One of the mechanisms by which bacteria acquire multidrug resistance (MDR) is by increasing the expression of multidrug efflux transporters (MDTs). In a spontaneous mutant of Bacillus subtilis isolated in the presence of an inhibitory concentration of rhodamine 6G, Bmr is overexpressed via intrachromosomal amplification of the bmr locus (17). Increased transcription also results in overexpression of the MDT. Puromycin- and lincomycin-resistant (PLR) mutants, isolated by growing B. subtilis 168 in the presence of high concentrations of puromycin and lincomycin, display increased expression of LmrB because of the inactivation of a negative transcriptional regulator, LmrA, or because of mutations in the 5′ untranslated region (5′ UTR), which is a binding site for the LmrA protein (16, 25). Expression of Bmr and Blt is regulated by the product of adjacent genes encoding the transcriptional activators BmrR and BltR, respectively (1, 2). In Escherichia coli, overexpression of the transcriptional regulators of a two-component regulatory system up-regulates the expression of several MDTs, resulting in an MDR phenotype (7, 12, 13).

The steady-state level of mRNA in a cell is a function of its rate of synthesis and degradation. An increase in the stability of MDT mRNA may result in a higher level of mRNA. Therefore, it is reasonable to expect that cells may acquire an MDR phenotype by stabilization of MDT mRNA. However, such an example has not been reported to date. In the present report, we describe the first example of increased stability of MDT mRNA (bmr3 mRNA) resulting in an MDR phenotype in B. subtilis.

Spontaneous mutants (PR mutants) isolated in the presence of high concentrations of puromycin showed an MDR phenotype. Spontaneous mutants isolated by growing B. subtilis 168 in Luria-Bertani (LB) medium containing high concentrations of puromycin (100 μg/ml) were found to be divided into two groups. One group of mutants showed high levels of resistance to lincomycin as well as puromycin (PLR mutants), whereas the other group (PR mutants) showed levels of lincomycin resistance similar to that of strain 168. Both groups of mutants expressed an MDR phenotype, although their drug specificities were somewhat different. It has previously been reported that PLR mutants have mutations in the lmrAB operon (16).

In the present study, a PR mutant was further characterized. Resistance to various drugs was assayed as described previously (20). As shown in Table 1, the PR mutant showed increased resistance to puromycin, norfloxacin, tosufloxacin, daunomycin, and ethidium bromide. Resistance levels to levofloxacin, lincomycin, tetraphenylphosphonium chloride, and rhodamine 6G were not significantly increased. The results indicated the possibility that expression of one of the MDTs was increased in the PR mutant.

Identification of the gene responsible for the MDR phenotype. In order to identify the gene responsible for the MDR phenotype, the PR mutant was transformed with pHV1248 (23). At a temperature inhibiting plasmid replication, a chloramphenicol resistance gene (cat) was inserted at random positions on the chromosome of the PR mutant by transposition. If insertion into the gene responsible for the MDR phenotype occurs, the cells become sensitive to puromycin. Therefore, we selected the chloramphenicol-resistant and puromycin-sensitive clones. One of the clones obtained (PR mutant with a cat insertion) showed wild-type levels of resistance to puromycin, norfloxacin, tosufloxacin, and daunomycin (Table 1), indicating that insertion of the cat gene inactivated the gene responsible for the MDR phenotype of the PR mutant.

Southern hybridization analysis showed that a 5.0-kb HindII fragment of chromosomal DNA from the PR mutant with a cat insertion contained the cat gene and flanking regions. This fragment was cloned into pUC18, and the partial DNA sequence of the insert was determined. The sequences obtained matched those of the bmr3 gene which encodes a third MDT of B. subtilis belonging to the major facilitator superfamily (21). A 1.8-kb fragment containing the bmr3 locus was amplified with High Fidelity Platinum Taq (Inverogen) with chromosomal DNA from the PR mutant used as a template and transformed into strain 168. The transformant selected by puromycin resistance showed the same MDR phenotype as the original PR
TABLE 1. MDR phenotypes of the PR mutant

