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Enhancement of heterologous protein expression in *Escherichia coli* by co-expression of nonspecific DNA-binding stress protein, Dps

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Abstract

The over-expression of foreign proteins imposes metabolic burden on host strains that may lead to reduced cell growth and even yield of target heterologous protein. We investigated the effect of co-expression of nonspecific DNA-binding protein, Dps, one of the many stress proteins against oxidative damage and nutrient starvation, on the over-expression of heterologous protein in *Escherichia coli* expression system. It was observed that the co-expression of recombinant Dps reduced the growth rate in minimal M9 medium. On the contrary, Dps had a positive effect on cell growth in rich LB medium. It was also observed that Dps was capable of enhancing the specific production of insoluble target foreign protein, baculoviral polyhedrin (Polh) and green fluorescent protein (GFP) fusion in both media, demonstrating that the co-expression of Dps has general positive effects on foreign protein production regardless of medium types. Even though the mechanism of Dps on foreign protein production remains unclear, the ability for significant enhancement of target protein production (about 46% of target protein fraction in total cellular proteins and about 2.5-fold increase in product yield) may be successfully applied in practical culture process.

Keywords: *Escherichia coli*; Nonspecific DNA-binding protein; Dps; Stress protein; Cellular stress; Foreign protein overproduction

1. Introduction

Cells undergo many changes including alterations in the patterns of gene expression as well as protein stability during their growth when exposed to chemical or physical stresses such as heat shock [1,2], oxygen radical [3–5], various toxic chemicals [6–8], viral infection [9], the presence of abnormal proteins [10,11], the over-expression of heterologous proteins [12–14], and nutrient limitation (carbon source, amino acid source, etc.) [15,16]. By changing the transcriptional pattern of genes and producing several stress proteins, cells can resist environmental stresses.

Nonspecific DNA-binding protein, Dps, one of the many stress proteins has ferritin-like structure and plays a protection role on DNA damage by substitute oxidation of Fe^{2+} to Fe^{3+} against oxygen radical or acetic stress produced from general metabolism [17]. Dps is highly produced not only from oxidative stress, but also under a stationary phase that imposes nutrient (amino acid or carbon source) starvation stress [18,19]. Dps is a major protein component of the nucleoid in the stationary phase [20]. Dps forms extremely stable complexes with DNA, without apparent sequence specificity. Dps causes compaction of genomic DNA and silencing of the genomic function [19,20].

Usually, overproduction of heterologous protein imposes metabolic burden stress. Subsequently, cell growth decreases and the final target protein yield is also reduced by this stress [21–24]. Therefore, it is important to reduce metabolic burden by foreign protein over-expression in order to obtain enhanced product yield. Also, it was reported that expression of Dps was down-regulated under the overproducing condition of insoluble recombinant protein in *Escherichia coli* [12]. In the present work, we investigated the effects of Dps co-expression on cell growth and foreign protein expression in *E. coli*. We used green fluorescent protein (GFP) that was fused with baculoviral polyhedrin (Polh) as a target foreign protein. This fusion protein has insoluble features in *E. coli* cells [25]. To our knowledge, the present work demonstrates, for the first time, the successful significant enhancement of foreign protein production using Dps co-expression strategy in recombinant *E. coli* expression system.

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2. Materials and methods

2.1. Strains and plasmids

Escherichia coli TOP10 (*F⁻ mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZ ΔM15 ΔlacX74 deoR recA1 araD139 Δ(ara-leu)7697 galU galK rpsL (StrR) endA1 nupG*) (Invitrogen, USA) was used for constructing recombinant plasmid and *E. coli* BL21 (*F′ ompT hsdSB* (r_B^- mB^-) *gal dcm*) (Novagen, USA) was used for expressing the proteins. Plasmid pMPL102 was used as a control vector that contains gfp_{uv} gene fused with $(His)_6$ affinity tag and *polh* gene ([21], Fig. 1). The *dps* gene sequence was obtained from GenBank (X69337; http://www3.ncbi.nlm.nih.gov/Entrez). The *Nhe*I–*Eco*RI digested PCR amplified *dps* from *E. coli* K12 (ATCC 29425) genomic DNA was subcloned in the pTrcHisC plasmid (Invitrogen). This plasmid was denoted pYS01. The *Pst*I–*Eco*RI digested PCR amplified *dps* including ribosome binding site (RBS) and $(His)_6$ affinity tag from pYS01 was subcloned in the plasmid pMPL102. This vector was named pYS02 (Fig. 1).

