J Biosci (2020)45:85
DOI: 10.1007/s12038-020-00060-3

Choline–betaine pathway contributes to hyperosmotic stress and subsequent lethal stress resistance in *Pseudomonas protegens* SN15-2

Danyan Tang, Xiaobing Wang, Jian Wang, Mengfei Wang, Yaping Wang and Wei Wang*

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China

**Corresponding author (Email, weiwang@ecust.edu.cn)*

MS received 23 December 2019; accepted 26 May 2020

Pseudomonas protegens SN15-2, a typical non-spore-forming rhizosphere bacterium, has excellent biocontrol capabilities; thus, it is necessary to explore the stress resistance of SN15-2. The choline–glycine betaine pathway is considered as an important mechanism by which bacteria adapt to stressful environments. In this work, we demonstrated that the expression of the *betA* and *betB* genes, which are involved in the choline–glycine betaine pathway in SN15-2, was highly increased by 12-fold and 26-fold, respectively, by hyperosmotic stress and choline treatment. The accumulation of betaine in SN15-2 (5.54 g/L) was significantly higher than that in the mutants *Δ betA* (3.44 g/L) and *Δ betB* (2.68 g/L) under hyperosmotic stress and choline treatment. Moreover, choline enhanced the growth of SN15-2 greatly, but it did not enhance the growth of *Δ betB* under hyperosmotic stress. Choline combined with hyperosmotic adaptation significantly increased the lethal stress resistance of SN15-2 while the resistance of *Δ betA* and *Δ betB* was significantly decreased. This research illuminated a strategy underlying the adaptation to osmotic stress in *P. protegens* and provided an effective method to improve the stress resistance of this species, thus provided a theoretical basis for the practical application of *P. protegens* SN15-2.

Keywords. Choline–betaine pathway; hyperosmotic; *Pseudomonas protegens* SN15-2; stress resistance

1. Introduction

Many *Pseudomonas* strains are characterized as important plant growth promoting bacteria (PGPB) (Frohlich *et al*. 2012; Fu *et al*. 2010; Haas and Defago 2005). PGPB have the ability to competitively colonize plant roots and stimulate plant growth as well as reducing the incidence of plant disease (Lugtenberg and Kamilova 2009). *P. protegens* SN15-2 which can prevent several plant diseases, was isolated from tomato roots in our laboratory, and its metabolites have been demonstrated to effectively inhibit the growth of *Ralstonia solanacearum* (Lou *et al*. 2018). Therefore, *P. protegens* SN15-2 has great potential in biological control.

High temperature or freezing are common adverse conditions during the formulating of bioagents in *P. protegens*. Previous studies have found that the adaptation to mild stresses including oxidative (Biryukova *et al*. 2007; Liu *et al*. 2012), temperature (Cheng *et al*. 2016; Dunlap *et al*. 2007), acid (Barbosa *et al*. 2015), and salt stress (Wang *et al*. 2010) can enhance the resistance of biocontrol agents to subsequent lethal stress. In many bacteria, researches have demonstrated that the subsequent stress resistance could be effectively improved when their normal growth is challenged with hyperosmotic stress (Guan *et al*. 2017). Excess NaCl significantly increased the resistance of *Listeria monocytogenes* to mild heat stress (Anderson *et al*. 1991). Moreover, the cell viability of *Pseudomonas fluorescens* EPS62e during freeze-drying could be significantly improved by hypertonic cultivation with lactose addition (Cabrefiga *et al*. 2014). There are two basic strategies by which microorganisms adapt to osmotic stress: the KCl accumulation

Published online: 23 June 2020

strategy and the compatible solute accumulation strategy (Galinski and Trüper 1994). Bacteria commonly accumulate potassium ions when confronted with osmotic stress (Kets *et al*. 1996; Whatmore and Reed 1990). Moreover, compatible solutes accumulated in bacteria can provide protection, including maintaining the appropriate cell volume, protecting intracellular macromolecules, and aid in adaptation to changes in highly osmotic environments (Rudulier *et al*. 1984; Vyrides and Stuckey 2017).

Glycine betaine is commonly considered to be an efficient osmo-protectant, it can protect against the water loss of cells and provide the driving force for cell growth (Velasco-García *et al*. 2006). However, few bacteria can accumulate glycine betaine through *de novo* synthesis, while most bacteria accumulate betaine through uptake of exogenous betaine or its precursor, choline. Therefore, the choline–glycine betaine pathway has been regarded as an important strategy in the adaptation to stressful environments in many bacteria (Kempf and Bremer 1998; Landfald and Strm 1986; Sand *et al*. 2014). A two-step oxidation reaction is involved in this pathway: First, choline is oxidized to glycine betaine aldehyde, and then glycine betaine aldehyde is oxidized to glycine betaine. The first step is catalyzed by choline dehydrogenase which is encoded by the gene *betA,* and the second step is catalyzed by betaine aldehyde dehydrogenase, which is encoded by the gene *betB* (Chen and Murata 2011; Lamark *et al*. 1991). Moreover, the *betBA* genes have been considered as important genes for betaine synthesis and tolerance to high osmotic pressure in *Pseudomonas putida* (Galvão *et al*. 2006).

Recently, it has been reported that choline oxidized to glycine betaine in *Pseudomonas aeruginosa* (Wargo 2013; Wargo and Matthew 2013) and *Pseudomonas syringae* (Beattie *et al*. 2016; Chen and Beattie 2007) in response to high-stress environments. However, there are still few related studies on *P. protegens*. Therefore, we investigated the contribution of the choline–glycine betaine pathway under hyperosmotic stress and subsequent lethal stress in *P. protegens* SN15-2 by constructing mutants.

