved Viability by Heterologous Expression of a Heat Shock Protein Gene from Carrot (*Daucus carota* L.)

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Additional index words. antifoam, *Daucus carota*, heat shock protein, heat stress, homologous recombination, lipoprotein gene promoter

Abstract. A small heat shock protein gene from carrot (*Daucus carota* L.), *Hsp17.7*, was inserted into the *Escherichia coli* chromosome by RecE/RecT-based homologous recombination to increase cell viability during industrial fermentation, which frequently encounters adverse growth conditions. DNA construct “lipoprotein (*Lpp*) gene promoter—*Hsp17.7* gene—flippase recombination target (Frt) cassette” flanked by the sequences of the insertion site of the *E. coli* chromosome (*yddE* pseudogene) was generated by polymerase chain reaction (PCR). The transformed *E. coli* cell lines that heterologously expressed *Hsp17.7* exhibited shorter lag phase, compared with control cell line under normal (37 °C), heat (45 °C), and antifoam conditions. Cell viability was higher in the transformed cell lines in the heat (50 °C, up to 2-fold) and cold (2 °C, up to 1.7-fold) conditions. The soluble protein levels were also higher in the transformed *E. coli* cell lines by up to 20%, compared with control cell line in both stress conditions. The stress-tolerant transgenic cell lines developed in this study can contribute to more efficient and cost saving industrial cultivation of *E. coli*, which is most frequently used for recombinant protein production.

Escherichia coli is an important industrial microorganism that is most widely used for recombinant protein production (reviewed in Jana and Deb, 2005). However, during mass production, bacterial cell growth can be hindered by a number of factors. For example, elevated temperatures in bioprocesses can lead to denaturation of cellular and/or recombinant proteins (Mordukhova et al., 2008). Furthermore, the agitation of the cell culture can introduce gases that cause foaming, which can adversely affect bacterial cell growth and recombinant protein expression (Routledge, 2012). To inhibit the formation of foam, antifoams are routinely added to culture media. However, they can change cellular membrane permeability resulting in decreased cell growth.

In the present study, we aimed to recombine a small heat shock protein gene, *Hsp17.7*, from carrot (*D. carota* ‘Mussangochoon’) into the *E. coli* chromosome to increase the tolerance to adverse cultural conditions. Small heat shock proteins (sHsps; 12–42 kDa in size) are found in virtually all living organisms during heat and other abiotic stress (Haslbeck and Vierling, 2015). They form oligomeric

complexes that consist of 12 to 24 subunits (Haslbeck et al., 2005). On exposure to stressful conditions, sHsps are dissociated into dimers and bind to protein substrates. Their primary function is that of a molecular chaperone that binds to partially unfolded protein substrates and prevents further denaturation and/or promotes correct refolding of the substrates. One of the characteristic structural features of sHsps is that they contain a conserved α -crystallin domain at the C-terminal end that plays important roles in oligomeric complex formation and molecular chaperone activity (Waters, 2013). The α - and β -crystallins are major proteins in the vertebrate eye lens, where they increase the refractive index and also play a protective role (Slingsby et al., 2013).

Plants have the greatest numbers of sHsps among all other organisms. There are 2 sHsps in *Archaea*, 2 in *E. coli*, 2 in *Saccharomyces cerevisiae*, 10 in *Homo sapiens*, and 16 in *Caenorhabditis elegans* (Haslbeck et al., 2005). However, there are more than 30 different *sHsp* genes in plants (Waters, 2013). Considering the sessile nature of plants, it is possible that diverse sHsps provide additional protection to plants against heat and other abiotic stress. Accordingly, plant sHsps can be valuable genetic resources to be used in the development of transgenic organisms with enhanced stress tolerance.

Hsp17.7 from carrot (*D. carota*), a model protein in this study, has been successfully

used to develop transgenic organisms with improved stress tolerance: transgenic carrot cell lines (Malik et al., 1999) and potato plants (Ahn and Zimmerman, 2006) overexpressing *Hsp17.7* exhibit enhanced tolerance to heat stress. Transgenic *E. coli* containing and expressing an *Hsp17.7* gene in a bacterial plasmid vector exhibit improved cell viability and soluble protein levels under heat (Kim and Ahn, 2009) and other abiotic stress (Lee and Ahn, 2013; Song and Ahn, 2010).