2.2. Media and cell culture

Luria–Bertani (LB) rich medium (5 mg ml^{-1} Yeast extract (Sigma, USA), 10 mg ml^{-1} Tryptone (Sigma), and 10 mg ml^{-1} NaCl) and M9 minimal medium (12.8 mg ml^{-1} $Na_2HPO_4{\cdot}7H_2O$, 3 mg ml^{-1} KH_2PO_4, 0.5 mg ml^{-1} NaCl, 1 g l^{-1} NH_4Cl, 3 μg ml^{-1} $CaCl_2$, 1 mM $MgSO_4$) were used for plasmid construction and cell culture. Two plasmids pMPL102 and pYS02 were introduced into the *E. coli* BL21. Cultures were performed in 60 ml LB and M9 media (with 0.5% (w/v) glucose) containing 50 μg ml^{-1} ampicillin (Sigma) using 250 ml flask at 37 °C and 250 rpm. The cultures were induced by the addition of 1 mM (as final concentration) isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma) to express Polh/GFP with or without Dps.

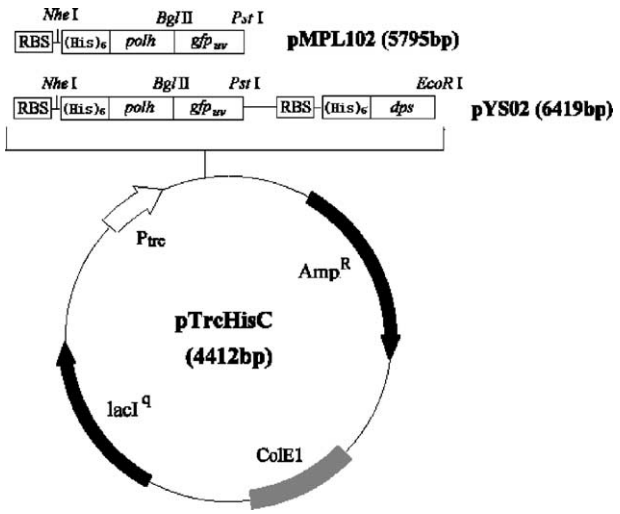

Fig. 1. Gene maps of recombinant plasmid pMPL102 and pYS02. Abbreviations: *polh*, polyhedrin gene; gfp_{uv}, GFPuv gene; *dps*, Dps gene; RBS, ribosome binding site; P_{trc}, *trc* promoter; Amp^R, ampicillin resistance gene; $lacI^q$, overexpressed *Lac* repressor; ColE1, replication origin.

2.3. Analytical methods

The samples were taken at every 1 h from each culture and then measured optical density at 600 nm (OD_{600}) on a UV-Vis spectrophotometer (Shimadzu, Japan). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting were performed to detect polyhedrin-GFP. The whole cell sample was mixed with sample buffer (0.5 M Tris–HCl (pH 6.8), 10% glycerol, 5% SDS, 5% β-mercaptoethanol (Bio-Rad, USA), and 0.25% bromophenol blue (Sigma)), incubated at 100 °C for 5 min for cell disruption, centrifuged briefly, and loaded onto a 12.5% slab gel. After electrophoresis, the gel was stained with coomassie blue (Bio-Rad) for SDS–PAGE. Monoclonal anti-$(His)_6$ antibody (Santa Cruz Biotechnology, USA) and monoclonal anti-mouse antibody conjugated

