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Poly-γ-glutamic acid production of *Bacillus subtilis* (natto) in the absence of DegQ: A gain-of-function mutation in *yabJ* gene

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Poly-γ-glutamic acid (γPGA) production by *Bacillus subtilis* is regulated by the quorum sensing system where DegQ transmits the cell density signal to a DNA-binding protein DegU. A mutation suppressing the γPGA-negative phenotype of *degQ* gene knock-out mutant (Δ*degQ*) was identified through whole genome sequencing. The mutation conferred an amino acid substitution of Ser103 to phenylalanine (S103F) in *yabJ* that belongs to the highly conserved YjgF/YER057c/UK114 family. Genetic experiments including LacZ-fusion assay of γPGA synthetic operon confirmed that the suppressor mutation (*yabJ*^{S103F}) was responsible for the recovery of γPGA production. The *yabJ* itself was not essential for the γPGA production and the mutant allele enabled γPGA production of the Δ*degQ* strain even in the presence of wild type *yabJ*. Thus, *yabJ*^{S103F} was a dominant positive allele. *degU-lacZ* fusion gene was hyper-expressed in cells carrying the *yabJ*^{S103F}, but disruption of *yabJ* did not affect the transcription level of the *degU-lacZ*. These observations suggested that YabJ acquired a function to stimulate expression of *degU* by the S103F mutation which is involved in the regulation of γPGA synthesis.

[**Key words:** YjgF/YER057c/UK114; YabJ; Gain-of-function mutation; *Bacillus subtilis*; Poly-γ-glutamic acid; DegQ]

Extracellular poly-γ-glutamic acid (γPGA) is mainly found in the genus *Bacillus* (1,2). It acts as a protective barrier against bacteriophages and host immune systems and also as a nutrient reservoir (1–5). In *Bacillus subtilis* the quorum sensing components ComQ, ComX, ComP, and ComA are essential for γPGA production in the stationary phase (6–9). A DNA-binding transcription factor, ComA, the cognate response regulator of the ComP–ComA two-component system, induces the expression of many genes in the stationary phase when it is activated by phosphorylation (10). Among the genes regulated by ComA, *degQ* gene plays an essential role in γPGA production (6). DegQ transmits the cell density signal to another two-component system, DegS–DegU, which is directly involved in the expression of the γPGA synthetic operon (*pgsBCA*): DegQ stabilizes DegS and DegU in the phosphorylated form (6,11), and phosphorylated DegU (DegU–P_i) is known to bind to the promoter region of *pgsBCA* (12,13).

We previously screened mutations suppressing the loss of *degQ* function to identify regulatory genes for γPGA synthesis, and nine suppressor mutants that enabled γPGA production in the absence of *degQ* were obtained (6). Among them, six (group 1) had a different mutation in the HisKA_3 phosphoacceptor domain of DegS (amino acid substitutions M195I, R208Q, P245S, L248F, D249G, and D250N) (6). In vitro phosphorylation experiments with ^{32}P-labeled ATP revealed that, unlike wild type, these mutant DegS and DegU were stabilize in the phosphorylated state which accelerated the γPGA synthesis and that the DegS suppressor mutations mimicked the function of DegQ (6).

In the stationary phase, DegU and DegU–P_i regulate different genes (11,14–18). For example, DegU–P_i is essential for γPGA synthesis, whereas expression of genetic competence requires non-phosphorylated DegU. Thus, the genetic competence of group 1 mutants is far lower than that of the parental strain, while DegU–P_i dependent exoenzymes such as alkaline protease and γ-glutamylpeptidase are up-regulated (6).

The mutations of the remaining three suppressor mutants (group 2) were not identified in the previous study due to a lack of genetic markers of the strain used (19). The group 2 suppressor mutant strains, Δ*degQ sup4* (NAFM734), Δ*degQ sup6* (NAFM736), and Δ*degQ sup8* (NAFM738), showed a partial recovery of γPGA production and mutations were not linked to the *degS–degU* loci (6). Genetic competence of the group 2 mutants was still comparable to that of the parental strain Δ*degQ* (NAFM73). Their exoenzyme level was higher than that of the parental Δ*degQ* strain but lower than that of group 1 mutants (6). The group 1 and the group 2 suppressor mutants both required *degU* to produce γPGA (6).

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In the present study we employed a whole genome resequencing approach to identify the group 2 suppressor mutations. Comparing the genome sequence of Δ*degQ sup4* (NAFM734), Δ*degQ sup6* (NAFM736), Δ*degQ sup8* (NAFM738), and the parental Δ*degQ* strain (NAFM73), it was found that the three group 2 mutants had a common nucleotide substitution in *yabJ* gene.

YabJ protein belongs to the YjgF/YER057c/UK114 protein family (20). Reportedly, the *yabJ* of *B. subtilis* is involved in repression of the purine biosynthetic gene *purA* (21) and currently annotated as the reactive intermediate deaminase A that hydrolyzes enamine and imine metabolic intermediates produced in the branched-chain amino acid biosynthesis pathway (22–26).

In the present study, involvement of *yabJ* gene in γPGA synthesis by *B. subtilis* was investigated. Repression of *purA* by adenine supply to the media was also examined in strains carrying the suppressor allele of *yabJ*.