2. Materials and methods

2.1 *Bacterial strains, media, and growth conditions*

P. protegens SN15-2 was cultured in LB medium (10 g tryptone, 5 g yeast extract, 10 g NaCl, and 1000 mL deionized water) at 28°C. *E.coil* was cultured in LB medium at 37°C. Before each experiment, we cultivated *P. protegens* SN15-2 and the mutants *Δ betA* and, *Δ betB,* in LB liquid medium overnight at 28°C, and 200 rpm. Then, SN15-2 cultures were inoculated into an Erlenmeyer flask (250 mL) containing 50 mL LB medium with 2% (v/v) inoculum at 28°C, and 200 rpm for 24 h. Kanamycin was used as an antibiotic at 50 μg/mL.

2.2 *RNA isolation and cDNA synthesis*

P. protegens SN15-2 was grown overnight at 28°C and 200 rpm in LB medium. Then, 2% (v/v) of the overnight culture was used to inoculate into fresh LB medium, LB medium plus 450 mM NaCl, LB medium plus 450 mM NaCl supplemented with 8 mM choline separately, and the cultures were incubated for 24 h (stationary phase) at 28°C. Total RNA from the cells was isolated using an RNA Pure Bacteria Kit (CWBIO) according to the manufacturer's instructions. Isolated RNA was quantified using a NanoDrop 2000c spectrophotometer (NanoDrop Scientific, Thermo). Purified RNA was used as a template to initiate the first-strand cDNA synthesis reaction with a Prime-Script^{TM} RT Reagent Kit (TaKaRa) according to the manufacturer's protocol and stored at −20°C until use.

2.3 *Gene expression analysis*

Transcriptional analysis was performed to assess the expression levels of the glycine betaine synthesis genes *betA* and *betB* of *P. protegens* SN15-2 in response to choline or hyperosmotic stress with or without choline using quantitative real-time RT-PCR (qRT-PCR). qRT-PCR analysis was carried out with a 20 μl reaction volume on a Real-Time PCR System (GenStar, China). The cycling conditions were as followed: 2 min at 95°C; 40 cycles of 15 s at 95°C and 30 s at 60°C. The relative expression of genes was determined using the comparative threshold cycle method with 16 s rRNA gene as a reference, and data analysis was performed with the $2^{-\Delta\Delta Ct}$ method. All primers are listed in table 1.

2.4 *Construction of deletion mutants*

An upstream fragment (689 bp) and a downstream fragment (873 bp) of the *betA* gene were amplified using the primer pairs betA-1/betA-2 and betA-3/betA-4 (table 1). The two fragments were cloned into the suicide vector p2P24-km (Li *et al*. 2018) using the Gibson

assembly method (Huang and Wilks 2017) to create p2P24-*betA*. A similar method was used to create p2P24-*betB*. The suicide plasmids p2P24-*betA* and p2P24-*betB* were introduced into strain SN15-2 by electroporation, and kanamycin-resistant colonies were plated on LB medium supplemented with 5% sucrose to generate the mutants *Δ betA* and *Δ betB*, respectively.

2.5 *Quantification of intracellular glycine betaine*

P. protegens SN15-2 wild type and mutant strains were incubated in LB medium plus 450 mM NaCl with or without supplementation with 8 mM choline at 28°C and 200 rpm for 24 h. Then, the cultures were centrifuged at 8,000 rpm for 10 min for collection of the bacteria and washed with sterile water three times to remove the residual culture medium. The initial OD of each treatment was adjusted to the same value of approximately 1.2. The cellular extraction was performed according to previous methods (Ma *et al*. 2017). Briefly, the supernatant was removed by centrifugation, and the cellular precipitate was subjected to three freeze-thaw cycles, then resuspended in ethanol. Then, the insoluble substances were removed by centrifugation. The ethanol was then removed from the extracted material by vacuum evaporation for further HPLC analysis. HPLC analysis was conducted at 230 nm using an Agilent C18 NH_2 column (250 × 4.6 mm with 5 μm particle size) and a 100 Å pore size on a DIONEX Ultimate 3000 analyzer at a flow rate of 1 mL/min. The mobile phase was a mixture of 95% acetonitrile and 5% water (v/v) in an isocratic elution mode over an 8 min total run time. The column temperature was maintained at 30°C, and the injection volume was 20 μL.

2.6 *Growth experiments*

To obtain growth curves, *P. protegens* SN15-2, *Δ betA* and *Δ betB* were grown in LB medium filled with 450 mM NaCl with or without 8 mM choline for 36 h. The cultures were maintained at 28°C and 200 rpm, and growth was monitored every 4 h by measuring the optical density at 600 nm. The assays were performed in triplicate, and the experiments were performed three times. The growth curves were fitted manually.

2.7 *Lethal stress*

P. protegens SN15-2 and its mutant strains were grown in LB liquid medium overnight at 28°C and 200 rpm. Then, the cultures were inoculated at 2% (v/v) into an Erlenmeyer flask containing 50 mL fresh LB, LB plus 450 mM NaCl, or LB plus 450 mM NaCl supplemented with 8 mM choline. The bacteria were cultured at 28°C and 200 rpm for 24 h, centrifuged at 8,000 rpm for 10 min to collect fresh bacteria, and washed with sterile water three times to remove the residual culture medium. The initial ODs were adjusted to the same value of approximately 1.2.

Five milliliters of the above samples was taken and immediately added to an Erlenmeyer flask with 45 mL sterile water that had been preheated in a water bath at 50°C. The time of addition was recorded as 0 min, and plate colony counts were carried out at the same time. Four minutes later, the colony counts were performed again. Cell counts were performed on LB plates after 24 h.