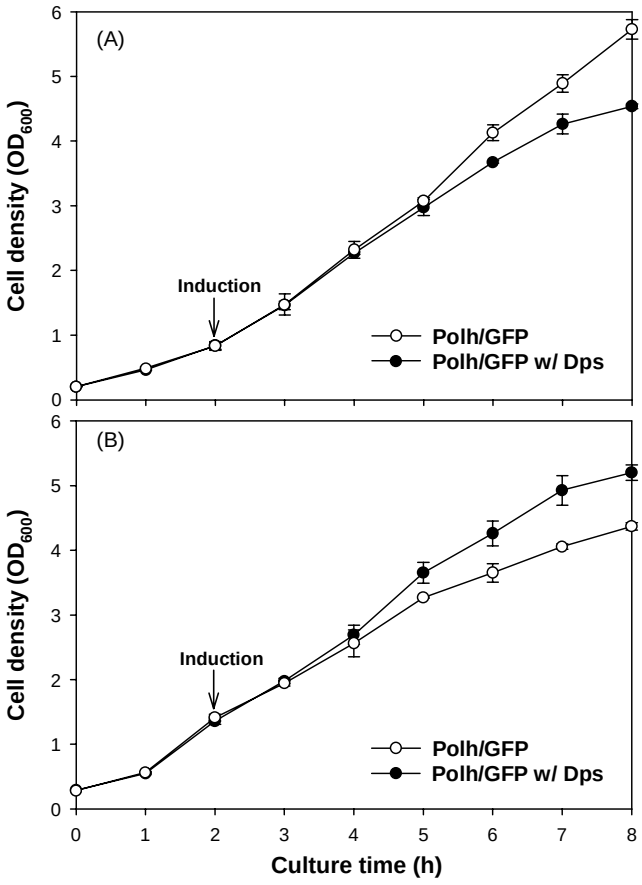

Fig. 2. Time profiles of cell growth in the culture of *E. coli* BL21 strains under Dps non-expressing (○) and expressing (●) conditions in (A) M9 minimal and (B) LB rich medium. Recombinant cells were cultured in 60 ml using 250 ml flasks at 37 °C and 250 rpm. Arrow indicates the point of induction with 1 mM IPTG.

with alkaline phosphate (Sigma) were used for Western blot analysis. The stained gel or nitrocellulose membrane was scanned, and the digitized image was stored and analyzed by Gel-Pro Analyzer software (Media Cybernetics, USA).

3. Results

3.1. Effect of Dps co-expression on cell growth

The profile of cell growth from *E. coli* BL21 transformed with the pYS02 plasmid that contains both target insoluble fusion protein Polh/GFP and Dps was compared to that from the pMPL102 that contains only target protein in two culture media, M9 minimal and LB rich (Fig. 2). In the case of M9 minimal medium, after induction, the growth rate of Dps co-expressed (Dps^+) strain became slower than that of Dps non-expressing (Dps^-) cells and subsequently, final cell density was lower (Fig. 2A). However, on the contrary, in the case of LB rich medium, cell growth as well as final cell density was higher in Dps^+ strain than that in Dps^- strain (Fig. 2B).

3.2. Effect of Dps co-expression on foreign protein production

We performed SDS–PAGE analyses using whole cell samples with the same optical density (Fig. 3) because fractionation was meaningless for totally insoluble Polh/GFP fusion protein as a target protein when expressed in *E. coli* cells (data not shown; [25]). The molecular weight of recombinant Dps is higher (20.2 kDa) than that (18.7 kDa) of natural Dps. Recombinant Dps bands were clearly observed in Dps^+ samples (lanes with positive in Fig. 3A) and its expression also slightly increased with the culture time. Because both target protein Polh/GFP and Dps have $(His)_6$ affinity tag at the N-terminus, we performed Western blot analysis using anti-$(His)_6$ antibody for confirmation (Fig. 4). As expected, only two bands for Polh/GFP target protein and Dps were revealed in Dps^+ strains (lanes with positive in Fig. 4).

We investigated the effect of Dps co-expression on production of target fusion protein Polh/GFP in both M9 minimal and LB rich media (Fig. 3). Surprisingly, in the case of M9 minimal medium, even though cell growth rate under co-expression of Dps was lowered, specific expression of Polh/GFP was much higher (lanes with positive in Fig. 3A) than that without Dps (lanes with negative in Fig. 3A). The difference of expression levels between the two strains became distinct 3 h after induction. In the case of rich LB medium, it was also shown that the expression level of target fusion protein was highly enhanced in Dps^+ environment (Fig. 3B). We depicted time profiles for fraction of Polh/GFP fusion protein in total cellular protein based on SDS–PAGE analyses (Fig. 5). When Dps was co-expressed in M9 medium, fraction of Polh/GFP band increased and the difference between fractions from Dps^+ and Dps^- strains

