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Overexpression of YbeD in *Escherichia coli* Enhances Thermotolerance ^S

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Heat-resistant microbial hosts are required for bioprocess development using high cell density cultivations at the industrial scale. We report that the thermotolerance of *Escherichia coli* can be enhanced by overexpressing *ybeD*, which was known to encode a hypothetical protein of unknown function. In the wild-type *E. coli* BL21(DE3), *ybeD* transcription level increased over five-fold when temperature was increased from 37°C to either 42°C or 46°C. To study the function of *ybeD*, a deletion strain and an overexpression strain were constructed. At 46°C, in comparison to the wild type, the *ybeD*-deletion reduced cell growth half-fold, and the *ybeD*-overexpression promoted cell growth over two-fold. The growth enhancement by *ybeD*-overexpression was much more pronounced at 46°C than 37°C. The *ybeD*-overexpression was also effective in other *E. coli* strains of MG1655, W3110, DH10B, and BW25113. These findings reveal that *ybeD* gene plays an important role in enduring high-temperature stress, and that *ybeD*-overexpression can be a prospective strategy to develop thermotolerant microbial hosts.

Keywords: *ybeD*, *Escherichia coli*, heat shock protein, thermotolerance

Introduction

Development of thermotolerant host strains is biotechnologically important as the heat produced during industrial high cell density cultures can heavily reduce the productivity of biomass and recombinant proteins [1–4]. When *Escherichia coli* cells are exposed to a high temperature, they overexpress heat shock proteins (HSPs) such as chaperones and proteases under the regulation of sigma factor σ^H encoded by *rpoH* [5, 6]. HSP synthesis confers thermotolerance at high temperatures and, thus, the overexpression of HSPs such as DnaK and GroEL has been commonly employed for recombinant protein production [7, 8]. However, HSP overexpression often results in reduced cell growth and poor quality recombinant protein production [7]. Therefore, it is important to identify new heat shock response genes that can be overexpressed without any negative side effects.

Previously, 28 σ^H-dependent promoter sequences were computationally predicted in the upstream of transcription units that were not previously reported to be related with HSPs [9]. Among them, five genes (*yadF*, *ybeD*, *glnS*, *yceJ*, and *xerD*) were transcribed either by σ^H and σ^D in the in vitro transcription assays. Considering that σ^D (encoded by predominant sigma factor gene of *rpoD*) transcribes most of the growth-related genes during exponential growth, expression of those genes whose expression also can be regulated by σ^H might be useful for bacterial growth during high temperatures. Particularly, *ybeD* was 9.5-fold highly expressed in the transcriptome of *E. coli* cells overexpressing σ^H [9], and showed the highest binding intensity in its promoter region in the genomic SELEX (Systematic evolution of ligands by exponential enrichment) study searching for binding sites of RNAP RpoH holoenzyme [10].

YbeD is a cytoplasmic protein which was determined from computational prediction by PSORT database [11] and from LC-MS/MS analysis of *E. coli* cytosolic fraction [12]. In *E. coli*, *ybeD* encodes a conserved hypothetical protein of unknown function. Protein structure analysis showed that *E. coli* YbeD had high structural similarity to the ACT domain of D-3-phosphoglycerate dehydrogenase, suggesting its possible role in the allosteric regulation of lipoic acid biosynthesis or the glycine cleavage system [13]. As described above, *ybeD* belongs to σ^H regulon, however, additional and extensive experimental verification for its

functional role in bacterial growth in high temperature is still required.

E. coli strains, especially BL21(DE3), have been widely used as an industrial host for overproducing various bioproducts using high cell density cultures [14, 15]. In this study, we investigated the usage of *ybeD* for conferring thermotolerance to *E. coli* BL21(DE3). We demonstrated that *ybeD*-overexpression enhanced *E. coli* growth at 46°C, while *ybeD*-deletion decreased growth at a high temperature. The effect of *ybeD*-expression on bacterial growth of other *E. coli* strains (MG1655, W3110, DH10B, and BW25113) were also investigated.

Materials and Methods

Strains, Plasmids, and Culture Conditions

E. coli strains and plasmids used in this study are listed in Table 1. *E. coli* BL21(DE3) was provided by F. William Studier, Brookhaven National Laboratory [16], and was used as a wild-type strain. Cells were grown aerobically in 125-ml flasks containing 25 ml of Luria-Bertani (LB) medium (10 g/l tryptone, 5 g/l yeast extract, and 10 g/l NaCl) with shaking at 200 rpm. The medium was supplemented with 1 mM L-rhamnose and 77 μM chloramphenicol. Cell growth was monitored by measuring absorbance at 600 nm (OD_{600}).

Construction of Gene Deletion and Overexpression Strains

All primers used in this study are listed in Table S1. The lambda Red recombinase system was used to delete the chromosomal *ybeD* in *E. coli* BL21(DE3) through homologous recombination using the linear PCR product as described previously [17]. Briefly, the chloramphenicol resistance (Cm^r) cassette was amplified from the plasmid pKD3 using ybeD-del-F/ybeD-del-R primers. The purified PCR product was electroporated into *E. coli* BL21(DE3) carrying pKD46. The Cm^r cassette was then removed using pCP20. The deletion was verified by colony PCR and subsequent electrophoresis through a 1.5% agarose gel, as well as by DNA sequencing.