Rather than using episomal expression vectors, such as plasmids, in this study, we introduced an *Hsp17.7* gene into the *E. coli* genome using RedE/RedT-mediated homologous recombination (Zhang et al., 2000). The insertion of a recombinant gene directly into the genome of the target organism has advantages over the use of episomal vectors: it provides experimental simplicity without having to undergo DNA restriction enzyme digestion and/or DNA ligation steps, which are prone to errors and DNA damage (Jacobus and Gross, 2015). Second, plasmids can carry up to 15 kb (Casali and Preston, 2003). However, DNA insert for homologous recombination can be generated by PCR without limitations on DNA sequences or sizes. Third, “plasmid instability” can cause losses in recombinant protein production (Friehs, 2004): the sequences of plasmids can be changed during replication, leading to possible losses in recombinant protein production and/or fragmented proteins. In general, plasmids are burdensome for the bacterial cells because plasmid maintenance and replication requires energy of the host cells (Silva et al., 2012). Accordingly, there is a preference toward plasmid free cells during cultivation, which will lead to decreased production of recombinant proteins. Accordingly, direct targeting of the *E. coli* chromosome appears to be an efficient alternative to using plasmid vectors. By selecting proper promoters, one can control the conditions of recombinant gene expression between inducible and constitutive modes. We examined whether transformed *E. coli* containing an *Hsp17.7* gene in its genome could be expressed by an *E. coli* promoter and increase cell viability under adverse culture conditions.

Materials and Methods

Insertion of an Hsp17.7 gene into the E. coli genome using homologous recombination. An *Hsp17.7* gene was inserted into the genome of *E. coli* BL21 (DE3) using the Quick and Easy *E. coli* Gene Deletion Kit (Gene Bridges GmbH, Heidelberg, Germany), which is based on RecE/RecT recombination (Zhang et al., 1998). The recombination in this study was targeted to the middle of the *yddE* pseudogene [National Center for Biotechnology Information (NCBI) accession no.: NC_012971.2] in the *E. coli* genome. To induce high levels of *Hsp17.7* expression in the transformed *E. coli*, the promoter region of *Lpp* gene (NCBI accession no.: NC_000913.2) was selected, because *Lpp* is one of the most abundant proteins in *E. coli* (Nakamura and Inouye, 1979).

Received for publication 26 Oct. 2015. Accepted for publication 13 Jan. 2016.

This research was supported by a 2015 Research Grant from Sangmyung University.

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The DNA construct was designed to contain an “*Lpp* gene promoter—*Hsp17.7* gene—Frt cassette” flanked by sequences of the insertion site, the *yddE* pseudogene. The Frt cassette contained a kanamycin selection marker. The stepwise generation of the DNA cassette is illustrated in Fig. 1. The *Lpp* gene promoter and *Hsp17.7* gene were amplified from the *E. coli* and carrot (*D. carota*) genomic DNAs, respectively, by PCR (1 cycle at 98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s) using *Lpp* gene promoter-specific and *Hsp17.7* gene-specific primers, respectively. The Frt cassette was also generated by PCR under the same conditions, with the exception of a longer extension step (72 °C) for 1 min 30 s due to its long length. Phusion High-Fidelity PCR Master Mix with HF buffer polymerase (New England Biolabs, Ipswich, UK) was used for the accurate amplification of the DNA constructs. The PCR-amplified *Lpp* gene promoter and *Hsp17.7* gene were combined by two-step extension PCR (1 cycle at 98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s, 72 °C for 30 s, and no annealing steps) without primers. The *Lpp* gene promoter, *Hsp17.7* fragment and Frt cassette were also combined by two-step extension PCR (1 cycle at 98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s, 72 °C for 1 min 45 s, and no annealing steps) without primers. For the two-step extension PCR, 50–100 ng of each DNA fragment were used. The sequences of all the primers used in this study are presented in Table 1. Is-*Lpp*_F and Frt-Is_R primers contained sequences of the insertion site, the *yddE* pseudogene. The sequence of the final DNA fragment, i.e., “*Lpp* gene promoter—*Hsp17.7* gene—Frt cassette” flanked by insertion site sequences, was confirmed by MacroGen (Seoul, Korea).