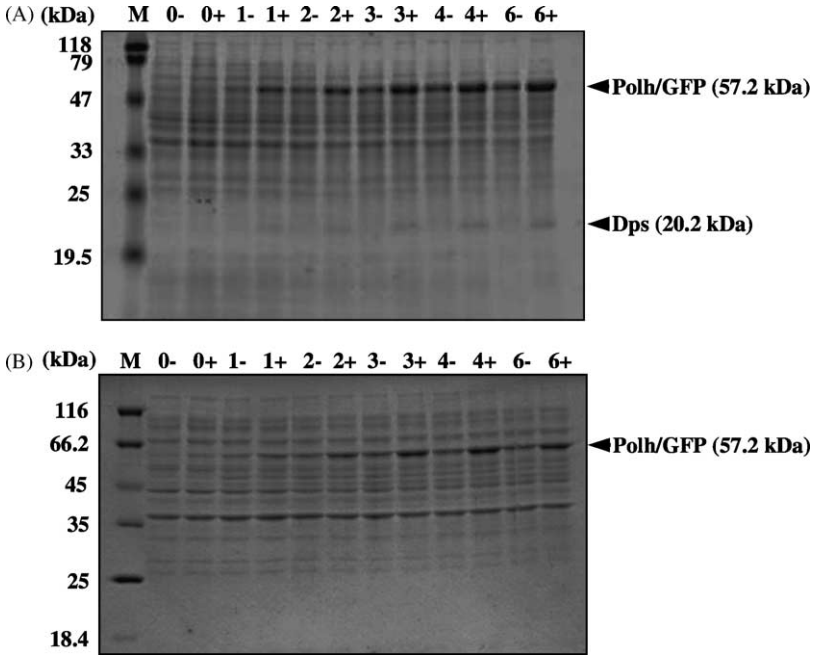

Fig. 3. SDS–PAGE analyses of *E. coli* BL21 strains under Dps non-expressing and expressing conditions that were cultured in (A) minimal M9 and (B) rich LB media. Lane M, protein molecular weight marker; lane 0−, Dps^- at 0 h after induction; lane 0+, Dps^+ at 0 h after induction; lane 1−, Dps^- at 1 h after induction; lane 1+, Dps^+ at 1 h after induction; lane 2−, Dps^- at 2 h after induction; lane 2+, Dps^+ at 2 h after induction; lane 3−, Dps^- at 3 h after induction; lane 3+, Dps^+ at 3 h after induction; lane 4−, Dps^- at 4 h after induction; lane 4+, Dps^+ at 4 h after induction; lane 6−, Dps^- at 6 h after induction; lane 6+, Dps^+ at 6 h after induction. Each lane had whole cell sample with same OD.

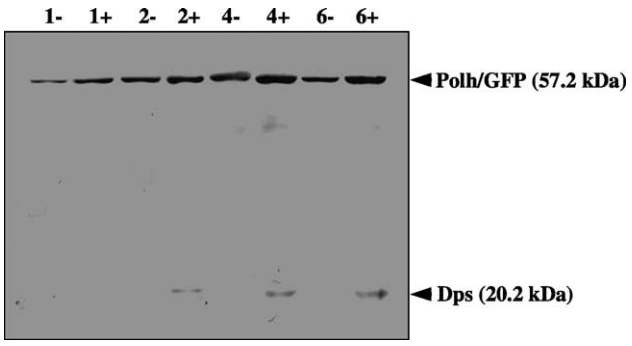

Fig. 4. Western blot analysis of *E. coli* BL21 strains under Dps non-expressing and expressing conditions using anti-$(His)_6$ antibody. Lane 1−, Dps^- at 1 h after induction; lane 1+, Dps^+ at 1 h after induction; lane 2−, Dps^- at 2 h after induction; lane 2+, Dps^+ at 2 h after induction; lane 4−, Dps^- at 4 h after induction; lane 4+, Dps^+ at 4 h after induction; lane 6−, Dps^- at 6 h after induction; lane 6+, Dps^+ at 6 h after induction. Each lane had whole cell sample with same OD that was cultured in LB medium.