For *ybeD*-overexpression, a pAR-*ybeD* plasmid was constructed by replacing *mlacI* in pAR-mLacI [18] with *ybeD*. The pAR-mLacI plasmid contains an L-rhamnose inducible promoter (P_{rhaBAD}) and a chloramphenicol resistance (Cm^R) gene. The *ybeD* was amplified by PCR using genomic DNA of *E. coli* BL21 (DE3) as the template and ybeD-over-F/ybeD-over-R primers. The pAR-mLacI was digested with NdeI and NarI restriction enzymes to delete *mlacI*, and then inserted with *ybeD*. The constructed plasmid (pAR-*ybeD*) was electroporated into *E. coli* strains.

Reverse Transcription PCR (RT-PCR) and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted using the mirVana miRNA Isolation Kit (Thermo Fisher Scientific Inc., USA) as previously described

Table 1. *E. coli* strains and plasmids used in this study.

Strain or plasmid	Description	Source or reference
Strains		
BL21(DE3)	Wild-type	Lab stock
BL21(DE3)Δ*ybeD*	*ybeD* null mutant of BL21(DE3)	This study
MG1655	Wild type	Lab stock
W3110	Wild type	KCTC 2223
DH10B	Wild type	KCTC 12020
BW25113	Wild type	Keio collection [30]
JW0014	BW25113 Δ*dnaJ*::Km^r	Keio collection
JW0426	BW25113 Δ*tig*::Km^r	Keio collection
JW2573	BW25113 Δ*clpB*::Km^r	Keio collection
JW0462	BW25113 Δ*htpG*::Km^r	Keio collection
JW4103	BW25113 Δ*groL*::Km^r	Keio collection
JW0626	BW25113 Δ*ybeD*::Km^r	Keio collection
Plasmids		
pACYC184	Low-copy-number cloning vector (Tet^r Cm^r) (referred to as CV)	NEB
pAR	pACYCDuet-1 derivative with NdeI/XhoI fragment of l-rhamnose inducible promoter amplified with primer pair RhamL-F and RhamL-R from *E. coli* K-12 MG1655 genomic DNA (gDNA)	[18]
pAR-*ybeD*	pAR derivative with NdeI/NarI fragment of wild-type *ybeD* amplified with primer pair ybeD-F and ybeD-R from *E. coli* BL21(DE3) genomic DNA (referred to as p*ybeD*)	This study

[19]. The purified RNA (1 μg/ml) was reverse transcribed to cDNA using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo, Japan). For RT-PCR, the synthesized cDNAs from each sample were amplified using T100 thermal cycler (BioRad, USA). The RT-PCR products were electrophoresed on a 1.5% agarose gel.

qRT-PCR was performed using the QuantStudio 3 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc., USA). Each qRT-PCR reaction containing 1 μl of diluted cDNA, 10 pmol of each primer, and 10 μl of iQ SYBR Green Supermix (Bio-Rad, USA) was performed on a 96-well plate for 40 cycles. Relative changes in target gene expression were calculated using the $2^{-\Delta\Delta C_T}$ method [20]. The 5S rRNA gene was used as an endogenous control for the normalization of target gene expression. The reference samples (37°C) were calculated as the $2^{-\Delta\Delta C_T}$ value of 1.

SDS-PAGE

Whole intracellular proteins were electrophoresed using tricine-SDS-PAGE (Tricine-sodium dodecyl sulfate–polyacrylamide gel electrophoresis), as described previously [21]. Briefly, cells were collected in reducing sample buffer (150 mM Tris/HCl, pH 6.8, 30% glycerol, 12% SDS, 0.1% bromophenol blue and 6% mercaptoethanol) and heated for 10 min at 95°C to prepare the whole protein lysates. The samples were separated by electrophoresis in 10% tricine-SDS-PAGE. The gel was stained with Coomassie blue.