MATERIALS AND METHODS

Bacterial strains and media *B. subtilis* (natto) strains used in this study are listed in Table 1. *B. subtilis* and *Escherichia coli* DH5α cells were transformed and selected on LB agar plates with appropriate antibiotics as described previously (6). GSP medium containing 1.5% (w/v) glucose, 1.5% (w/v) sodium L-glutamate, and 1.5% (w/v) phytone peptone (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was used for γPGA production. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to media at 1 mM, if necessary.

Whole genome sequencing The entire nucleotide sequence of genomic DNA was obtained using Illumina technology (Genome Analyzer IIx; Illumina, San Diego, CA, USA). Genomic DNA libraries for the parental *ΔdegQ* strain (NAFM73) and the suppressor mutant strains, *ΔdegQ sup4* (NAFM734), *ΔdegQ sup6* (NAFM736), and *ΔdegQ sup8* (NAFM 738) were individually prepared using Covaris instruments (Covaris Inc., Woburn, MA, USA), Paired-End DNA Sample Prep Kits, and Multiplexing Sample Preparation Oligonucleotide Kits (Illumina). The amplified libraries were sequenced as 58-bp paired-end short reads, assembled, and mapped on the published genome sequence of *B. subtilis* (natto) strain with the Burrows-Wheeler alignment program, as described previously (27,28). Variant calls and SNP/Indel detection were performed for each strain separately using the Genome Analysis Toolkit version 3.2-2 (https://software.broadinstitute.org/gatk/), as described previously (28).

TABLE 1. Strains used in this study.

Strain or plasmid	Genotype and description	Reference or source
B. subtilis (natto)		
NAFM5[a]	WT, natto fermenting strain, *bio*, γPGA -positive	3
NAFM73	NAFM5 derivative; Δ*degQ*, γPGA-negative	6
NAFM734	NAFM73 derivative; *sup4*	6
NAFM736	NAFM73 derivative; *sup6*	6
NAFM738	NAFM73 derivative; *sup8*	6
NAFM79	*amyE*::*pgsB-lacZ*	7
NAFM227	Δ*degQ*, Δ*yabJ*	This study
NAFM233	Δ*degQ*, *aprE*::P_{yabJ}-*yabJ*	This study
NAFM234	Δ*degQ*, *aprE*::P_{yabJ}-*yabJ*^{S103F}	This study
NAFM235	Δ*degQ*, Δ*yabJ*, *aprE*::P_{yabJ}-*yabJ*	This study
NAFM236	Δ*degQ*, Δ*yabJ*, *aprE*::P_{yabJ}-*yabJ*^{S103F}	This study
NAFM237	Δ*degQ*, *amyE*::*pgsB-lacZ*	This study
NAFM238	Δ*degQ*, Δ*yabJ*, *amyE*::*pgsB-lacZ*	This study
NAFM241	Δ*degQ*, Δ*yabJ*, *aprE*::P_{yabJ}-*yabJ*, *amyE*::*pgsB -lacZ*	This study
NAFM242	Δ*degQ*, Δ*yabJ*, *aprE*::P_{yabJ}-*yabJ*^{S103F}, *amyE* ::*pgsB-lacZ*	This study
NAFM243	NAFM734 derivative, *amyE*::*pgsB-lacZ*	This study
NAFM246	Δ*yabJ*	This study
NAFM249	Δ*yabJ*, *amyE*::*pgsB-lacZ*	This study
NAFM250	Δ*yabJ*, *aprE*::P_{yabJ}-*yabJ*	This study
NAFM251	Δ*yabJ*, *aprE*::P_{yabJ}-*yabJ*^{S103F}	This study
NAFM254	Δ*degQ*, Δ*yabJ*, *aprE*::P_{spac}-*yabJ*^{S103F}	This study
NAFM255	Δ*degQ*, Δ*yabJ*, *aprE*::P_{spac}-*yabJ*	This study
NAFM296	NAFM5 derivative, *amyE*::*degU-lacZ*	This study
NAFM295	NAFM73 derivative, *amyE*::*degU-lacZ*	This study
NAFM297	NAFM246 derivative, *amyE*::*degU-lacZ*	This study
NAFM299	NAFM736 derivative, *amyE*::*degU-lacZ*	This study
NAFM300	NAFM227 derivative, *amyE*::*degU-lacZ*	This study
NAFM305	NAFM254 derivative, *amyE*::*degU-lacZ*	This study

[a] *B. subtilis* (natto) NAFM5 derived from a commercial natto-fermenting strain Miyagino.

Construction of *yabJ* mutant strains The kanamycin-resistance cassette excised from pDG780 (29) as SmaI-HincII fragment was inserted between the 5′ *yabJ* fragment (from nucleotide [nt] −620 to +110, translation start point = nt +1) and the 3′ *yabJ* fragment (from nt +125 to +790) that were tandemly cloned into the multiple cloning site of pBluescript (SK+). This plasmid was linearized with ScaI digestion and used to disrupt the *yabJ* gene. The correct disruption of *yabJ* by double-crossover recombination was verified by Southern blot analysis.