Transformation of the Red/ET plasmid into *E. coli*. The Red/ET expression plasmid to facilitate homologous recombination was inserted into *E. coli* BL21 (DE3) according to the protocol of the Quick and Easy *E. coli* Gene Deletion Kit (Gene Bridges GmbH). The *E. coli* cells were cultured at 37 °C overnight with shaking. The bacterial culture was diluted 1:1000 with Luria-Bertani (LB) medium and cultured until the o.d.₆₀₀ reached 0.6 and centrifuged at 1360 g_n (4 °C) for 20 min. The resulting bacterial cell pellet was dissolved in 1 mL of 10% glycerol and centrifuged under the abovementioned conditions and followed by the removal of the supernatant. After three rounds of washing, 30 µL of bacterial cells were mixed with 20 ng·µL⁻¹ Red/ET expression plasmid and placed on ice for 30 min followed by electroporation (1800 V, 25 µF, 200 ohm; Gene Pulser X; Bio-Rad, Hercules, CA). After the addition of super optimal medium with glucose (SOC medium, 1 mL), the cell cultures were then incubated at 30 °C for 70 min with shaking. The bacterial cells were then spread on solid LB medium containing 50 µg·µL⁻¹ ampicillin and incubated overnight.

Fig. 1. Schematic diagram of the DNA construct generation. The lipoprotein gene [*Lpp* gene; National Center for Biotechnology Information (NCBI) accession no.: NC_000913.2] promoter, an *Hsp17.7* gene from carrot (*Daucus carota* L.; NCBI accession no.: X53851) and flippase-recombinase targets (Frt) cassette were generated by polymerase chain reaction (PCR) using three sets of specific primers whose sequences are shown in Table 1. The first and last primers (Is-*Lpp*_F and Frt-Is_R) contained sequences for insertion sites (*yddE* pseudogene; NCBI accession no.: NC_012971.2). The DNA fragment was extended by PCR without annealing steps. The final DNA construct was introduced into the *Escherichia coli* genome using RecE/RecT-based homologous recombination as described in “Materials and Methods”. Is = insertion site (*yddE* pseudogene); Lpp = lipoprotein; Hsp = heat shock protein; Frt = flippase-recombinase targets; F = forward; R = reverse.

Table 1. Sequences of the primers used to generate the DNA construct containing *Hsp17.7* gene for homologous recombination into the *E. coli* genome.

Primers ^a	Sequences ^b
Is- <i>Lpp</i> _F	5'- <u>CCGGATCTTCCACAATACCAATCGCAGGCGA</u> <u>GAACATGCGACCCTGTAATATTGCTTT</u> -3'
	Is (<i>yddE</i>) Lpp promoter
Lpp-Hsp17.7_R	5'- <u>TGGAATGATCGACATTATTAATACCCTCTA</u> -3'
	Hsp17.7 Lpp promoter
Lpp-Hsp17.7_F	5'- <u>TAGAGGGTATTAATAATGTCGATCATTCCA</u> -3'
	Lpp promoter Hsp17.7
Hsp17.7-Frt_R	5'- <u>GAGAATAGGAACCTCTTAACCAGAAATATC</u> -3'
	Frt cassette Hsp17.7
Hsp17.7-Frt_F	5'- <u>GATATTCTTGGTTAAGAAGTTCCTATTCTC</u> -3'
	Hsp17.7 Frt cassette
Frt-Is_R	5'- <u>GGTACGCCGGGCTTTGAAGTCCGCTGGAGGGT</u> <u>GAAGTACGCGCAATACGACTCACTATAGGGC</u> -3'
	Is (<i>yddE</i>) Frt cassette

^aIs = insertion site (*yddE* pseudogene); Lpp = lipoprotein; Hsp = heat shock protein; Frt = flippase-recombinase targets; F = forward; R = reverse.

^bThe nucleotide sequences are underlined to show where the sequences were originated from.

Introduction of an *Hsp17.7* gene into the *E. coli* genome. A surviving colony was chosen and cultured in LB medium containing 50 µg·µL⁻¹ ampicillin overnight at 37 °C with shaking, diluted at 1:1000 with fresh LB medium with ampicillin, and cultured at 37 °C with shaking until the o.d.₆₀₀ reached 0.6. L-arabinose (Sigma-Aldrich, St. Louis, MI) was added to a final concentration of 0.4%, and the bacterial cells were cultured at 37 °C for 1 h with shaking followed by centrifugation at 1360 g_n (4 °C) for 20 min. The cell pellet was then washed three times with 1 mL of 10% glycerol. Competent *E. coli* cells (30 µL in 10% glycerol) were mixed with 3 µL of the DNA construct “*Lpp* gene promoter—*Hsp17.7* gene—Frt

cassette” flanked by sequences of the insertion site (*yddE* pseudogene; Fig. 1) and incubated on ice for 30 min followed by electroporation (1800 V, 25 µF, 200 ohm). SOC medium (1 mL) was added, and the bacterial samples were cultured at 37 °C for 3 h with shaking followed by spreading on solid LB medium containing kanamycin (15 µg·µL⁻¹ final concentration). The bacterial samples were then incubated at 37 °C overnight, and the surviving colonies were obtained.