Results and Discussion

Sequence Analysis of *ybeD*

In *E. coli* BL21(DE3), *ybeD* is 264 bp long, and is located between *dacA*, encoding D-alanyl-D-alanine carboxypeptidase for peptidoglycan biosynthesis, and *lipB*, encoding lipoyl (octanoyl) transferase catalyzing the first step of lipoic acid biosynthesis (Fig. 1A) [13]. BLASTN and BLASTP searches of *ybeD* against the NCBI nonredundant (nr) database did not produce any significant alignments with proteins of known function (E-value < 10), only generating hits to DUF493 family proteins or putative lipoate regulatory proteins. The *ybeD* homologs were found in all of the 88 finished genomes of *E. coli* strains from BLASTN and BLASTP searches in the Integrated Microbial Genomes with Microbiome Samples (IMG/M) server [22] (Table S2); their amino acid sequences and sizes were exactly the same with those of the queried *ybeD* of *E. coli* BL21(DE3). Moreover, at the nucleotide level, the sequence identities were 100% except for homologs from seven *E. coli* genomes that had one-nucleotide mismatches. Only one copy of *ybeD* is present in the *E. coli* genome; however, *E. coli* K-12 DH10B has two copies of *ybeD* in a large region precisely duplicated in tandem [23].
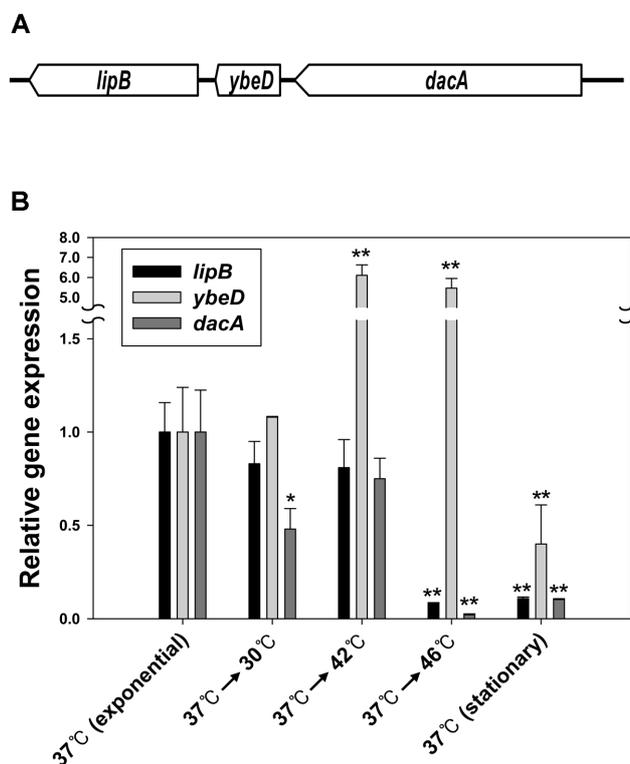
Fig. 1. Relative gene expression of *dacA*, *ybeD*, and *lipB* in wild-type BL21(DE3) at various temperatures.
(**A**) Genetic organization of *dacA*, *ybeD*, and *lipB*. (**B**) Transcription ratio. Wild-type cells growing exponentially at 37°C (1.0 in OD$_{600}$) were shifted to 30°C, 42°C, or 46°C shaking incubator for 1h. In addition, the exponentially growing cells were allowed to reach stationary phase at 37°C. The expression levels of wild-type cells growing exponentially at 37°C were set as a relative expression of 1. The error bar denotes the standard deviation of the mean from four independent qRT-PCR reactions. Asterisks indicate significant difference from the exponential growth at 37°C (*p-value <0.05, **p-value <0.01).

Construction of *ybeD*-Deletion and *ybeD*-Overexpression Strains

We constructed a *ybeD*-overexpression plasmid (pAR-*ybeD*, referred to as p*ybeD* hereafter) (Fig. S1), which was transformed into BL21(DE3). We constructed a *ybeD* null mutant (BL21(DE3)Δ*ybeD*) in which only its structural gene was deleted using the lambda Red recombinase system [17]. The deletion was confirmed by RT-PCR analysis of Δ*ybeD* exponentially growing at 37°C (Fig. S2A). The *lipB* was found to still be expressed, verifying that the *ybeD*-deletion did not affect *lipB* expression.

YbeD protein expressions in BL21(DE3), BL21(DE3)Δ*ybeD*, and BL21(DE3)/p*ybeD* were demonstrated by Coomassie

blue staining of the 10% tricine-SDS-PAGE [21] (Fig. S2B). Whole intracellular proteins from an equal amount of cells (1.0 in OD_{600}) were loaded in each lane of the SDS-PAGE gel and electrophoresed. For each strain, cells growing at 37°C were compared between cultures before and after the addition of 1 mM rhamnose. On the gel, YbeD migrated at ~14 kDa, a value which is approximately the molecular mass previously estimated by gel filtration (~13 kDa) [13]. The gel image showed that a large amount of YbeD was expressed only when rhamnose was added to the cultures of *ybeD*-overexpressing strains (WT/p*ybeD* and Δ*ybeD*/p*ybeD*).

Overexpression and deletion of a gene often causes a toxic effect, resulting in morphological changes such as filamentation [24, 25]. To know whether *ybeD* expression influences the cell morphology, *E. coli* cells with overexpression and deletion of *ybeD* were cultured overnight in LB medium at 37°C and 46°C, and were observed under microscope (Fig. S3). Compared to wild-type BL21(DE3), no obvious differences in cell morphology were found for *ybeD*-deletion and *ybeD*-overexpression strains. The microscopic observation indicates that neither deletion nor overexpression of *ybeD* have any effect on cell morphology under normal and heat shock conditions.

It should be mentioned that we deliberately used LB complex medium rather than minimal medium [26]. Growth ability of *E. coli* strains at high temperatures varies with growth medium [26]. It is well known that when *E. coli* cells grown in minimal medium are transferred to high temperatures, intracellular methionine concentration becomes limited, resulting in cessation of cell growth [27]. The methionine deficiency is mainly caused by temperature sensitivity of the homoserine trans-succinylase catalyzing the first step in the methionine biosynthetic pathway [28]. In our preliminary experiment, BL21(DE3) cultured in glucose-containing M9 minimal medium grew at only up to 42°C of mild heat shock condition, primarily due to inactivation of the methionine biosynthetic enzyme. Thus, in the present study, LB rich medium was used to avoid growth defect caused by biosynthetic insufficiency and to investigate temperature-dependent growth control by *ybeD* expression at the severe heat shock temperature of 46°C.

Temperature-Dependent Expression of *ybeD*

Previously, *ybeD* and *lipB* were highly expressed in *E. coli* overexpressing *rpoH*, and were suggested to be σ^H regulon members [9]. Thus, it is logical to expect that *ybeD* expression varies in a temperature-dependent manner. To address this, we measured the mRNA levels of *lipB*, *ybeD*, and *dacA* at different temperatures by qRT-PCR. *E. coli* cells

growing exponentially at 37°C (1.0 in OD_{600}) were shifted to shaking incubators set at 30°C, 42°C, or 46°C. At 37°C, the cells reached the final density of 4.93 in OD_{600}. Culture temperature shifts from 37°C to 30°C, 42°C, or 46°C decreased cell growth to give final cell densities of 4.3, 4.4, and 2.3 in OD_{600}, respectively.