Wild type *yabJ* and *yabJ*^{S103F} were introduced to the *aprE* loci using the pAPNC213 plasmid (30): The *yabJ* fragment containing its own promoter region (P_{yabJ}) was amplified using the following primers: 5′-GAATTCGTCAAACGTACTCAT-TATTGATGAC-3′ (corresponding to nt −267 to −242) and 5′-TAGGATATAGTTACA-CAATTAGG-3′ (complementary to nt +452 to +475) and the genomic DNA obtained from strains, Δ*degQ* (NAFM73) and Δ*degQ sup4* (NAFM734). The amplified fragments were inserted into pAPNC213 vector at the SmaI site. Plasmids created were digested with ScaI to transform *B. subtilis* cells by double-crossover recombination at the *aprE* locus (30). To construct strains that had *yabJ* under an IPTG inducible promoter (P_{spac}), the *yabJ* fragment (−21 to +475) was amplified using the oligonucleotide primers 5′-AAAGAATGGAGAGACAGAATCATG-3′ (corresponding to nt −21 to +3) and 5′-TAGGATATAGTTACACAATTAGG-3′ (complementary to nt +452 to +475), and then introduced into the *aprE* locus using pAPNC213, as described above.

Reporter assay with *lacZ* fusion The *pgsB–lacZ* fusion of the γPGA synthetic operon was previously constructed (7). To construct the *purA–lacZ* fusion gene, the promoter region of adenylosuccinate synthase gene (*purA*) was amplified using the primers 5′-TAGATCTCTCATGCCGATTATCATTTGGAATTC-3′ and 5′-TAGATCTGTTAACCTTTCAAAACGATTCTATTC-3′ (BglII sites are underlined) and then ligated into the BamHI site of pDG1661 (31). To construct the *degU–lacZ* fusion gene, the promoter region of *degU* was amplified with a primer pair 5′-GCATGAATTCGGCCTGATTCCAACTTTAA-3′ and 5′-GCATAAGCTTCTGATGGTCGTCGATAAT-3′ (EcoRI and HindIII sites are underlined) and introduced to pDG6013 as described previously (7). The *lacZ*-fusion genes were introduced to *amyE* locus and the β-galactosidase activity was measured as described previously (7).

Western blot analysis Cells were inoculated into GSP liquid medium, grown at 37°C with shaking (150 rpm) and harvested in the stationary phase ($OD_{600} \cong 1.5$). The cells were then suspended in STE buffer (100 mM NaCl, 10 mM Tris–HCl [pH 7.5], 1 mM EDTA) containing lysozyme (50 μg ml^{-1}) and incubated at 37°C for 30 min. The cell lysate was mixed with SDS (final concentration 1%) and heated at 95°C for 10 min. Cell debris was removed by centrifugation (13,800 ×*g*, 10 min, RT). For each sample, 50 μg of protein was subjected to SDS-PAGE (Mini-Protean TGX, Bio-Rad, Hercules, CA, USA). The fractionated proteins were transferred to a polyvinylidene fluoride membrane (Fluoro Trans, 0.2 μm; Pall Corporation, Port Washington, NY, USA), and protein bands that reacted with anti-DegU antiserum were detected using an ECL Prime Western blocking kit (GE Healthcare, Little Chalfont, UK) and X-ray films (RX-U; FujiFilm, Tokyo, Japan) according to the manufacturer's instructions. Recombinant DegU protein was expressed in *E. coli* cells and purified as described previously (6) to immunize rabbits (Protein Purify Co., Ltd., Isesaki, Japan).

Others KOD polymerase (KOD-plus, Toyobo Co., Osaka, Japan) was used for PCR amplification of DNA fragments according to the manufacturer's instructions. Nucleotide sequences of amplified DNA fragments subcloned in plasmids were verified using an ABI 310 DNA sequencer and the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). γPGA produced to medium was purified and subjected to agarose gel electrophoresis followed by staining with methylene blue as described (3). The swarming ability of *B. subtilis* cells was assessed as described previously (32). CLUSTALW and ESPript were used for multiple amino acid sequence alignments (33,34). Chemicals not mentioned were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

RESULTS

Suppressor mutants had a common mutation in the *yabJ* gene The suppressor strains Δ*degQ sup4* (NAFM734), Δ*degQ sup6* (NAFM736), and Δ*degQ sup8* (NAFM738) were subjected to whole genome sequencing analysis using Illumina-based technology. Variations were found in the assembled sequence reads mapped to the reference genome: NAM734 and NAFM736 had four mutations and NAFM738 had three mutations (Supplemental Table S1). The read depth at the mutation points was 49–381. Mutation points of the three suppressor mutants differed each other, which implied that suppressor mutants were

not siblings. A total of seven mutations were found in ORFs of *yabJ*, *yloB*, *comEB*, *phoA*, *yqjV*, *ybgE*, *yczC* and two were found in the intergenic region (Supplemental Table S1). Among them, only one mutation (C to T change in the coding region of the *yabJ* gene) was common to all the three mutants. This mutation conferred serine to phenylalanine substitution at the residue 103 (S103F mutation). The read depth at the S103F mutation was at least 82 (Supplemental Table S1). The S103F mutation of *yabJ* was further examined by genetic experiments.