Confirmation of the *Hsp17.7* gene insertion and protein expression in the transformed *E. coli*. Cell cultures were initiated from 10 surviving colonies, and genomic DNA was extracted using the ExiPrep Bacteria Genomic DNA kit (Bioneer, Daejeon,

Fig. 2. Heterologous expression of Hsp17.7 in transformed *Escherichia coli*. (A) Genomic DNA was extracted from the transformed *E. coli* and control cell lines and subjected to polymerase chain reaction analysis using *Hsp17.7* gene-specific primers. The size of the band was ≈ 500 bp. (B) The proteins were extracted from the transformed *E. coli* and control cell lines when the o.d.₆₀₀ reached 0.6. Equal amounts of protein (30 μ g per sample) were resolved on a 17% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and stained using Coomassie blue dye. (C) Equal amounts of proteins (30 μ g per sample) were resolved on a 17% SDS-PAGE gel and subjected to an immunoblot analysis using a polyclonal antibody raised against Hsp17.7. The size of the band was ≈ 18 kDa. The experiment was repeated three times, and representative images are shown. T1, T3, T6, and T7 = transformed cell lines heterologously expressing Hsp17.7; C = control cell line containing pRed/ET, which facilitates homologous recombination.

Fig. 3. Enhanced growth of the transformed *Escherichia coli* cell lines heterologously expressing Hsp17.7 under heat and antifoam conditions. Transformed *E. coli* and control cell lines were cultured at (A) an optimal growth temperature (37 °C) and (B) an elevated temperature (45 °C) for 10 h. The bacterial cell lines were also cultured in the presence of an antifoam (Ppg 2000), (C) at 1 mL·L⁻¹ and (D) at 2 mL·L⁻¹. Cell growth was examined by measuring the o.d.₆₀₀ at 1 h intervals. The error bars indicate the standard errors of the means. T1, T3, T6, and T7 = transformed cell lines heterologously expressing Hsp17.7; C = control cell line containing pRed/ET, which facilitates homologous recombination.

Korea). PCR (1 cycle at 98 °C for 30 s, followed by 35 cycles of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s) was performed on the genomic DNA with *Hsp17.7* gene-specific primers (Lpp-Hsp17.7_F and Hsp17.7-Frt_R; Table 1) using the Accu-power ProFi Taq PCR Premix (Bioneer). The control cell line contained only the pRed/ET plasmid.

To examine the heterologous accumulation of Hsp17.7 protein in the transformed *E. coli*, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Laemmle, 1970) and immunoblot analyses were performed as previously described (Ko et al., 2015). Briefly, transformed *E. coli* cells were cultured overnight, diluted at 1:1000 with LB medium containing kanamycin (15 μ g· μ L⁻¹), and cultured at 37 °C until the o.d.₆₀₀ reached 0.6. After centrifugation at 1360 g_n at 4 °C for 20 min, the resulting pellet was dissolved in 3 mL protein extraction buffer (25 mM Tris-HCl pH 7.5, 300 mM NaCl, and 3 mM β -mercaptoethanol) and sonicated (420 W and 20 kHz for a total of 4 min and 40 s with repeated bursts of 10 s of ultra-sonication and 30 s pauses) using a Sonosmasher (S&T Science, Seoul, Korea) and followed by centrifugation at 20,900 g_n for 1 h. The resulting supernatant was quantified by Bradford assay (1976). Equal amounts of the bacterial proteins (30 μ g) were resolved on a 17% SDS-PAGE gel and either stained with Coomassie blue dye (Bio-Rad) or transferred to a nitrocellulose membrane (Hybond ECL; GE Healthcare, Piscataway, NJ) followed by an immunoblot analysis with a polyclonal antibody raised against Hsp17.7 using the ECL Plus system (GE Healthcare). The experiment was repeated three times.