Wild-type cells growing for 1 h after the temperature shifts were subjected to transcriptional analysis (Fig. 1B). Compared to wild-type BL21(DE3) growing exponentially at 37°C, the relative expression levels of *dacA*, *ybeD*, and *lipB* were determined by qRT-PCR. This analysis observed that *ybeD* expression levels had increased significantly (> 5-fold, *p*-value $< 6 \times 10^{-5}$) during temperature upshifts from 37°C to either 42°C or 46°C; however, little change was observed upon temperature downshift from 37°C to 30°C. The expression pattern of *ybeD* suggested that *ybeD* encodes a heat-responsive protein. The expression patterns of *dacA* and *lipB* were similar: they decreased marginally when temperature change was mild (from 37°C to either 30°C or 42°C), and decreased significantly (< 0.1-fold, *p*-value $< 5 \times 10^{-4}$) upon severe heat stress (from 37°C to 46°C). Moreover, their expression levels were considerably reduced in stationary growth at 37°C. Considering that
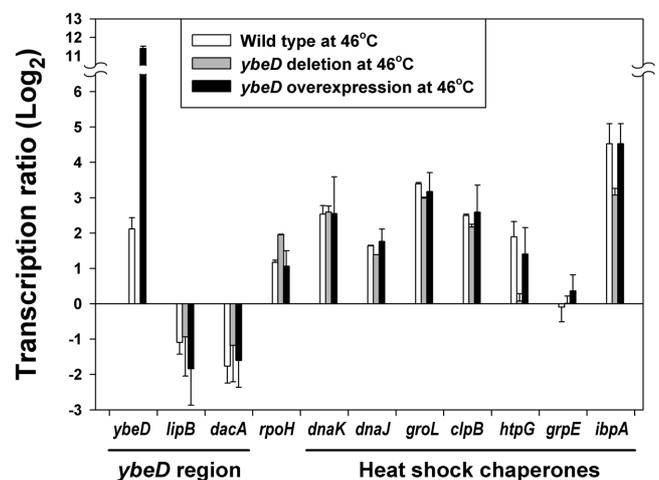
Fig. 2. Relative gene expression of heat shock chaperone genes of wild type, *ybeD*-deletion, and *ybeD*-overexpression strains at 46°C.

BL21(DE3) strains growing exponentially at 37°C (1.0 in OD_{600}) were shifted to 46°C shaking incubator for 1 h. The qRT-PCR analyses were performed for strains of wild type (WT/CV, denoted as white bars), *ybeD*-deletion (Δ*ybeD*/CV, gray bar), and *ybeD*-overexpression (Δ*ybeD*/p*ybeD*, black bars). Log2-transformed gene expressions are given as relative values to those from WT exponentially growing at 37°C. The error bar denotes the standard error of the mean from three biological replications of the qRT-PCR.

dacA is involved in peptidoglycan biosynthesis and *lipB* is implicated in biosynthesis of lipoic acid, which is used as a cofactor for central metabolic pathways [29], the gene expression levels of *dacA* and *lipB* seemed to vary in a growth stage-dependent manner.

We were intrigued by the remarkable increase in *ybeD* transcription of the wild-type cells during temperature upshift from 37°C to 46°C (Fig. 1B). Next, we investigated the effect of *ybeD* expression on the expression of heat shock genes (Fig. 2). qRT-PCR analysis was performed on total RNA isolated from wild-type (WT) BL21(DE3) transformed with the empty plasmid (pACYC184, referred to as CV hereafter), *ybeD*-deletion strain harboring CV (Δ*ybeD*/CV), and *ybeD*-overexpressing strain (Δ*ybeD*/p*ybeD*). Those cells growing for 1 h after temperature upshift from 37°C to 46°C were subjected to transcription analyses of the heat-inducible sigma factor gene (*rpoH*) and heat shock chaperone genes (*dnaK*, *dnaJ*, *groL*, *clpB*, *htpG*, *grpE*, and *ibpA*). As compared to case of the wild type at 37°C, expressions of the heat shock chaperone genes, except *grpE*, were highly increased in the wild type and *ybeD*-deletion/

overexpression strains at 46°C. The increased expression levels of the chaperone genes were similar among the strains, except for no increased *htpG* expression in Δ*ybeD*. This observation raises a question about whether YbeD is a functionally redundant heat shock protein or whether it acts in alternative ways, which requires the omics-based systems approach.