One milliliter of the above samples was transferred to sterile tubes, and the cell suspensions were frozen at −20°C for 1 h and thawed at room temperature. The plate colony counts of *P. protegens*, *Δ betA* and *Δ betB* were analyzed immediately. The count method was as described above, and the cell counts were performed in triplicate.

The damage index (DI) was used to evaluate the heat or cold resistance of *P. protegens* SN15-2, *Δ betA* and *Δ betB*. The DI calculation method was described in previous research (Chen *et al.* 2019), and the DI was inversely proportional to the heat or cold stress resistance of the strains.

2.8 *Statistical analysis*

To test the significance of the effect of treatments on the gene expression level, the content of betaine and the DI of *P. protegens* SN15-2, *Δ betA,* and *Δ betB* under different environmental conditions, a one-way ANOVA of variance was analyzed and mean separations were performed by Waller-Duncan test with SPSS software (Version 21, SPSS Inc.). All results were considered statistically significant at $P < 0.05$.

3. Results

3.1 *High osmotic stress combined with choline strongly induced the expression of betA and betB in P. protegens SN15-2*

The expression of the *betA* gene (figure 1A), either in LB medium with choline or under hyperosmotic stress, did not show a significant difference from that under the control (LB medium). However, the expression of

Table 1. Primer pairs of target genes used in this study

Primer	Sequence	Description
betA-1	CGTTGTAAAACGACGGCCAGTGCCAAGCTTGTTCACCAGGCGTTCATCC	Deletion of gene *betA*
betA-2	GATTACATCATTGTGGGTGCCGGATGCGCGATGTGACTCC	
betA-3	GGAGTCACATCGCGCATC CGGCACCCACAATGATGTAATC	
betA-4	CAGCTATGACCATGATTACGAATTCCCGCTTTCGAAGCCAAGATC	
betB-1	CGTTGTAAAACGACGGCCAGTGCCAAGCTTTCGAAGAGGATCTTGTCGGTC	Deletion of gene *betB*
betB-2	CGCCACTTTCGATGCCATCACATCAAGTCGGTGCAGGTC	
betB-3	GACCTGCACCGACTTGATG TGATGGCATCGAAAGTGGCG	
betB-4	CAGCTATGACCATGATTACGAATTCCATCTGCGAGCAGGAGAAACT	
betA-F	TCGGTCTGCACCTCGATGCC	Gene expression of *betA*
betA-R	ACATGGCCACCGAGCAGGAC	
betB-F	CAGCGGGATCTGCTCGCCTT	Gene expression of *betB*
betB-R	CCATGGAGCGTTCGCGCATC	
16S-F	CTACGGCTACCTTGTTAC	Gene expression of *16S*
16S-R	GATGGATTGGTGCCTTCG	

betA increased by 12-fold, demonstrating that *betA* was highly induced by hyperosmotic stress with choline addition. Additionally, the expression of *betB* with choline exposure (figure 1B) did not show a significant difference with the control but increased significantly under hyperosmotic stress. Moreover, the expression of *betB* increased 26-fold under hyperosmotic stress combined with choline.

3.2 *Glycine betaine showed intracellular accumulation in P. protegens SN15-2*

The intracellular betaine of *P. protegens* SN15-2 and the mutants *Δ betA* and *Δ betB* under different conditions were analyzed by HPLC (figure 2). The content of betaine in the wild type, which was grown in a hyperosmotic environment with choline addition, reached 5.54 g/L. The betaine accumulation in *Δ betA* (3.44 g/L) and *Δ betB* (2.68 g/L) was significantly lower than that in the wild type under hyperosmotic stress combined with choline. In addition, the accumulation of betaine in *Δ betB* was significantly lower than that of *Δ betA* under the combination of hyperosmotic stress and choline, and showed no significant difference with that in the wild type in hyperosmotic environment without choline.

3.3 *Choline, as an osmoprotectant, can efficiently improve the growth of P. protegens SN15-2 under hyperosmotic stress*

Hyperosmotic stress (450 mM NaCl) significantly reduced the growth of *P. protegens* and its mutants *Δ betA* and *Δ betB* compared with LB medium (data not shown). Both the wild type and mutant strains showed a similar growth pattern under hyperosmotic stress regardless of choline addition (figure 3). Choline enhanced the growth of the wild type under hyperosmotic stress, and the maximum OD_{600} of approximately 2.19 was observed after incubation for 32 h. However, the addition of choline slightly enhanced the growth of *Δ betA* compared with that of the wild type but impaired the growth of *Δ betB* in the hyperosmotic environment.

3.4 *Hyperosmotic adaptation combined with choline can efficiently improve the resistance of P. protegens SN15-2 to lethal stress*

To investigate whether the choline–betaine pathway contributed to the subsequent lethal stress resistance, an assay of the DI under heat shock (50°C, 4 min) or cold stress (20°C, 1 h) was conducted. Under heat shock (figure 4A), the DI of the wild type grown under hyperosmotic stress with choline (1.31) was significantly lower than that in LB medium with (1.63) or without 450 mM NaCl (2.32). Additionally, the DI of *Δ betB* (1.87) was significantly higher than that of the wild type, but the DI of *Δ betA* (1.54) did not show a significant difference with that of the wild type under high osmotic pressure combined with choline. Furthermore, the DI of *Δ betB* was significantly higher than that of *Δ betA* under the hyperosmotic environment with choline addition.