| Drug         | Relative resistance<sup>a</sup> | PR mutant | PR:cat | TF-5‘ UTR |
|--------------|---------------------------------|-----------|--------|-----------|
| Puromycin    | 54.1                            | 0.9       | 52.6   |           |
| Norfloxacin  | 8.7                             | 1.1       | 9.0    |           |
| Tosufloxacin | 5.4                             | 1.0       | 5.4    |           |
| Daunomycin   | 2.7                             | 0.9       | 2.9    |           |
| Ethidium bromide | 2.6                          |           | 2.5    |           |
| Levofloxacin | 1.3                             |           | 1.4    |           |
| Lincomycin   | 1.4                             |           | 1.4    |           |
| TPP<sup>b</sup> | 1.2                          |           | 1.2    |           |
| Rhodamine 6G | 1.1                             |           | 1.1    |           |

<sup>a</sup> Relative resistance was determined by dividing the 50% inhibitory concentration (IC<sub>50</sub>; the drug concentration required to inhibit growth by 50%) of various mutants by the IC<sub>50</sub> of strain 168. PR:cat, PR mutant with cat inserted; TF-5‘ UTR, 5‘ UTR transformants (see text for details).

<sup>b</sup> TPP, tetraphenylphosphonium chloride.

mutant. These results indicate that the bmr3 gene was responsible for the MDR phenotype of the PR mutant.

**Mutations found in the bmr3 gene.** The nucleotide sequence of a 1.8-kb fragment of the PR mutant containing the bmr3 gene was determined and compared to that of strain 168, which has been previously reported (21). As members of a European-Japanese cooperative B. subtilis genome sequencing project, Yamane et al. determined the nucleotide sequence for this region (accession no. D50453). In a comparison of the sequences of the 1.8-kb region containing the bmr3 gene determined by us (accession no. D50098) and by Yamane et al., many discrepancies (at more than 20 sites) were found. For example, Yamane et al. reported that the bmr3 gene encodes a protein containing 315 amino acids (24), whereas based on our data, it encodes a protein containing 536 amino acids (21). We confirmed that our sequence is correct at each discrepant site, and consequently our sequence data were adopted for this study.

As shown in Fig. 1, two nucleotide changes were found in the 5‘ UTR. One change was C to T at the –18 position and the other was T to A at the +4 position, both relative to the initiation site of transcription (see below). Two amino acid changes, A274T and A285E, which are located in the 9th of 14 transmembrane segments of the Bmr3 protein, were also found. In order to determine whether these two amino acid changes affect the MDR phenotype of the PR mutant, a 0.9-kb fragment containing the 5‘ UTR and the coding region up to the 67th amino acid were amplified with High Fidelity Platinum Taq (Invitrogen), with chromosomal DNA from the PR mutant used as a template, and transformed into strain 168 cells. The transformants showed the same MDR phenotype as the original PR mutant (Table 1). No difference in drug specificity between the transformants and the original PR mutant was determined by us (accession no. D50098) and by Yamane et al., sequences of the 1.8-kb region containing the bmr3 gene resulted in the MDR phenotype and that the two amino acid changes had no effect on the drug specificity of the Bmr3 efflux protein.

### bmr3 mRNA

**bmr3 mRNA increased more than 20-fold in the PR mutant.** In order to examine whether the expression of the bmr3 gene increased at a transcriptional level, Northern hybridization analysis was carried out with total RNA isolated from strain 168 and PR mutant cells as described previously (19). In strain 168, the bmr3 gene was transcribed as a 1.6-kb monocistronic mRNA whose expression level was dependent on growth phase: high level in early log phase and low level in late log phase (21). In the PR mutant, the cellular level of bmr3 mRNA increased about 24-fold in early-log-phase cells (Fig. 2). In late-log-phase cells, the cellular level of bmr3 mRNA decreased in the PR mutant. However, the difference between levels in early and late log phase was only 3.5-fold, compared to 20-fold in the wild-type strain. No experimental data have been obtained to explain this observation.