Enhanced Cell Growth at High Temperature by *ybeD* Overexpression

Next, we tested the effect of *ybeD*-overexpression on bacterial growth at a high temperature. Growth of *ybeD*-overexpressing strain (BL21(DE3)/p*ybeD*) was monitored at temperatures of optimal growth (37°C) and of severe heat shock (46°C), and were compared with those of the wild-type (BL21(DE3)/CV) and its *ybeD* deletion mutant (Δ*ybeD*/CV) (Fig. 3A). At 37°C, the growth behaviors were similar between wild type and *ybeD*-overexpressing strains, while the *ybeD*-deletion strain exhibited slow cell growth and lower final cell density; however, the decreases were minimal. At 46°C, cell growth was highest in *ybeD*-
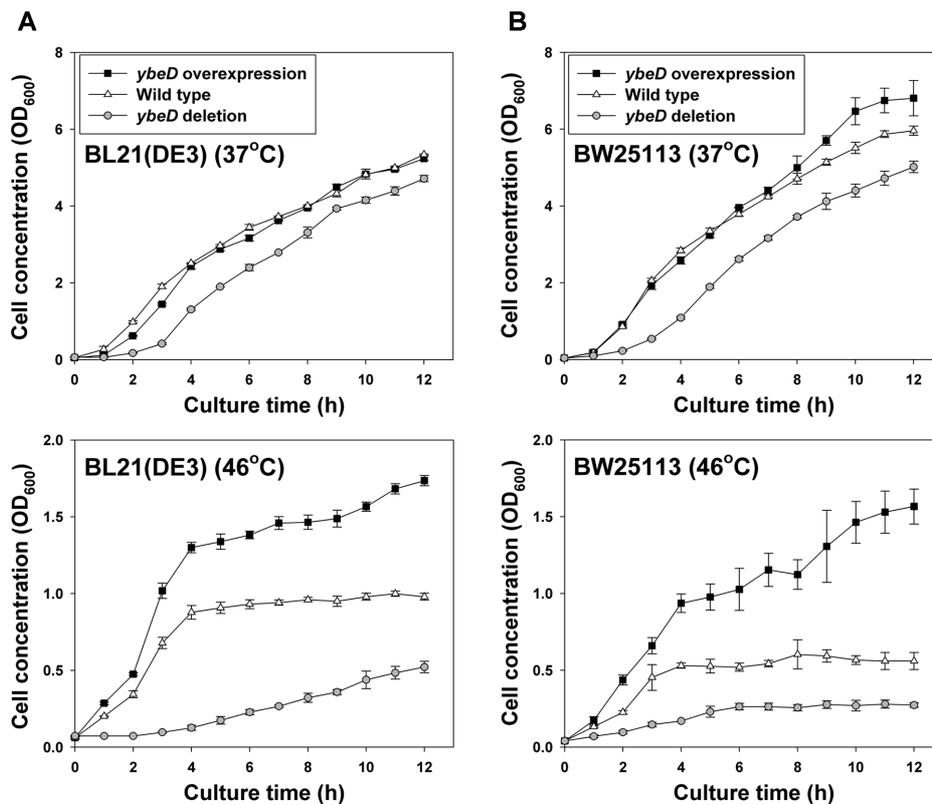
Fig. 3. Growth curves of *ybeD*-deletion/overexpression strains cultured at 37°C and 46°C.

Wild-type *E. coli* strains are (**A**) BL21(DE3) and (**B**) BW25113. Strains are wild type (WT/CV, denoted as △), *ybeD*-overexpression (WT/p*ybeD*, ■), and *ybeD*-deletion (Δ*ybeD*/CV, ●). The error bar denotes the standard deviation of the mean from three independent cultivations.

overexpressing strain (*ybeD*-overexpression (1.8 in OD$_{600}$) > wild type (1.0) > *ybeD*-deletion (0.45)). The growth comparison indicates that *ybeD*-overexpression contributed to the acquired thermotolerance.

To generalize *ybeD*-overexpression responsible for thermotolerance in *E. coli*, growth experiments were performed with the other four *E. coli* strains of BW25113, MG1655, W3110, and DH10B. We obtained BW25113 and BW25113Δ*ybeD* from Keio collection [30], and constructed a *ybeD*-overexpressing BW25113 (BW25113/p*ybeD*). Their growth patterns at 37°C and 46°C were similar to the case of BL21(DE3) strains, and the final cell density in BW25113/p*ybeD* was 2.8-fold and 5.7-fold higher than those in BW25113 and BW25113Δ*ybeD*, respectively (Fig. 3B). As for MG1655, W3110, and DH10B, each wild-type strain (WT/CV) and its *ybeD*-overexpressing strain (WT/p*ybeD*) were cultured at 37°C and 46°C (Fig. S4). Overnight cultures of WT/p*ybeD* at 46°C reached the final cell density higher than their counterpart WT/CV: about four-fold for MG1655 and W3110, and two-fold in the case of DH10B. Marginal growth improvements (< 15%) were observed for the cultures at 37°C. All the growth curve experiments indicate that thermotolerance by *ybeD*-overexpression is a common effect in *E. coli* strains, although the extent varied with *E. coli* strains.

Effect of *ybeD*-Overexpression in Mutants Deficient of Chaperon Genes on Bacterial Growth

E. coli strains possess a variety of heat shock chaperones [6]. Among them, GroEL and GroES are the only molecular chaperone essential for cell growth at all temperatures [31]. To know if the growth improvement by *ybeD*-overexpression also occurs in the different deletion background of heat shock proteins, we transferred p*ybeD* into single-deletion mutant of chaperone genes, which was grown and compared with the corresponding deletion background. To this end, in-frame deletion mutants of chaperone genes (*dnaJ*, *clpB*, *htpG*, and *tig*) were obtained from Keio collection of single-gene deletion mutants of *E. coli* K-12 BW25113 [30]. The gene deletions were confirmed by PCR. We attempted to grow BW25113Δ*dnaK* from Keio collection, however, we failed to get a colony. Instead, we tested the mutant deficient of *tig* encoding trigger factor (TF) because the ribosome-associated chaperone is one of the major chaperones. TF was also reported to have partially overlapping function with DnaK, although it is not a *rpoH* regulon member [32].