increased with the culture time until 4 h post-induction time (Fig. 5A). At the 4 h post-induction time, fraction (44.6%) of fusion protein under Dps^+ was about 1.49-fold higher than that (30%) under Dps^-. In the case of LB medium, the fraction of target fusion band was also enhanced and it reached a maximum at the 4 h post-induction time (Fig. 5B). The fraction of target protein under Dps^+ was 45.9% and showed about 1.72-fold higher than that (26.7%) under Dps^-. Specific amounts of target Polh/GFP fusion protein were normalized based on maximum specific protein amount from SDS–PAGE analyses (Fig. 6) because quantification of target protein amount was not possible from lack of standard Polh/GFP protein. In both media, specific Polh/GFP amount of Dps^+ strain was much higher (1.7- to 2.1-fold) during the entire post-induction time. Also, we found that production rates of GFP fusion protein were high under Dps^+ environment compared to Dps^- condition. At the time for maximum production, specific yield (per OD_{600}) of Polh/GFP fusion protein was enhanced about 2.1-fold regardless of medium used. However, cell density cultured in minimal M9 medium was reduced under Dps^+ environment and volumetric product yield has more important meaning than the specific one in preparative culture, we calculated enhancement ratio for volumetric yield of target fusion protein. By co-expression of Dps, volumetric product yield was about 1.6-fold higher in minimal M9 medium and about 2.5-fold higher in rich LB medium.

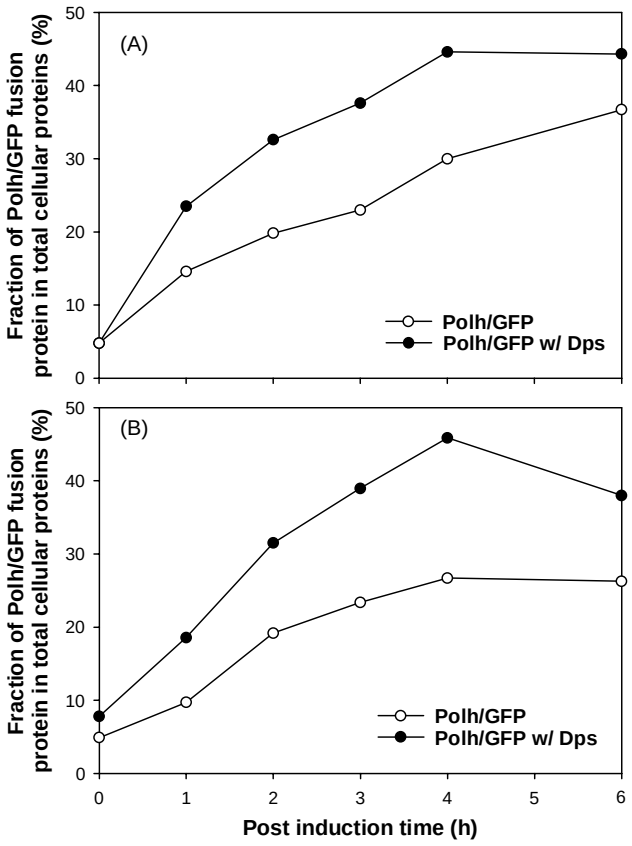

Fig. 5. Time profiles for Polh/GFP fusion protein fraction in total cellular proteins under Dps non-expressing (○) and expressing (●) conditions in (A) minimal M9 and (B) rich LB media. Duplicate analyses were performed for each whole cell sample with same OD of 5 based on SDS–PAGE (Fig. 4); average values of the duplicates were reported.