Under cold stress, the DI of the wild type under different conditions showed the same trend as that under heat shock (figure 4B). However, the DI values of *Δ betA* (1.03) and *Δ betB* (1.33) were all

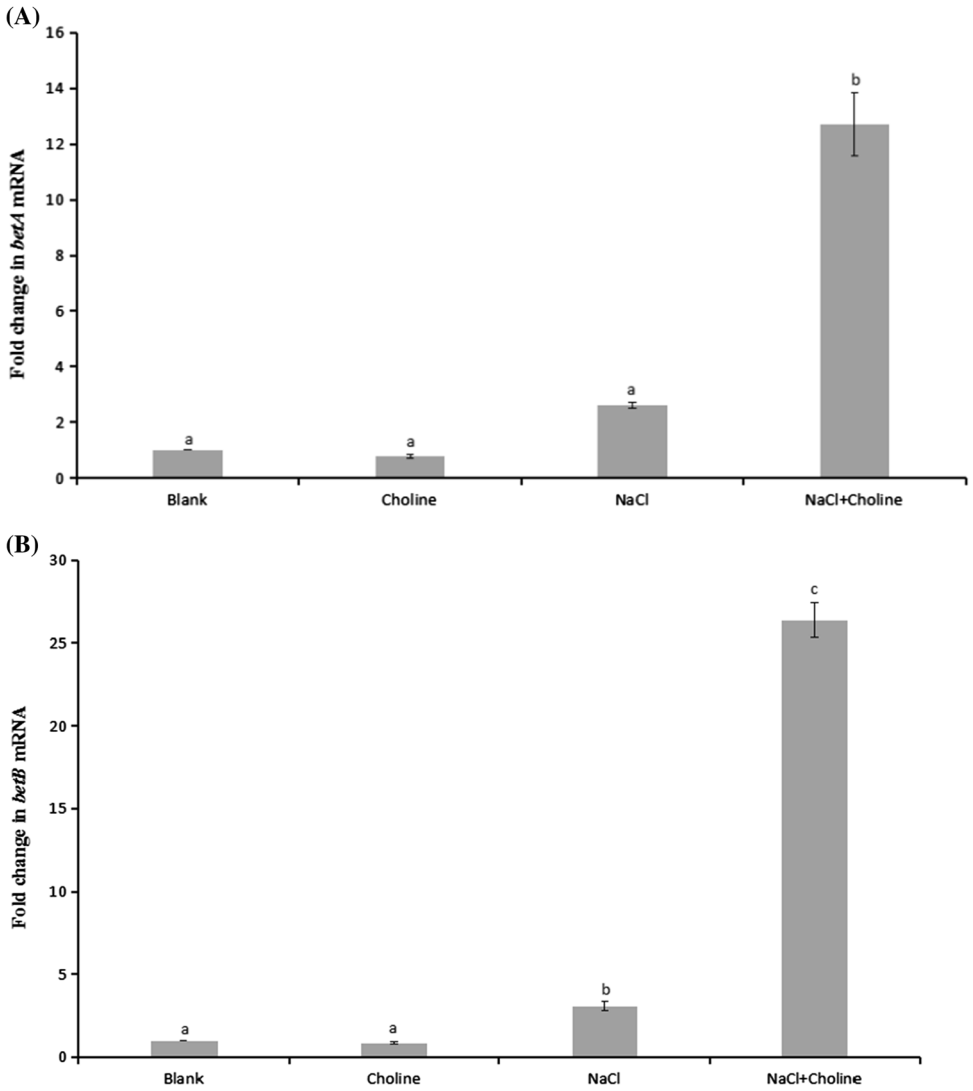

Figure 1. Expression of gene *betA* and *betB* in SN15-2 in different condition. (**A**) The expression of *betA* in *P. protegens* SN15-2 at 24 h, 28°C. (**B**) The expression of *betB* in *P. protegens* SN15-2 at 24 h, 28°C. Values are expressed relative to the *betA* and *betB* transcript level at common LB medium without addition. The expression of indicated genes was normalized by that of 16sRNA. Values are the means of three replicates. Bars with the same letter in the same panel do not differ significantly ($P < 0.05$) according to the Waller-Duncan test. Error bars indicate standard deviations of the means (n = 3).

significantly higher than that of the wild type (0.82) under the hyperosmotic environment combined with choline.

4. Discussion

P. protegens SN15-2 encounters various challenges during processing, including high and freezing temperatures. However, as a non-spore-forming bacteria, the stress resistance of *P. protegens* is poor, which leads to its failure as a successful biological control agent. The induction of the choline–glycine betaine pathway has been demonstrated that it could affect the stress resistance of many bacteria, but few relevant details have been reported for *P. protegens* SN15-2. Therefore, our research explored the contribution of this pathway under hyperosmotic stress and lethal stress in *P. protegens* SN15-2 with great application potential.

The analysis of gene transcription demonstrated that the expression of *betA* and *betB* was not induced by choline alone. Hyperosmotic stress alone could not