Growth of *ybeD*-overexpression in each of the deletion backgrounds was compared with those of the corresponding deletion background and Keio collection parent strain BW25113 (Fig. 4). At 37°C, the growth levels of *ybeD*-overexpressing strains were comparable to those of the corresponding deletion background and the wild-type strains. At 46°C, *ybeD*-overexpressing strains exhibited better growth than their deletion backgrounds, especially in the case of Δ*dnaJ*. However, the growth improvement was only the recovery level of the wild-type growth, and even *ybeD*-overexpression in Δ*tig* did not make any appreciable growth improvement. Considering that *ybeD*-overexpression in wild-type BW25113 led to much higher growth yield (2.8-fold) than BW25113 (Fig. 3B), the growth improvements by *ybeD*-overexpression in deletion backgrounds of the chaperone genes were rather marginal. These might imply that YbeD interacts cooperatively with members of heat shock proteins for the thermotolerance.

Effect of *ybeD*-Expression under Different Stress Conditions

Heat shock proteins provide a fundamental protection mechanism, and they are highly induced when cells are exposed to many stressful conditions such as oxidative stress and foreign protein overexpression [33]. It was reported that the protein abundance of YbeD increased 16-fold in *E. coli* overexpressing membrane proteins [34]. We investigated the effect of *ybeD*-expression on bacterial growth under different stress conditions of temperature, pH, and osmolality (Fig. 5). Wild-type BL21(DE3) (WT/CV), *ybeD*-deletion mutant (Δ*ybeD*/CV), and *ybeD*-overexpressing strain (WT/p*ybeD*) were grown in LB media for 24 h, and their final cell densities were measured. The tested culture conditions were different combinations of values in temperature (23°C, 37°C, and 46°C), pH (5.5, 7.0, and 8.0), and osmolality (0.17 M and 0.5 M). Under all the tested culture conditions, Δ*ybeD*/CV exhibited lower final cell densities than WT/CV. WT/p*ybeD* showed better growth only at 46°C than WT/CV. These observations might imply that function of *ybeD* gene is closely related to cell growth and is especially beneficial to growth at high temperatures.

In this study, we found that *ybeD*-overexpression promoted cell growth at a high temperature. In wild-type *E. coli* BL21(DE3), *ybeD* was highly expressed when cells were exposed to high temperatures. At 46°C, *ybeD*-deletion reduced the final cell density, while *ybeD*-overexpression increased the cell yield. However, at 37°C, the *ybeD* manipulations had little effect on cell growth. Co-expression of bacterial chaperones, mainly DnaK and GroEL, have been widely used for overexpression of recombinant proteins [8]. However, overexpression of chaperones can lead to metabolic burden, often resulting in
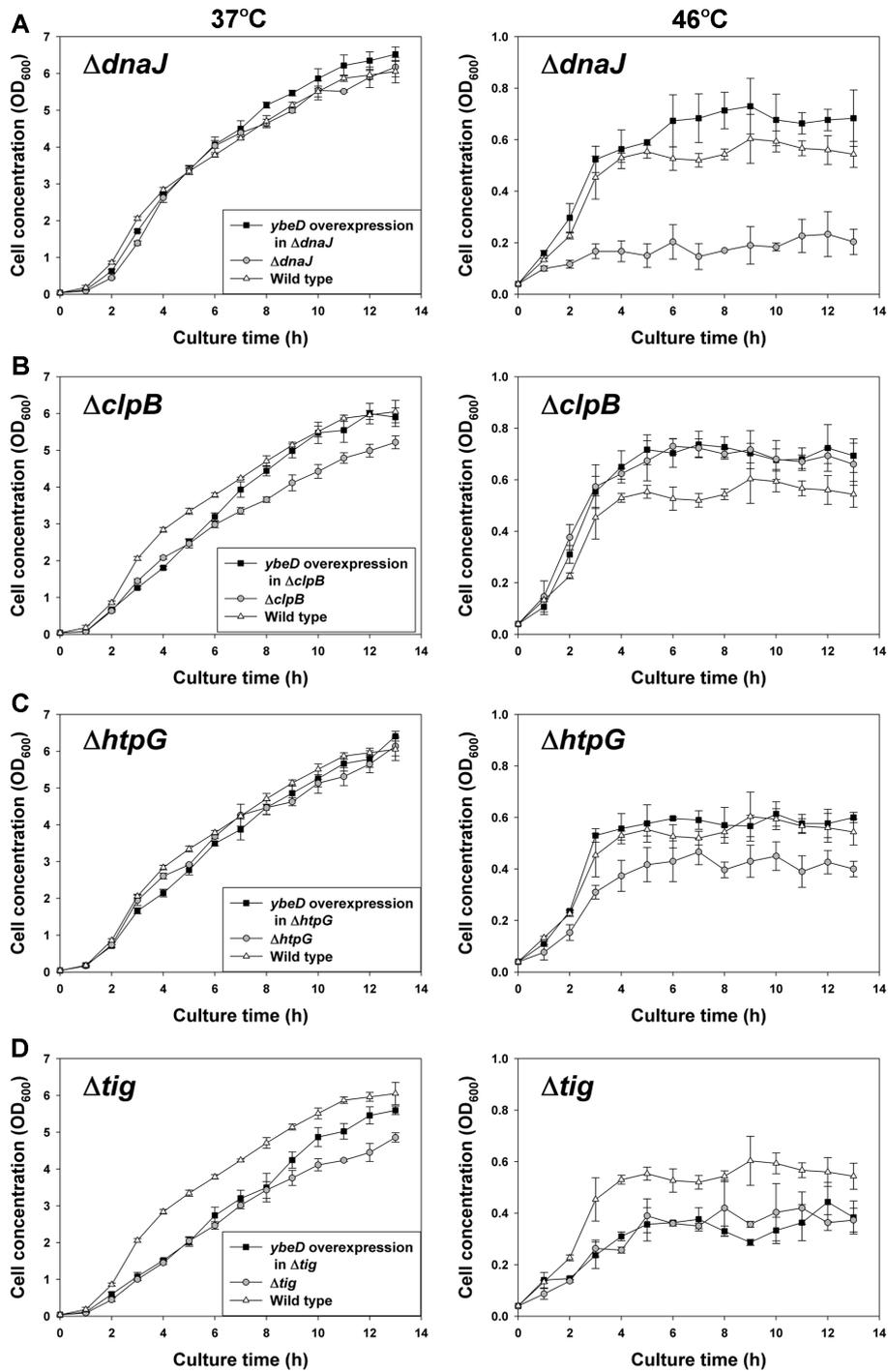
Fig. 4. Effect of *ybeD*-overexpression in deletion backgrounds of chaperone genes on bacterial growth at 37°C and 46°C. Deletion backgrounds are (**A**) Δ*dnaJ*, (**B**) Δ*clpB*, (**C**) Δ*htpG*, and (**D**) Δ*tig*. Shown are strains of wild-type BW25113 (WT/CV, denoted as △), deletion mutant (Δ*gene*/CV, ●), and *ybeD*-overexpression in each deletion background (Δ*gene*/p*ybeD*, ■). The error bar denotes the standard deviation of the mean from three independent cultivations.