The *yabJ* ^{S103F} allele dominantly suppressed the Δ*degQ* phenotype *B. subtilis* (natto) cells actively produce extracellular γPGA and have a mucoid phenotype which is clearly distinguished from γPGA-negative cells. Disruption of *degQ* abolishes γPGA production by hampering the cell density signal transduction that is essential for expression of the γPGA synthetic operon *pgsBCA* (6,12,35). We introduced wild type and the mutant *yabJ* genes at the *aprE* locus of the Δ*degQ* strain (NAFM73), creating strains Δ*degQ* P_{yabJ}-*yabJ* (NAFM233) and Δ*degQ* P_{yabJ}-*yabJ*^{S103F} (NAFM234). Production of γPGA was observed only when the S103F mutant *yabJ* was introduced. The Δ*degQ* P_{yabJ}-*yabJ*^{S103F} strain has the authentic wild type *yabJ*, indicating that the suppressor allele *yabJ*^{S103F} is dominant active. We created strains whose authentic *yabJ* was disrupted and the *yabJ* ^{S103F} was inducible (Δ*degQ* Δ*yabJ* P_{spac}-*yabJ*^{S103F}, NAFM254). This strain produced γPGA only when 1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to the medium (Fig. 1, lanes 11 and 12). The control strain, which had wild type *yabJ* instead of the *yabJ*^{S103F}, did not produce γPGA even in the presence of IPTG (Fig. 1, lanes 17 and 18).

Expression of the γPGA synthetic operon was also examined in the suppressor mutants. PgsB-LacZ activity was not detected in the Δ*degQ* strain (NAFM73), and it was recovered when *yabJ*^{S103F} was expressed (Table 2). The *yabJ* itself was not required for the *pgsB-lacZ* expression (Table 2). However, the amount of γPGA produced by Δ*yabJ* strain (NAFM246) was less than the wild type (Fig. 1). Both the wild type *yabJ* and the suppressor allele *yabJ*^{S103F} complemented this defect (Fig. 1, lanes 15 and 16).

TABLE 2. PgsB-LacZ fusion activities.

Strain and genotype	PgsB-LacZ β-galactosidase activity (Miller U)[a]
NAFM79, WT	143 ± 5
NAFM237, Δ*degQ*	<5
NAFM243, Δ*degQ sup4*	12 ± 2
NAFM249, Δ*yabJ*	99 ± 1
NAFM238, Δ*degQ* Δ*yabJ*	<5
NAFM241, Δ*degQ* Δ*yabJ aprE*::P_{yabJ}-*yabJ*	<5
NAFM242, Δ*degQ* Δ*yabJ aprE*::P_{yabJ}-*yabJ*^{S103F}	58 ± 6

Cells were grown in GSP medium for 24 h.
[a] Average values of 3 independent experiments are indicated with the standard deviation.

All the *yabJ*^{S103F} suppressor mutants created and the *yabJ* knockout mutant grew normally in LB and GSP liquid media.

Expression level of *degU* was increased in cells harboring the *yabJ* ^{S103F} allele The γPGA production lost by disrupting *degQ* was recovered by the suppressor mutation *yabJ*^{S103F}, but *degU* was still required for the recovery (6). In other words, the *yabJ*^{S103F} dominant allele was able to bypass the DegQ function, but not that of DegU. We examined DegU expression levels by monitoring *degU-lacZ* expression (Fig. 2). The *degU-lacZ* expression level was