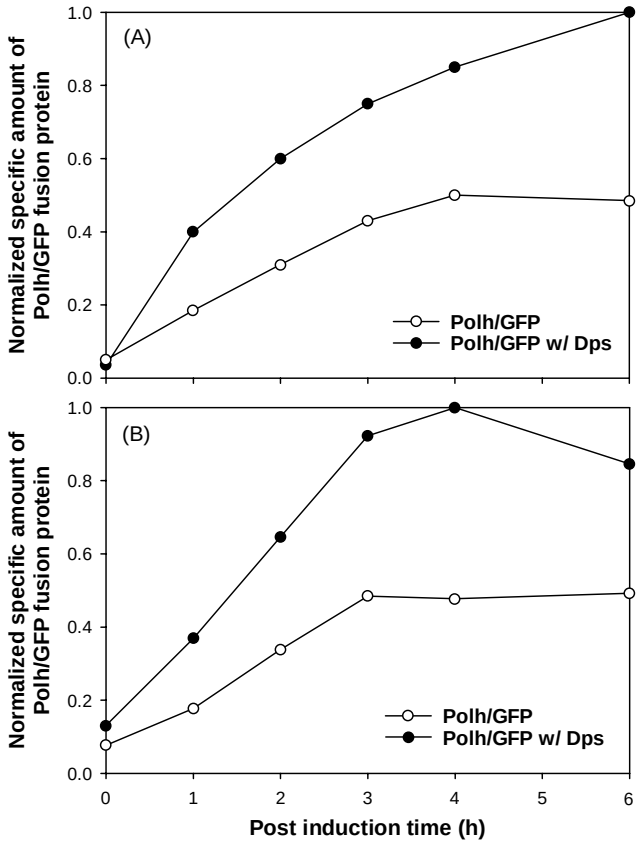

Fig. 6. Time profiles for normalized specific amount of Polh/GFP fusion protein under Dps non-expressing (○) and expressing (●) conditions in (A) minimal M9 and (B) rich LB media. Duplicate analyses were performed for each whole cell sample with same OD of 5 based on SDS–PAGE (Fig. 4); average values of the duplicates were reported.

4. Discussion

While co-expression of nonspecific DNA-binding stress protein, Dps, helped significant enhancement (over twofold) of target heterologous protein, Polh/GFP fusion protein, in *E. coli* cells grown in all types of medium tested, it reduced the growth rate in minimal M9 medium and stimulated cell growth in rich LB medium. Expression of recombinant universal stress protein, UspA, whose synthesis is induced by growth inhibition, also showed similar results on cell growth in two medium types [26]. It has been proposed that UspA inhibits the growth of cells in minimal medium when it is induced to physiological levels and drastically reduces the cell's ability to adapt to upshift conditions unless amino acids are included. Because Dps is also induced in the stationary phase, which is a state of cell growth inhibition by depletion of nutrient, we can surmise that Dps might have similar mechanism on change of cell growth according to medium type. Also, we can think of another possible explanation for this; Dps made significant enhancement of target protein production and this highly enhanced product yield might impose a big burden in minimal M9 medium that tends to have quick limitation of nutrients, but not in rich LB medium.

Even though Dps co-expression had different effects on cell growth in two types of media, it showed a huge impact on product yield in all media. However, the mechanism of Dps co-expression on enhancement of heterologous protein in *E. coli* cells remains unclear. Therefore, detailed investigations are necessary to understand Dps effect. It was also recently reported that Dps (cloned from *Corynebacterium glutamicum*) expression increased the efficiency of L-lysine fermentation by recombinant coryneform bacteria even though they did not show the suggested mechanism of Dps [27]. Therefore, the ability of Dps for significant enhancement of target protein production (about 46% of target protein fraction in total cellular proteins and about 2.5-fold increase in maximum product yield) may be successfully applied in practical culture process.

Two possible explanations might be surmised for the mechanism of Dps on enhancement of foreign protein production in *E. coli* system. First, Dps might directly give a resistant ability to host strain against the stress from over-expression of foreign protein. It was generally known that Dps can protect DNA damage from oxidative stress [3–5,17]. We have checked DNA damage (cleavage) under foreign protein overproduction environment, but it looked like the DNA was not affected (data not shown). Also, there were no reports on the relationship between oxidative stress and foreign protein overproduction. It was also widely known that Dps is expressed during the stationary phase suffering nutrient depletion [18–20]. It might be thought that amino acid or carbon source is getting more deficient in minimal media as cells are growing and recombinant proteins are overproduced. In this case, we can surmise that Dps might contribute to the mechanism coping with such nutritional stress and therefore, cells might produce more foreign protein. However, Dps had a similar degree of effect (based on unit cell) on product yield in rich LB medium that has sufficient amino acids and carbon source and might cause very minimal nutritional stress on the host. Therefore, we can disregard possibilities of correlation of Dps effect with oxidative or nutritional stress. In addition, it was also reported that Dps stress protein is induced under acetic [5] or osmotic stress [28]. Therefore, even though it is not clear yet, it can be thought that Dps might have a general resistant function on several stresses including foreign protein over-expression. Second, it was reported that over-expression of a stress protein resulted in alterations in global protein synthesis [26]. Therefore, we can surmise that alterations in global protein synthesis by Dps co-expression caused positive effects on target foreign protein production. To prove this theory, investigations for global patterns of protein synthesis under Dps co-expression are necessary using modern molecular biological techniques such as 2D protein gel electrophoresis and DNA microarray.

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