slow cell growth and reduced cell yield [7]. Results obtained in this study reveal that *ybeD* encodes heat shock protein whose expression is required for overcoming high-temperature stress. Thus, *ybeD* expression can be modulated
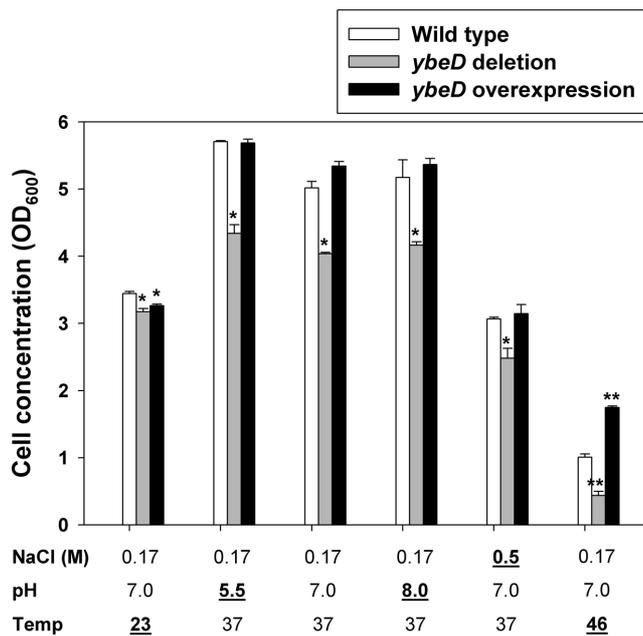
Fig. 5. Final cell densities of wild-type BL21(DE3) and its *ybeD*-deletion/overexpression strains under stress conditions of temperature, pH, and osmolality.

Cells were grown for 24 h in LB media under different culture conditions. Different combinations of values in temperature, pH, and osmolality are denoted under grouped bars, with high or low values underlined. The error bar denotes the standard deviation of the mean from three independent cultivations. Asterisks indicate significant difference between *ybeD*-deletion (or overexpression) strain and the wild-type strain at each culture condition (*p-value <0.05, **p-value <0.01).

to develop thermotolerant microbial hosts for industrial applications.

Acknowledgments

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Ezemaduka AN, Yu J, Shi X, Zhang K, Yin CC, Fu X, *et al.* 2014. A small heat shock protein enables *Escherichia coli* to grow at a lethal temperature of 50°C conceivably by maintaining cell envelope integrity. *J. Bacteriol.* **196:** 2004-2011.

2. Liu D, Lu Z, Mao Z, Liu S. 2009. Enhanced thermotolerance of *E. coli* by expressed OsHsp90 from rice (*Oryza sativa* L.). *Curr. Microbiol.* **58:** 129-133.

3. Gasser B, Saloheimo M, Rinas U, Dragosits M, Rodriguez-Carmona E, Baumann K, *et al.* 2008. Protein folding and conformational stress in microbial cells producing recombinant proteins: a host comparative overview. *Microb. Cell Fact.* **7:** 11.

4. Zhang X, Liu Y, Genereux JC, Nolan C, Singh M, Kelly JW. 2014. Heat-shock response transcriptional program enables high-yield and high-quality recombinant protein production in *Escherichia coli. ACS Chem. Biol.* **9:** 1945-1949.

5. Arsene F, Tomoyasu T, Bukau B. 2000. The heat shock response of *Escherichia coli. Int. J. Food Microbiol.* **55:** 3-9.

6. Gross CA. 1996. Function and regulation of the heat shock proteins, pp. 1382-1399. *In* Neidhardt FC (ed.), *Escherichia coli and Salmonella*, Ed. ASM Press, Washington.

7. Martinez-Alonso M, Garcia-Fruitos E, Ferrer-Miralles N, Rinas U, Villaverde A. 2010. Side effects of chaperone gene co-expression in recombinant protein production. *Microb. Cell Fact.* **9:** 64.