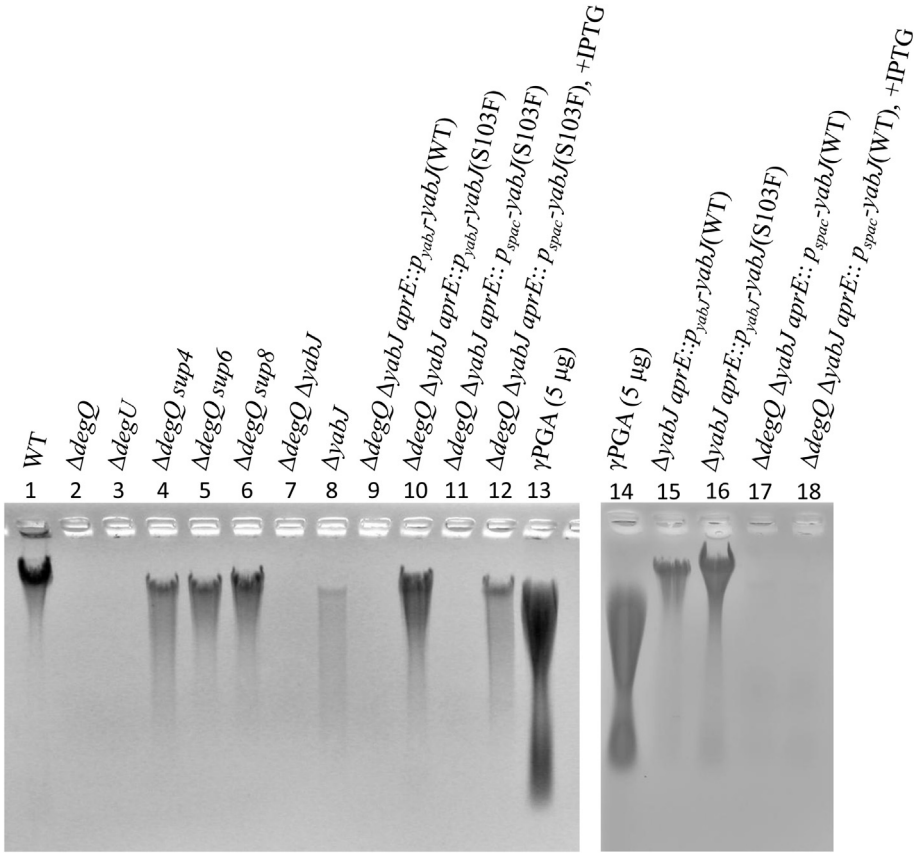

FIG. 1. γPGA production by wild type and mutants of *B. subtilis* (natto) strains. Cells were propagated in GSP medium for 24 h at 37°C and γPGA produced to medium was purified and subjected to agarose gel electrophoresis followed by staining with methylene blue as described previously (3). Genotypes of strains were indicated on the lane numbers.

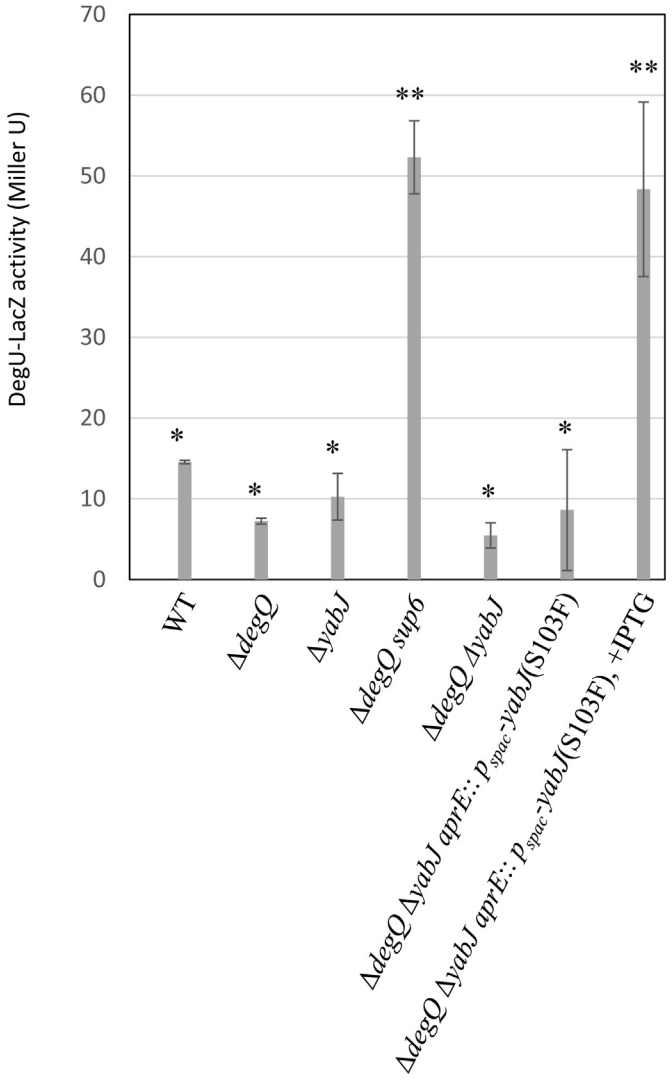

FIG. 2. Expression of *degU-lacZ* fusion gene in *yabJ* mutants. A portion (0.5 ml) of overnight preculture of *B. subtilis* cells carrying the *degU-lacZ* fusion gene was inoculated to LB medium (100 ml), and cells were propagated with vigorous shaking at 37°C for 8 h. The β-galactosidase activity of DegU-LacZ was measured as described in the Materials and methods section. Error bars indicate the standard deviation of mean values of biologically duplicated measurements. Genotype of the strains examined are indicated. Different symbols (asterisk and double asterisk) indicate significant difference between determined values ($p < 0.01$).