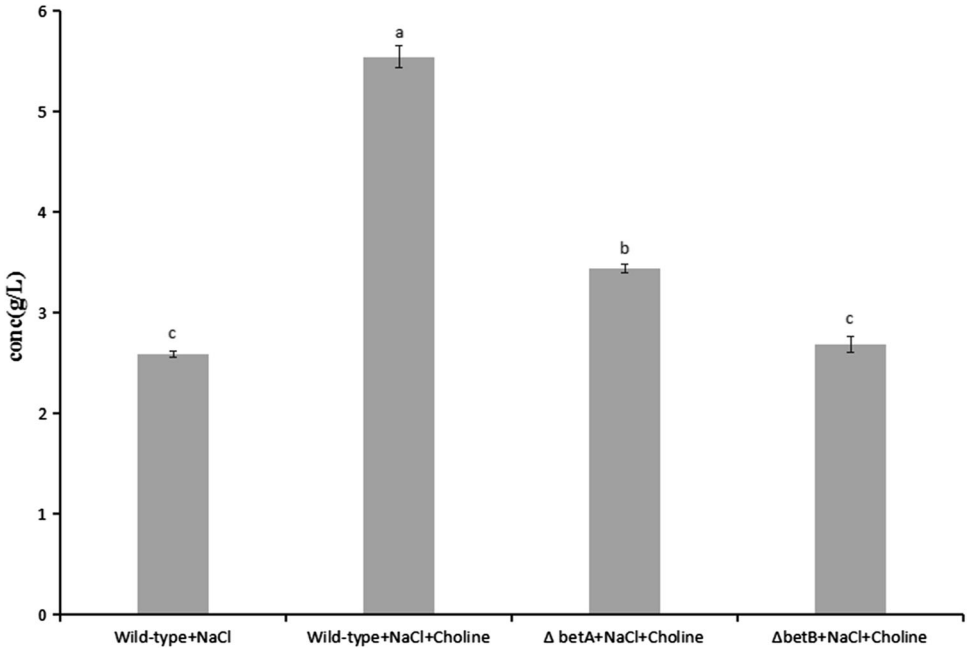

Figure 2. Quantitative analysis of intracellular betaine in SN15-2 and *Δ betA, Δ betB* through HPLC. Same letters suggest no significant difference ($P < 0.05$) according to the analysis of Waller-Duncan test. Error bars indicate standard deviations of the means (n = 3).

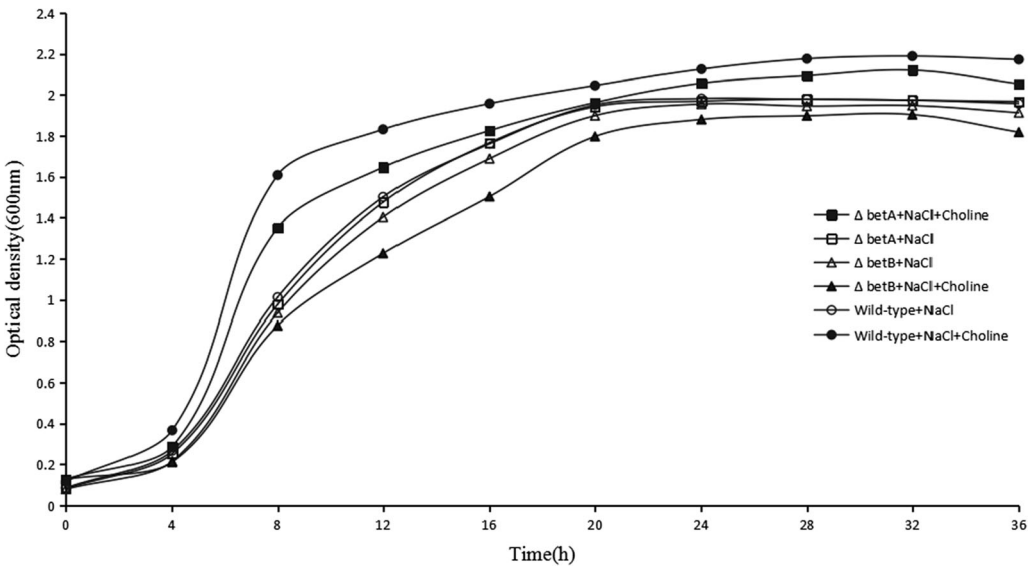

Figure 3. Optical density of cultures of SN15-2 and *Δ betA, Δ betB.* The bacteria cultures were inoculated in LB medium containing 450 mM NaCl with or without choline at 28°C, and 200 rpm for 36 h.

induce the expression of *betA* but *betB* was significantly induced ($p < 0.05$). Hyperosmotic stress combined with choline could highly induced the expression of *betA* (12-fold) and *betB* (26-fold) especially for *betB* (figure 1). Previous research has also demonstrated that choline uptake was stimulated markedly in cells that were grown in the presence of both choline and high salt in *Acinetobacter baylyi* (Sand *et al*. 2014). These