8. Kolaj O, Spada S, Robin S, Wall JG. 2009. Use of folding modulators to improve heterologous protein production in *Escherichia coli. Microb. Cell Fact.* **8:** 9.

9. Nonaka G, Blankschien M, Herman C, Gross CA, Rhodius VA. 2006. Regulon and promoter analysis of the *E. coli* heat-shock factor, σ^{32}, reveals a multifaceted cellular response to heat stress. *Genes Dev.* **20:** 1776-1789.

10. Shimada T, Tanaka K, Ishihama A. 2017. The whole set of the constitutive promoters recognized by four minor sigma subunits of *Escherichia coli* RNA polymerase. *PLoS One* **12:** e0179181.

11. Peabody MA, Laird MR, Vlasschaert C, Lo R, Brinkman FS. 2016. PSORTdb: expanding the bacteria and archaea protein subcellular localization database to better reflect diversity in cell envelope structures. *Nucleic Acids Res.* **44:** D663-668.

12. Ishihama Y, Schmidt T, Rappsilber J, Mann M, Hartl FU, Kerner MJ, *et al.* 2008. Protein abundance profiling of the *Escherichia coli* cytosol. *BMC Genomics* **9:** 102.

13. Kozlov G, Elias D, Semesi A, Yee A, Cygler M, Gehring K. 2004. Structural similarity of YbeD protein from *Escherichia coli* to allosteric regulatory domains. *J. Bacteriol.* **186:** 8083-8088.

14. Yoon SH, Jeong H, Kwon S-K, Kim JF. 2009. Genomics, biological features, and biotechnological applications of *Escherichia coli* B: "Is B for better?!", pp. 1-17. *Systems Biology and Biotechnology of Escherichia coli*, Ed. Springer, Berlin, Germany

15. Choi JH, Keum KC, Lee SY. 2006. Production of recombinant proteins by high cell density culture of *Escherichia coli Chem. Eng. Sci.* **61:** 876-885.

16. Studier FW, Moffatt BA. 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189:** 113-130.

17. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia* coli K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **97:** 6640-6645.

18. Kim SK, Lee DH, Kim OC, Kim JF, Yoon SH. 2017. Tunable control of an *Escherichia coli* expression system for the overproduction of membrane proteins by titrated expression of a mutant *lac* repressor. *ACS Synth. Biol.* **6:** 1766-1773.

19. Kim S, Jeong H, Kim EY, Kim JF, Lee SY, Yoon SH. 2017. Genomic and transcriptomic landscape of *Escherichia coli* BL21(DE3). *Nucleic Acids Res.* **45:** 5285-5293.

20. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. *Methods* **25:** 402-408.

21. Schagger H. 2006. Tricine-SDS-PAGE. *Nat. Protoc.* **1:** 16-22.

22. Chen IA, Markowitz VM, Chu K, Palaniappan K, Szeto E, Pillay M, *et al.* 2017. IMG/M: integrated genome and metagenome comparative data analysis system. *Nucleic Acids Res.* **45:** D507-d516.

23. Durfee T, Nelson R, Baldwin S, Plunkett G, 3rd, Burland V, Mau B, *et al.* 2008. The complete genome sequence of *Escherichia coli* DH10B: insights into the biology of a laboratory workhorse. *J. Bacteriol.* **190:** 2597-2606.

24. Bukau B, Walker GC. 1989. Cellular defects caused by deletion of the *Escherichia coli dnaK* gene indicate roles for heat shock protein in normal metabolism. *J. Bacteriol.* **171:** 2337-2346.

25. Teleha MA, Miller AC, Larsen RA. 2013. Overexpression of the *Escherichia coli* TolQ protein leads to a null-FtsN-like division phenotype. *Microbiologyopen* **2:** 618-632.

26. Herendeen SL, VanBogelen RA, Neidhardt FC. 1979. Levels of major proteins of *Escherichia coli* during growth at different temperatures. *J. Bacteriol.* **139:** 185-194.

27. Ron EZ, Davis BD. 1971. Growth rate of *Escherichia coli* at elevated temperatures: limitation by methionine. *J. Bacteriol.* **107:** 391-396.

28. Ron EZ, Shani M. 1971. Growth rate of *Escherichia coli* at elevated temperatures: reversible inhibition of homoserine trans-succinylase. *J. Bacteriol.* **107:** 397-400.

29. Jordan SW, Cronan JE, Jr. 2003. The *Escherichia coli lipB* gene encodes lipoyl (octanoyl)-acyl carrier protein:protein transferase. *J. Bacteriol.* **185:** 1582-1589.

30. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, *et al.* 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2:** 2006 0008.

31. Fayet O, Ziegelhoffer T, Georgopoulos C. 1989. The *groES* and *groEL* heat shock gene products of *Escherichia col*i are essential for bacterial growth at all temperatures. *J. Bacteriol.* **171:** 1379-1385.

32. Teter SA, Houry WA, Ang D, Tradler T, Rockabrand D, Fischer G, *et al.* 1999. Polypeptide flux through bacterial Hsp70: DnaK cooperates with trigger factor in chaperoning nascent chains. *Cell* **97:** 755-765.

33. Wick LM, Egli T. 2004. Molecular components of physiological stress responses in *Escherichia coli*. *Adv. Biochem. Eng. Biotechnol.* **89:** 1-45.

34. Wagner S, Baars L, Ytterberg AJ, Klussmeier A, Wagner CS, Nord O, *et al.* 2007. Consequences of membrane protein overexpression in *Escherichia coli*. *Mol. Cell. Proteomics* **6:** 1527-1550.