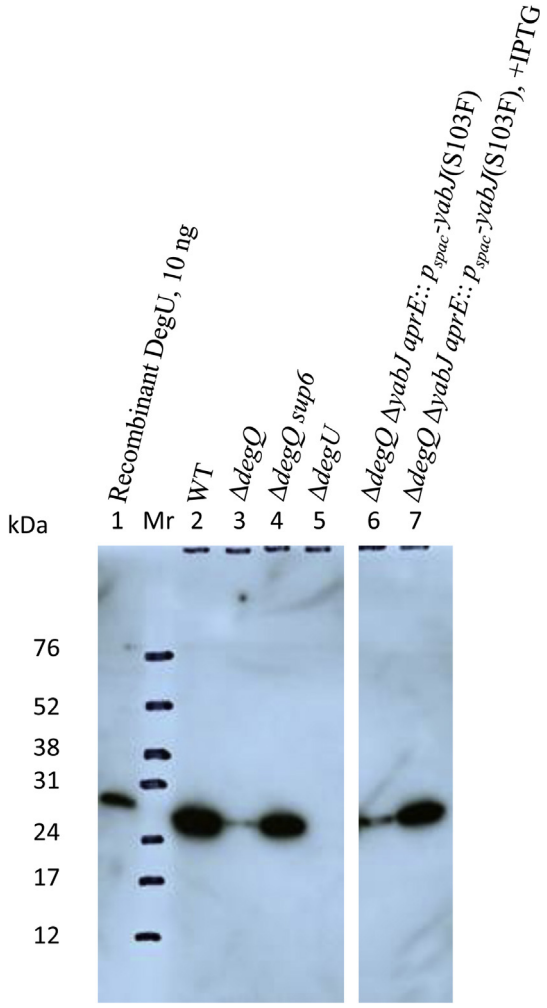

FIG. 3. Western blot analysis of DegU. Protein samples (50 μg) from *B. subtilis* (natto) cells were subjected to Western blot analysis with anti-DegU rabbit antiserum. Lane 1, control (recombinant DegU, 10 ng); Mr, molecular mass marker; lane 2, wild type (NAFM5); lane 3, Δ*degQ* (NAFM73); lane 4, Δ*degQ sup6* (NAFM736); lane 5, Δ*degU* (NAFM104); lane 6, Δ*degQ* Δ*yabJ aprE*::P_{spac}–$yabJ^{S103F}$ (NAFM254); lane 7, NAFM254 + IPTG.

higher in cells harboring the $yabJ^{S103F}$ than wild type and the parental Δ*degQ* strain. Indeed, the strain NAFM305 (Δ*degQ* Δ*yabJ* P_{spac}-$yabJ^{S103F}$) that carries the $yabJ^{S103F}$ under inducible promoter (P_{spac}) showed the high expression of *degU-lacZ* only when IPTG was added to the medium (Fig. 2). Disruption of *degQ* and *yabJ* did not significantly affect the *degU-lacZ* expression level (Fig. 2).

Cellular amount of DegU protein was further examined by Western blot analysis (Fig. 3). DegU, the DNA-binding transcriptional regulator of *pgsBCA*, was clearly detected in the wild type, but at the low level in Δ*degQ* strain (Fig. 3, lanes 2 and 3). Introduction of the $yabJ^{S103F}$ to the Δ*degQ* strain increased the amount of DegU to the level comparable to wild type (Fig. 3, lane 4). No band was observed for the negative control (Δ*degU* strain) (Fig. 3, lane 5), which indicated that the antiserum used was specific to DegU, at least in the conditions employed. Furthermore, the upregulation of DegU was observed for the strain Δ*degQ* Δ*yabJ aprE*::P_{spac}-$yabJ^{S103F}$ (NAFM254) only when IPTG was added to the medium (Fig. 3, lanes 6 and 7).

DISCUSSION

By whole genome resequencing, a mutation in *yabJ* (S103F mutation) was commonly found in genomes of the group 2 suppressor mutants that produces γPGA in the absence of DegQ (Supplemental Table S1). The three suppressor mutants, Δ*degQ sup4* (NAFM734), Δ*degQ sup6* (NAFM736), and Δ*degQ sup8* (NAFM738) which were subjected to the genome resequencing analyses, showed very similar phenotypes, including recovered γPGA production, level of genetic competence and exoenzyme production, and colony morphology (6). These results indicated that the S103F mutation of *yabJ* is responsible for their phenotype. We experimentally confirmed that $yabJ^{S103F}$ suppressed the loss of *degQ* function (Fig. 1, Table 2). Other mutations found in the

genome resequencing analysis including those in *comEB*, *phoA*, and *yczC* were assumed to have minimal influence on γPGA production (Supplemental Table S1).

The amino acid sequence around Ser103 is highly conserved in the YjgF/YER057c/UK114 family and includes Arg102, the putative catalytic residue for deamination (Fig. 4). The residues Ser103 and Arg102 are located in the cavity between the subunits of homo-trimeric YabJ (36). Metabolites carrying a carboxylate group are supposed to be incorporated into this cavity as putative substrates (37). Thus, the mutation occurred in a structurally important point that is open and accessible for substrates and other compounds (36).

It was found that the cellular amount of DegU was largely reduced by the disruption of *degQ* and it was recovered by the *yabJ*^{S103F} mutation (Fig. 3), which may explain the recovery of γPGA. The *yabJ*^{S103F} mutation appeared to enhance the DegU production at the transcriptional level (Fig. 2). The *degU* is a positively autoregulated gene (10). Therefore, actual stimulation of *degU* transcription by the *yabJ*^{S103F} could be much higher than that observed for the *lacZ*-fusion assay. The *degU-lacZ* expression level was not significantly affected by the disruption of *degQ* (Fig. 2). Thus, DegQ might be involved in post-translational DegU turnover. Collectively, the transcriptional upregulation of *degU* by YabJ^{S103F} was thought to overcome the instability of DegU in the cells lacking the *degQ*.

YabJ of *B. subtilis* belongs to the YjgF/YER057c/UK114 family. This family is highly conserved in all domains of cellular organism (20), but biological function of this family has been enigmatic although it is annotated as a deaminase (20,22,24,26,38–40).