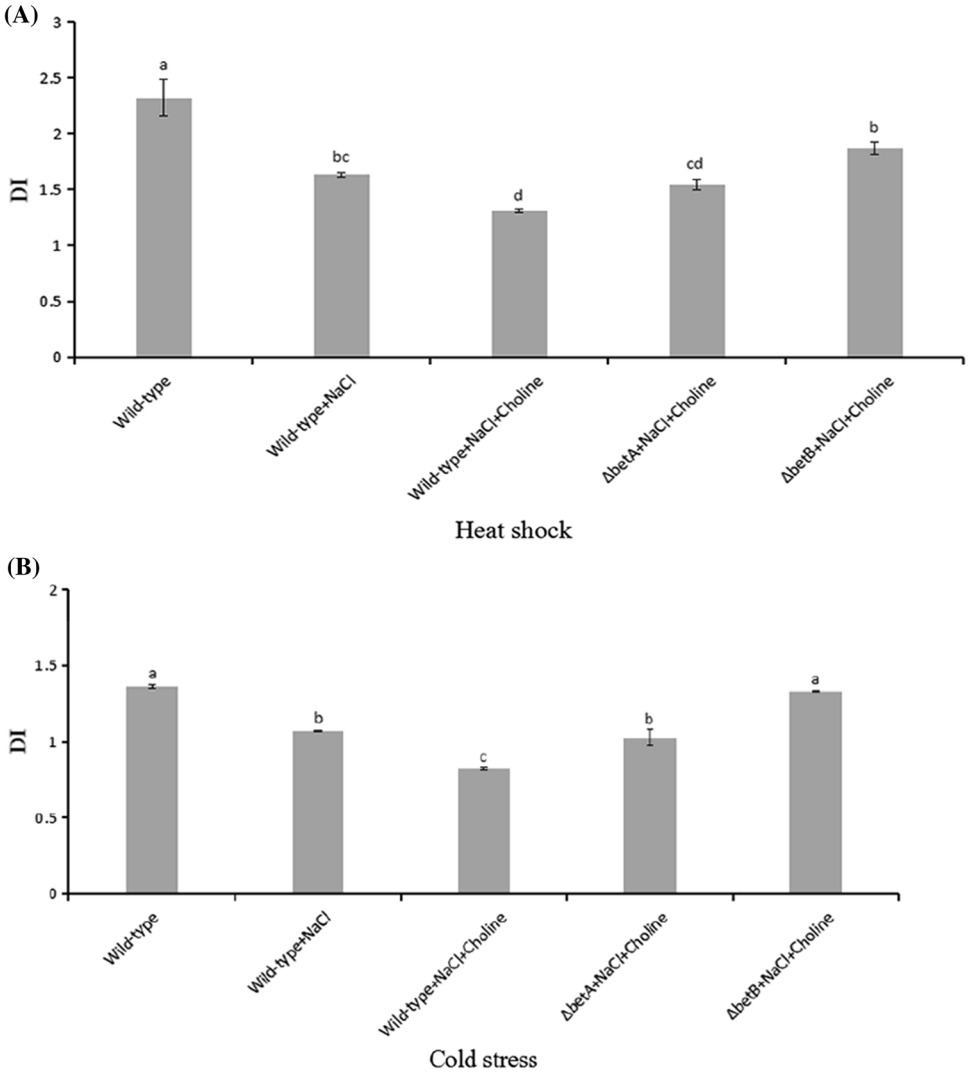

Figure 4. The assay of stress resistance in SN15-2 and *Δ betA, Δ betB*. (**A**) Damage index of wild and mutant strains under heat shock (50°C, 4 min). (**B**) Damage index of wild type and mutant strains under cold stress (−20°C, 1 h). The Damage Index (DI) to evaluate the stress tolerance of *P. protegens* SN15-2 and mutants. The method to calculate damage index: $DI = \log N_0/N_f$, N_0 = initial cell count; N_f = final cell count. Error bars indicate standard deviations of the means (n = 3). Same letters indicate not significant difference ($P < 0.05$) according to the Waller-Duncan test.

results suggested that the expression of the *betB* gene was significantly induced by hyperosmotic stress and that *betA* and *betB* were both promoted by hyperosmotic stress combined with choline.

Interestingly, the results of HPLC analysis (figure 2) showed that cells grown in hyperosmotic conditions without choline could accumulate betaine. The reason for the slight betaine accumulation under hyperosmotic conditions was due to the presence of yeast extract in the LB medium. Previous research found that yeast extract contains large amounts of glycine betaine (Galinski and Trüper 1994); the largest osmo-protectant transport systems in bacteria contain binding protein-dependent ATP-binding cassette (ABC) transporters, including *OpuBC* transporters. *OpuBC* functioned as the primary or sole transporter for glycine betaine under high osmolarity in *P. syringae* (Chen and Beattie 2007). According to our transcriptome data (data not shown), the *OpuBC* family, including the *OpuC* transporter, was upregulated during long-term

hyperosmotic cultivation so that glycine betaine could be transported from the growth medium to the intracellular spaces.

Moreover, our data demonstrated that the content of betaine under hyperosmotic stress combined with choline was significantly higher than that under hyperosmotic stress alone, and the results of qRT-PCR also showed that the betaine synthesis genes *betA* and *betB* were strongly induced in a hyperosmotic environment supplemented with choline. As a result, the ability to catabolize choline to glycine betaine was promoted by hyperosmotic stress combined with choline in *P. protegens* SN15-2. In addition, with the transport of betaine from the growth medium, cells under hyperosmotic stress combined with choline accumulated the most betaine.

The contents of betaine in *Δ betA* and *Δ betB* were significantly lower than that in the wild type, which demonstrated that the *betA* and *betB* genes are critical for the synthesis of glycine betaine in *P. protegens* SN15-2. The betaine content in *Δ betB* was significantly lower than in *Δ betA* in the hyperosmotic environment with choline and showed no significant difference with that of the wild type under hyperosmotic stress. These results suggest that the *betB* gene may play an important role in the choline–glycine betaine pathway. This is mainly due to betaine aldehyde dehydrogenase (BADH), which is encoded by *betB* and catalyzes the last, irreversible step in the synthesis of glycine betaine from choline. This reaction is an obligatory step in the assimilation of carbon and nitrogen when bacteria are grown in the presence of choline (Velasco-García *et al*. 1999).

Based on these data, we discovered that choline can protect *P. protegens* SN15-2 from hyperosmotic stress (figure 3). However, the growth of *Δ betA* was slightly enhanced by choline, while the growth of *Δ betB* was not enhanced by choline under hyperosmotic stress. This means that the choline–glycine betaine pathway is very important in the *P. protegens* adaptation to hyperosmotic stress, and previous research has also demonstrated that this pathway is very important for increasing the survival of many bacteria in hyperosmotic environment (Boch *et al*. 1994; Landfald and Strm 1986). Additionally, the growth of *Δ betB* was impaired by choline under hyperosmotic stress, mainly due to the BADH substrate, betaine aldehyde, which accumulated in the *betB* mutant when choline is present, has a toxic effect on its growth (Sage *et al*. 1997).