Cell growth under heat and antifoam conditions. Transformed *E. coli* expressing Hsp17.7 and control cell lines were cultured at 37 °C overnight, diluted 1:1000 as stated above, and then cultured under heat (45 °C) and antifoam [polypropylene glycol (Ppg) 2000 up to 2 mL·L⁻¹; Routledge, 2012] conditions with shaking. The o.d.₆₀₀ was measured at hour intervals for up to 10 h. The experiment was repeated three times with three replicates.

Cell viability of the transformed *E. coli* under heat and cold conditions. Transformed *E. coli* expressing Hsp17.7 and control cell lines were cultured at 37 °C overnight and diluted at 1:1000 as stated above. When the o.d.₆₀₀ reached 0.6, the bacterial cells were then transferred to heat (50 °C) or cold (2 °C) conditions and cultured for 60 min and 10 d, respectively. The cell cultures were then diluted to 1:1000 with LB medium containing kanamycin (15 μ g· μ L⁻¹) and spread on LB medium containing kanamycin. After overnight incubation at 37 °C, the numbers of surviving colonies were counted and the survival percentages were calculated. The experiment was repeated three times with five replicates.

Molecular chaperone function of heterologous Hsp17.7 under heat and cold stress. Overnight cell culture, protein extraction, and quantification of the transformed *E. coli* expressing Hsp17.7 and the control

Fig. 4. Enhanced cell viability in the transformed *Escherichia coli* heterologously expressing Hsp17.7 in the heat and cold conditions. Transformed *E. coli* and control cell lines were cultured until the o.d.₆₀₀ reached 0.6 and then exposed to (A) heat (50 °C) or (B) cold (2 °C) conditions for 60 min and 10 d, respectively. The *E. coli* cell cultures were diluted 1:10⁶ with fresh Luria-Bertani (LB) medium containing kanamycin (15 µg·µL⁻¹) and spread on solid LB medium containing kanamycin. After overnight incubation at 37 °C, the numbers of the surviving colonies were counted, and the survival percentages were calculated. The error bars indicate the standard errors of the means. T1, T3, T6, and T7 = transformed cell lines heterologously expressing Hsp17.7; C = control cell line containing pRed/ET, which facilitates homologous recombination.

cell lines were performed as stated above. Equal amounts of bacterial protein (2 mg) were incubated in heat (52 °C) or cold (2 °C) conditions for 60 min or 10 d, respectively. The protein samples were then centrifuged at 20,900 g_n for 1 h, and the amount of soluble protein in the supernatants was quantified by Bradford assay (1976). The percentages of soluble proteins in the stressful conditions were calculated.

The experiment was repeated three times with five replicates.

Results and Discussion

Insertion of an Hsp17.7 gene into the E. coli genome using homologous recombination. *E. coli* is one of the most widely used microorganisms for the production of recombinant proteins. In most cases, episomal vectors are

used to carry and express recombinant genes. However, there have been improvements in the approaches to directly engineering the bacterial genome using homologous recombination (Zhang et al., 2000). These approaches allow for DNA constructs to be inserted without the restriction of insertion sites, which enables various types of chromosomal DNA modifications, such as gene cloning, gene deletion, and artificial chromosome generation. In *E. coli*, bacteriophage-encoded recombination proteins (RecE and RecT) efficiently recombine sequences with homologies as short as 35 to 50 bases (Zhang et al., 1998).

A *sHsp* gene from carrot (*D. carota*), *Hsp17.7*, was inserted into the *E. coli* genome using RecE/RecT-mediated homologous recombination. To maximize the heterologous expression of Hsp17.7 in transformed *E. coli*, we used the promoter of *Lpp* gene, which is expressed very abundantly in *E. coli* (Nakamura and Inouye, 1979). The DNA construct “*Lpp* gene promoter—*Hsp17.7* gene—FRT cassette” flanked by sequences of the insertion site (*yddE* pseudogene) was successfully generated by PCR and inserted into *E. coli* BL21 (DE) by electroporation. The treated *E. coli* cells were then spread on LB plates containing kanamycin. After overnight incubation, 10 colonies were selected and subjected to PCR analysis with *Hsp17.7*-specific primers. Only four colonies exhibited positive bands at ≈500 base pair (bp) (*Hsp17.7* gene = 474 bp; Fig. 2A), which suggested the successful insertion of the *Hsp17.7* gene. DNA sequencing further confirmed that the DNA construct containing an *Hsp17.7* gene was successfully inserted into the target insertion site (data not shown).