In higher organisms, this family has been reported to include a molecular chaperone *in vitro* (41), an endoribonuclease (39,42), a calpain activator (43), a translation inhibitor (44), and a plant antiviral protein (45). Knockout mutants of YjgF/YER057c/UK114 family gene were created in microbes including *Lactococcus lactis* subsp. *lactis* (46), *B. subtilis* (21,47,48), *Saccharomyces cerevisiae* (49,50), *Schizosaccharomyces pombe* (51), *E. coli* and *Salmonella enterica* serovar Typhimurium (25,52–55), and *Staphylococcus aureus* (56). The phenotype of these null mutants varies depending on the species studied. Deletion of N-terminal 39-amino-acid of *yabJ* in *B. subtilis* disturbed the repression of purine biosynthesis by adenine supplementation (21,48). In case of *E. coli* K12 Δ*rutC* mutant is not able to utilize uridine as the sole nitrogen source (54). The *E. coli* BL21 Δ*ridA* is more sensitive to HClO than the wild type (38). Yeast *Δmmf1* cells show a lack of the transaminase activity for isoleucine and lose mitochondrial genome on glucose-containing media, which is recoverable by the addition of isoleucine to the medium (49,50). *S. enterica* Typhimurium *ΔridA* mutants require isoleucine for growth in pyruvate medium and glucose-serine medium (52). In *S. aureus*, the bicistronic *yabJ-spoVG* operon that is positively regulated by sigma B was examined for the virulence development, but no obvious phenotype was reported for the *ΔyabJ* mutant (56). *L. lactis* subsp. *lactis ΔaldR* exhibits reduced growth in the absence of isoleucine and appears to have a defect in the degradation of metabolic intermediates in the isoleucine biosynthetic pathway (46). These lines of experimental evidence obtained by knockout mutants suggested that YjgF/YER057c/UK114 family is involved in the detoxification of enamine/imines metabolic intermediates (22). However, RidA of *E. coli* BL21 and *S. aureus* appears to perform a molecular chaperon activity when it is modified by *N*-chlorination (38,40).