Under heat shock or cold stress, the DI of the hyperosmosis-adapted wild type significantly decreased compared to that in normal medium, which indicated that the hyperosmotic adaptation of cells could improve the lethal stress resistance in *P. protegens* SN15-2. This is mainly because adaptation enhances resistance to stresses and promotes the survival of microorganisms under various adverse environments (Koga *et al.* 2002). Research has found that nutrient-starved cells of *Vibrio parahaemolyticus* triggered cross-protection against heat, osmotic or H_2O_2 stresses (Koga and Takumi 1995). In addition, stress response mechanisms are triggered in *E. coli* exposed to challenging environmental conditions, including osmotic stress (Rodríguez-González *et al*. 2011).

Furthermore, hyperosmotic adaptation combined with choline significantly decreased the DI and increased the survival rate of the wild type compared with cells exposed to hyperosmotic adaptation alone. This result suggested that hyperosmotic adaptation combined with choline is an efficient method to improve the lethal stress resistance of *P. protegens* SN15-2. Moreover, the DI values of *Δ betA* and *Δ betB* were higher than that of the wild type, and the stress resistance of *Δ betB* was lower than that of *Δ betA* in a hyperosmotic environment combined with choline. These results revealed that the choline–glycine betaine pathway could contribute to stress resistance and that *betB* likely plays a more important role than *betA* in this pathway.

In conclusion, our data show that the firmly established genes *betA* and *betB* are two critical genes in the choline–glycine betaine pathway and that the *betB* gene may play an especially important role in this pathway (figure 2). This pathway is stimulated by hyperosmotic stress and promoted by choline (figure 1). Moreover, our results demonstrated that the choline–glycine betaine pathway contributed to the adaptation of osmotic stress (figure 3) and lethal stress resistance (figure 4), and hyperosmotic adaptation combined with choline could significantly improve stress resistance (figure 4). Our findings provide new insight into how adaptation to hyperosmotic stress may be acquired and how lethal stress resistance may be efficiently improved through the choline–glycine betaine pathway, which is very important for practical applications of *P. protegens* SN15-2. However, more efficient methods for improving stress resistance need to be explored in *P. protegens* SN15-2 in the future.

Funding

This work was supported by the National Key Research and Development Program of China under

Grant number 2017YFD0200400; the Project of Prospering Agriculture through Science and Technology of Shanghai, China under Grant number Hu NongKeChuangZi (2018) No. 2–5.

References

Anderson WA, Hedges ND, Jones MV and Cole MB 1991 Thermal inactivation of *Listeria monocytogenes* studied by differential scanning calorimetry. *J. Gen. Microbiol.* **137** 1419–1424

Barbosa J, Borges S and Teixeira P 2015 Influence of sublethal stresses on the survival of lactic acid bacteria after spray-drying in orange juice. *Food Microbiol.* **52** 77–83

Beattie GA, Chen C, Nielsen L and Freeman BC 2016 Interstrain variation in the physiological and transcriptional responses of *Pseudomonas syringae* to osmotic stress; in *Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria* (eds) J Frans and D Bruijn (John Wiley & Sons, Inc) pp 647–656

Biryukova EN, Medentsev AG, Arinbasarova AY and Akimenko VK 2007 Adaptation of the yeast *Yarrowia lipolyticato* to heat shock. *Microbiology* **76** 158–163

Boch J, Kempf B and Bremer E 1994 Osmoregulation in *Bacillus subtilis*: synthesis of the osmoprotectant glycine betaine from exogenously provided choline. *J. Bacteriol.* **176** 5364–5371

Cabrefiga J, Francés J, Montesinos E and Bonaterra A 2014 Improvement of a dry formulation of *Pseudomonas fluorescens* EPS62e for fire blight disease biocontrol by combination of culture osmoadaptation with a freeze-drying lyoprotectant. *J. Appl. Microbiol.* **117** 1122–1131

Chen C and Beattie GA 2007 Characterization of the osmoprotectant transporter OpuC from *Pseudomonas syringae* and demonstration that cystathionine-beta-synthase domains are required for its osmoregulatory function. *J. Bacteriol.* **189** 6901–6912

Chen J, Wang X, Tang D and Wang W 2019 Oxidative stress adaptation improves the heat tolerance of *Pseudomonas fluorescens* SN15-2. *Biol. Control* **138** 104070

Chen TH and Murata N 2011 Glycine betaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant Cell Environ.* **34** 1–20

Cheng Z, Chi M, Li G, Chen H, Sui Y, Sun H, Wisniewski M, Liu Y *et al.* 2016 Heat shock improves stress tolerance and biocontrol performance of *Rhodotorula mucilaginosa*. *Biol. Control* **95** 49–56

Dunlap CA, Evans KO, Bart T, Teun B and Schisler DA 2007 Osmotic shock tolerance and membrane fluidity of cold-adapted *Cryptococcus flavescens* OH 182.9, previously reported as *C. nodaensis*, a biocontrol agent of *Fusarium* head blight. *FEMS Yeast Res.* **7** 449–458

Frohlich A, Buddrus-Schiemann K, Durner J, Hartmann A and von Rad U 2012 Response of barley to root colonization by *Pseudomonas* sp. DSMZ 13134 under laboratory, greenhouse, and field conditions. *J. Plant Interact.* **7** 1–9

Fu Q, Liu C, Ding N, Lin Y and Guo B 2010 Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium *Pseudomonas* sp. DW1 on growth of eggplant (*Solanum melongena* L.) seedlings under salt stress. *Agric. Water Manag.* **97** 1994–2000

Galinski EA and Trüper HG 1994 Microbial behaviour in salt-stressed ecosystems. *FEMS Microbiol. Rev.* **15** 95–108

Galvão TC, De Lorenzo V and Cánovas D 2006 Uncoupling of choline -O- sulphate utilization from osmoprotection in *Pseudomonas putida*. *Mol. Microbiol.* **62** 1643–1654