To verify the Hsp17.7 protein accumulation in the transformed *E. coli* cell lines, proteins were extracted and subjected to SDS-PAGE and immunoblot analysis using a polyclonal antibody raised against Hsp17.7. The overall protein pattern was similar in the transformed *E. coli* cell lines and control cell line that contained the pRed/ET plasmid only (Fig. 2B). Immunoblot analysis revealed the heterologous expression of Hsp17.7 in the transformed *E. coli* cell lines (Fig. 2C). Protein bands appeared at ≈18 kDa.

Our results revealed that the *Hsp17.7* gene was successfully integrated into the *E. coli* genome through RecE/RecT-based homologous recombination and successfully expressed into the protein. Our study provides valuable information about the genomic DNA manipulation and recombinant protein production in *E. coli*. Other recombinant genes will also be able to be introduced into the *yddE* pseudogene site in the *E. coli* genome and expressed under the control of the *Lpp* gene promoter. To further increase recombinant gene expression, it is possible to screen more insertion sites in the *E. coli* chromosome. We examined two other insertions sites [in front of the *rhsE* gene (NCBI accession no.: NC_000913.3) and in the middle of the *ydbA* pseudogene (NCBI accession no.: NC_284775.0)] in the *E. coli* genome to express *Hsp17.7* gene. This cassette was successfully inserted into the *E. coli* genome; however,

Fig. 5. Enhanced protein solubility in the transformed *Escherichia coli* heterologously expressing Hsp17.7 in heat and cold conditions. The transformed *E. coli* and control cell lines were cultured until the o.d.₆₀₀ reached 0.6. The total proteins were extracted using ultra-sonication. Equal amounts of bacterial protein (2 mg) were incubated in (A) heat (52 °C) or (B) cold (2 °C) conditions for 60 min or 10 d, respectively. After ultra-centrifugation, the amounts of soluble proteins in the supernatants were quantified, and the percentages of soluble protein were calculated. The error bars indicate the standard errors of the means. T1, T3, T6, and T7 = transformed cell lines heterologously expressing Hsp17.7; C = control cell line containing pRed/ET, which facilitates homologous recombination.

the protein did not accumulate (data not shown), which suggests that the recombination site is very important for heterologous gene expression. In addition, by modifying the promoter sequence, it will also be possible to further increase the expression level of

the recombinant gene and control the gene expression in terms of whether it is constitutive or conditional. For example, using the heat shock gene promoter from one of the *Hsp* genes in *E. coli* would allow for recombinant gene expression only under stressful conditions.

Increased cell growth of transformed E. coli under heat and antifoam conditions. To examine whether the genomic insertion of the *Hsp17.7* gene and its heterologous expression could confer tolerance to adverse conditions, the growth of the transformed *E. coli* and control cell lines were measured under heat and antifoam conditions. Under the normal growth condition (37 °C), all of the transformed cell lines that heterologously expressed Hsp17.7 exhibited shorter lag phase compared with the control (Fig. 3A). An elevation in the temperature (45 °C) decreased the growth of all of the cell lines examined (Fig. 3B). However, the transformed *E. coli* cell lines also exhibited shorter lag phase than the control. The addition of an antifoam agent (Ppg 2000) decreased the growth of all the cell lines examined (Fig. 3C and D). However, at 1 and 2 mL·L⁻¹ Ppg 2000, which are typical concentrations in *E. coli* culture when the addition of Ppg 2000 is needed, the transformed *E. coli* cell lines exhibited shorter lag phase than the controls. In the transformed *E. coli* cell lines in the presence of 2 mL·L⁻¹ Ppg 2000 treatment, the lag phase was shorter by up to ≈2 h than the control cell line (Fig. 3D). During stationary phase, the o.d.₆₀₀ of the transformed *E. coli* cell lines was higher than that of the control by 0.3–0.5 in all of the growth conditions examined.

Our result suggests that heterologously expressed Hsp17.7 functions to improve the growth of the transformed *E. coli* cell lines even in the absence of stress. Previous studies have reported that some sHSPs were expressed under normal growth conditions, mostly at specific developmental stages of plants, such as during embryogenesis, seed maturation, pollen development, and fruit maturation (reviewed in Waters, 2013). Hsp17.7 was also accumulated in dry seeds, seedlings (up to 14 d after imbibition), and flowers under normal growth conditions (Kim and Ahn, 2010). In addition to preventing protein denaturation under stress conditions, molecular chaperones, such as sHSPs, play a role in maintaining protein homeostasis in nonstressed cells. They facilitate the folding of newly synthesized proteins, the assembly/disassembly of other macromolecular complexes, and the translocation of proteins across membranes of intracellular compartments (Cox et al., 2014). It is possible that shorter lag phase of transformed *E. coli* cell lines in the absence of stress, compared with the control (Fig. 3A), may result from higher levels of protein homeostasis by heterologously expressed Hsp17.7.