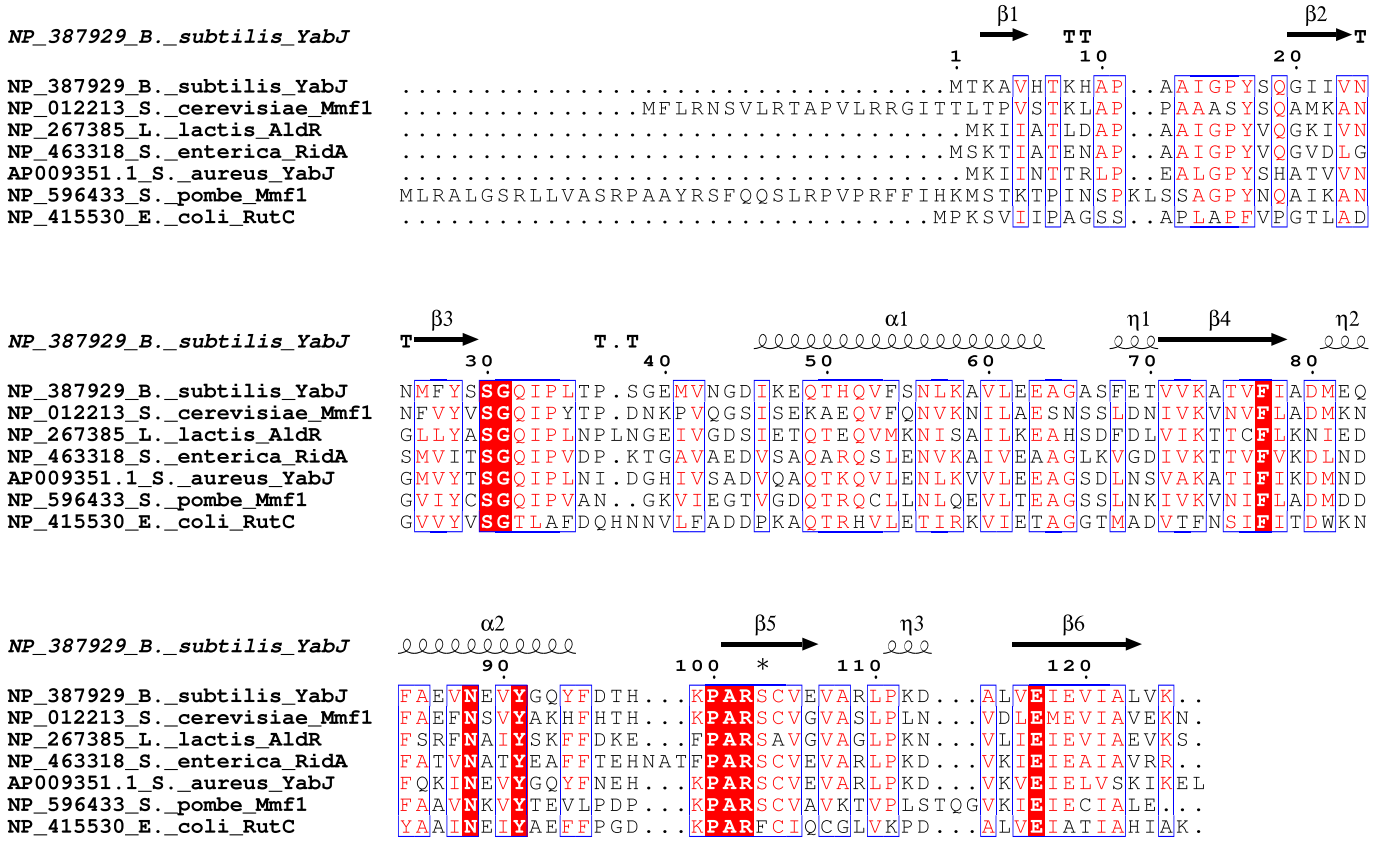

FIG. 4. Alignment of amino acid sequences of YjgF/YER057c/UK114 family proteins of bacteria and yeast. Amino acid sequences of YabJ (*B. subtilis*), RidA (*S. enterica*), AldR (*L. lactis*), Mmf1 (*S. cerevisiae* and *S. pombe*), RutC (*E. coli*), and YabJ (*S. aureus*) were aligned by CLUSTALW (33). Graphic presentation was performed by ESPript (34). The secondary structures of the trimer YabJ is shown above aligned amino acid sequences. Asterisks indicate the serine residue that was mutated to phenylalanine in the suppressor mutants.

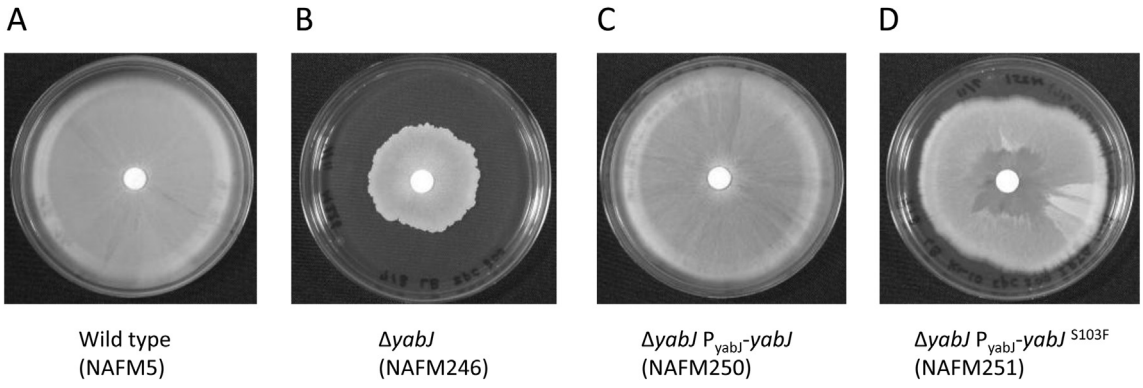

FIG. 5. Swarming motility of Δ*yabJ* mutant cells. Cells were grown in LB liquid medium overnight at 37°C with vigorous shaking. A portion of cell culture (5 μl) was put on a sterile filer paper placed in the center of LB soft agar (0.8%) plates and incubated for 24 h at 37°C. (A) Wild type; (B) Δ*yabJ*; (C) Δ*yabJ aprE*::P_{yabJ}-*yabJ*; (D) Δ*yabJ aprE*::P_{yabJ}-*yabJ* ^{S103F}. The diameter of the agar plate used was 9 cm.

YabJ might be involved in the γPGA synthesis in response to stresses including those of enamine/imine toxic metabolic intermediates and high salt conditions. Indeed, some *Bacillus* produce γPGA in response to environmental stress. For example, γPGA synthesis by *B. subtilis*, *Bacillus megaterium*, and *Bacillus licheniformis* is salt-inducible (57–59). Reactive oxygen species (e.g., H_2O_2) and alkaline pH stress also enhance the γPGA synthesis (60,61).

Transcriptional regulation of *purA* (the adenylosuccinate synthetase gene) by adenine and guanosine is important to balance the synthesis of AMP and GMP from IMP (21). The S103F mutation in *yabJ* had no effect to the regulation of *purA* by supplementation of adenine and guanosine (Table S2). Probably *yabJ* differently regulates the *degU* and *purA* responding to variable environmental signals.

The PgsB-LacZ level of Δ*yabJ* strain (NAFM246) was comparable to that of the wild type (Table 2), but the actual amount of γPGA produced by the Δ*yabJ* strain was less than that produced by the wild type (Fig. 1). *B. subtilis* (natto) cells show swarming motility and colonies of cells those actively swarm spread rapidly on agar plate (31,62). The Δ*yabJ* strain was defective in the swarming motility (Fig. 5). The swarming motility and the γPGA production are genetically related and some swarming-negative mutants are also γPGA-negative (32,63). The defective ability to swarm might negatively affect the γPGA production. This awaits further experimental elucidation.

In conclusion, we identified a gain-of-function mutation of *yabJ* gene (*yabJ*^{S103F}) by screening mutants that suppressed the γPGA-negative phenotype of *B. subtilis* (natto) cells that have a defect in cell density signal transduction. The expression level of *degU* which directly regulates the γPGA-synthetic operon was largely increased in cells carrying the *yabJ* ^{S103F}. The upregulation of DegU level by the *yabJ*^{S103F} may explain the recovery of γPGA.

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