Guan N, Li J, Shin H-d, Du G, Chen J and Liu L 2017 Microbial response to environmental stresses: from fundamental mechanisms to practical applications. *Appl. Microbiol. Biotechnol.* **101** 3991–4008

Haas D and Defago G 2005 Biological control of soil-borne pathogens by *fluorescent pseudomonads*. *Nat. Rev. Microbiol.* **3** 307–319

Huang W and Wilks A 2017 A rapid seamless method for gene knockout in *Pseudomonas aeruginosa*. *BMC Microbiol.* **17** 1–8

Kempf B and Bremer E 1998 Stress responses of *Bacillus subtilis* to high osmolarity environments: Uptake and synthesis of osmoprotectants. *J. Biosci.* **23** 447–455

Kets EPW, de Bont JAM and Heipieper HJ 1996 Physiological response of *Pseudomonas putida* S12 subjected to reduced water activity. *FEMS Microbiol. Lett.* **139** 133–137

Koga T, Katagiri T, Hori H and Takumi K 2002 Alkaline adaptation induces cross-protection against some environmental stresses and morphological change in *Vibrio parahaemolyticus*. *Microbiol. Res.* **157** 249–255

Koga T and Takumi K 1995 Nutrient starvation induces cross protection against heat, osmotic, or H_2O_2 challenge in *Vibrio parahaemolyticus*. *Microbiol. Immunol.* **39** 213–215

Lamark T, Kaasen I, Eshoo MW, Falkenberg P, McDougall J and Strom AR 1991 DNA sequence and analysis of the bet genes encoding the osmoregulatory choline—glycine betaine pathway of *Escherichia coli*. *Mol. Microbiol.* **5** 1049–1064

Landfald B and Strm AR 1986 Choline–glycine betaine pathway confers a high level of osmotic tolerance in *Escherichia coli*. *J. Bacteriol.* **165** 849–855

Rudulier DL, Strom AR, Dandekar A, Smith L and Valentine R 1984 Molecular biology of osmoregulation. *Science* **224** 1064–1068

Li X, Gu G, Chen W, Gao L, Wu X and Zhang L 2018 The outer membrane protein OprF and the sigma factor SigX regulate antibiotic production in *Pseudomonas fluorescens* 2P24. *Microbiol. Res.* **206** 159–167

Liu J, Wisniewski M, Droby S, Norelli J, Hershkovitz V, Tia S and Farrell R 2012 Increase in antioxidant gene

transcripts, stress tolerance and biocontrol efficacy of *Candida oleophila* following sublethal oxidative stress exposure. *FEMS Microbiol. Ecol.* **80** 578–590

Lou H, Wang X, Chen J, Wang B and Wang W 2018 Transcriptomic response of *Ralstonia solanacearum* to antimicrobial *Pseudomonas fluorescens* SN15-2 metabolites. *Can. J. Microbiol.* **64** 816–825

Lugtenberg B and Kamilova F 2009 Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* **63** 541–556

Ma Y, Wang Q, Gao X and Zhang Y 2017 Biosynthesis and uptake of glycine betaine as cold-stress response to low temperature in fish pathogen *Vibrio anguillarum*. *J. Microbiol.* **55** 44–55

Rodríguez-González O, Walkling-Ribeiro M, Jayaram S and Griffiths MW 2011 Cross-protective effects of temperature, pH, and osmotic and starvation stresses in *Escherichia coli* O157: H7 subjected to pulsed electric fields in milk. *Int. Dairy J.* **21** 953–962

Sage AE, Vasil AI and Vasil ML 1997 Molecular characterization of mutants affected in the osmoprotectant-dependent induction of phospholipase C in *Pseudomonas aeruginosa* PAO1. *Mol. Microbiol.* **23** 43–56

Sand M, Stahl J, Waclawska I, Ziegler C and Averhoff B 2014 Identification of an osmo-dependent and an osmo-independent choline transporter in *Acinetobacter baylyi*: implications in osmostress protection and metabolic adaptation. *Environ. Microbiol.* **16** 1490–1502

Velasco-García R, Mújica-Jiménez C, Mendoza-Hernández G and Muñoz-Clares RA 1999 Rapid purification and properties of betaine aldehyde dehydrogenase from *Pseudomonas aeruginosa*. *J. Bacteriol.* **181** 1292–1300

Velasco-García R, Villalobos MA, Ramírez-Romero MA, Mujica-Jimenez C, Iturriaga G and Munoz-Clares RA 2006 Betaine aldehyde dehydrogenase from *Pseudomonas aeruginosa*: cloning, over-expression in *Escherichia coli*, and regulation by choline and salt. *Arch. Microbiol.* **185** 14–22

Vyrides I and Stuckey DC 2017 Compatible solute addition to biological systems treating waste/wastewater to counteract osmotic and other environmental stresses: a review. *Crit. Rev. Biotechnol.* **37** 865–879

Wang Y, Wang P, Xia J, Yu T, Lou B, Wang J and Zheng X 2010 Effect of water activity on stress tolerance and biocontrol activity in antagonistic yeast *Rhodosporidium paludigenum*. *Int. J. Food Microbiol.* **143** 103–108

Wargo MJ 2013 Choline catabolism to glycine betaine contributes to *Pseudomonas aeruginosa* survival during murine lung infection. *PLoS One* **8** e56850

Wargo MJ and Matthew J 2013 Homeostasis and catabolism of choline and glycine betaine: lessons from *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol.* **79** 2112–2120

Whatmore AM and Reed RH 1990 Determination of turgor pressure in *Bacillus subtilis*: a possible role for K^+ in turgor regulation. *J. Gen. Microbiol.* **136** 2521–2526

Corresponding editor: Ashis Kumar Nandi