Under heat stress, the heterologous expression of sHsps in *E. coli* increased cell growth and viability. The overexpression of sHsps in *E. coli*, IbpA and IbpB, increased viability under heat and oxidative stress (Kitagawa et al., 2000). The sHsp from *C. elegans* Hsp17 increased the viability of the transformed *E. coli* by maintaining the cell membrane stability (Ezemaaduka et al., 2014). However, most of these studies used episomal vectors to carry and express

recombinant *sHsp* genes. Our study reported that a *sHsp* gene from plants can successfully be inserted into the *E. coli* genome and expressed to confer tolerance to adverse growth conditions, such as heat and antifoam.

Increased cell viability and protein solubility by Hsp17.7. The primary function of Hsps is to act as a molecular chaperone to maintain the solubility of cellular proteins, which contributes to increased cell viability under stressful conditions (Sun et al., 2002). In this study, the transformed *E. coli* cell lines containing the *Hsp17.7* gene from carrot (*D. carota*) and the control cell line were subjected to heat and cold conditions. Colony forming units and protein solubility were measured to examine whether the heterologously expressed Hsp17.7 could increase the survival rates of the transformed *E. coli* and function as a molecular chaperone to maintain the soluble protein levels under adverse growth conditions.

Elevations in temperature (50 °C) decreased the cell viabilities of all of the examined cell lines (Fig. 4A). However, the transformed cell lines heterologously expressing Hsp17.7 exhibited more than 2-fold greater levels of surviving cells, compared with the control cell line. At 45 min, the transformed cell line T1 exhibited a 46% survival rate, compared with the 22% in the control. Extended cold treatment (2 °C) decreased the viability of the control cell line to 75% after 10 d (Fig. 4B). However, the transformed cell lines gradually increased their cell survival rates to 133% (cell line T1).

Both thermal stresses decreased soluble protein levels in the transformed *E. coli* and the control cell line (Fig. 5). However, the transformed *E. coli* cell lines exhibited levels of soluble proteins that were up to 20% greater in both conditions. Our results revealed that heterologously expressed Hsp17.7 from carrot (*D. carota*) could function as a molecular chaperone to increase protein solubility in heat and cold stress conditions, possibly resulting in increased cell viability. Purified Hsp17.7 protected the model substrate malate dehydrogenase at 50 °C in vitro, which proved its molecular chaperone function (data not shown).

Unlike the heat stress, which caused decreases both in cell viability and protein solubility of transformed *E. coli* heterologously expressing Hsp17.7, under the cold condition, there was a slight increase in viability of transformed *E. coli* cell lines (up to 133%, Fig. 4B), although protein solubility decreased to 73% (Fig. 5B). Low temperatures mainly cause a decrease in membrane fluidity and in transcription/translation efficiencies by stabilization of secondary structures of nucleic acids (reviewed in Phadtare, 2004). It is possible that heterologously expressed Hsp17.7 may play a role in these processes in the transformed *E. coli* cell lines under cold stress.

In this study, an *Hsp17.7* gene from carrot (*D. carota*) was successfully inserted into the

E. coli genome using homologous recombination and expressed under the control of the *Lpp* gene promoter from *E. coli*. The insertion of a recombinant gene into the *E. coli* genome provided more stable recombinant gene expression compared with the use of episomal vectors. The information about the insertion site (*yddE* gene) and promoter (*Lpp* gene promoter) could be used to express other recombinant genes. Furthermore, by manipulating the insertion site and the promoter sequences, it will be possible to control the expression level (high or low) and/or the expression condition (constitutive or conditional) of the recombinant gene. The heterologous expression of Hsp17.7 increased the growth, cell viability, and protein solubility in the transformed *E. coli* in heat, cold, and antifoam conditions, which suggests that Hsp17.7 is a useful genetic resource that confers tolerance to adverse growth conditions. The transformed *E. coli* cell lines developed in this study could be used in large-scale culture for industrial production and provide higher cell growth and viability.

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