The primer extension analysis was carried out to determine the start site of the transcription. Total RNA (40 μg) and 2 pmol of a rhodamine X-isothiocyanate (XRITC)-labeled primer (5‘-XRITC-CAGGACCCAAATTTTGGAAAGCC) complementary to the sequence located 48 to 23 bp downstream from the putative initiation codon of the bmr3 gene were mixed, made up to a total volume of 17 μl with distilled water, heated at 95°C for 5 min, and incubated at 40°C for 60 min. The

FIG. 1. Mutations detected in the PR mutant. Putative promoter −35 and −10 consensus sequences and Shine-Dalgarno sequences are shown as boxes in black. The transcription initiation site is marked +1. The horizontal arrows indicate inverted repeat sequences. (A) Mutations in the 5‘ UTR. Nucleotide changes detected in the PR mutant are indicated by filled arrows. (B) Amino acid changes in the bmr3 coding region. Both amino acids are located in the ninth transmembrane segment of Bmr3.
first-strand cDNA was synthesized by use of an avian myeloblastosis virus reverse transcriptase first-strand cDNA synthesis kit (LIFE SCIENCE, Inc.) in accordance with the manufacturer’s instruction. Extension products were subjected to gel electrophoresis (5% polyacrylamide sequencing gel), alongside sequence ladders obtained with the same primer, and analyzed with an FMBIO-100 Fluor Bio image analyzer (Hitachi Software Engineering Co., Ltd.). The results showed that transcription was initiated from an adenosine residue located 83 bp upstream from the initiation codon in both strain 168 and the PR mutant (Fig. 3). Typical σ<sup>70</sup>-dependent −35 and −10 consensus sequences were found in the upstream region, as shown in Fig. 1.

Expression of pbmr3-lacZ transcriptional fusion genes. To determine whether only one or both of the C-to-T (−18) and T-to-A (+4) changes in the PR mutant were responsible for the increased expression of the bmr3 gene, a 386-bp fragment encompassing positions −103 to +283 of the bmr3 gene was transcriptionally fused to the lacZ gene. In order to construct 386-bp fragments having a base change of C to T at the −18 position, T to A at the +4 position, or both, in vitro mutagenesis was carried out with a Quick Change site-directed mutagenesis kit (Stratagene, La Jolla, Calif.) in accordance with the method described by the manufacturers. The primers used were as follows: for the C-to-T change at the −18 position, 5′-CTTGGTGAACAAGCATGCAGTCCTCTTCTATAAAAAT

![Diagram](https://via.placeholder.com/150)

**FIG. 3.** Mapping of the 5′-end of bmr3 mRNA by primer extension analysis. Total RNA was isolated from an early-log-phase (OD<sub>530</sub>, 0.5) culture of strain 168 and the PR mutant grown in LB medium. Forty micrograms of total RNA was used for primer extension. The amount of sample loaded on lane 2 was 1/10 of that loaded on lane 1. The potential transcription start site is marked with arrows. Lanes: 1, strain 168; 2, PR mutant; A, C, G, and T, dideoxy sequencing ladder obtained with the same primer used for primer extension.

**TABLE 2.** Expression of pbmr3-lacZ transcriptional fusion genes

| Strain | β-Galactosidase activity (U) <sup>a</sup> |
|--------|----------------------------------|
| 168 amyE::pbmr3(wild type)-lacZ | 1.05 |
| 168 amyE::pbmr3(PR mutant)-lacZ | 49.0 |
| 168 amyE::pbmr3(T−A)-lacZ | 2.37 |

<sup>a</sup> β-Galactosidase activity was measured in cells at mid-log phase. One unit of β-galactosidase activity was defined as the amount of enzyme that catalyzed the production of 1 μmol of o-nitrophenol per optical density unit at 530 nm.
of 168 amyE::pbmr3(wild type)-lacZ, whereas the β-galactosidase activity of 168 amyE::pbmr3(C→T)-lacZ was only twice that of the wild-type fusion strain (Table 2). These results indicate that the T-to-A(+) change in the PR mutant was mainly responsible for increased expression of the pbmr3 gene at a transcriptional level, although the C-to-T(−18) change together with T-to-A(+) change resulted in about a fourfold increase in expression.

**Increased stability of pbmr3 mRNA in the PR mutant.** We examined potential secondary structures of pbmr3 mRNA in the 83-bp 5′ UTR and found that the 5′-proximal region of pbmr3 mRNA can fold to form stem-loop structures without any unpaired nucleotides at the 5′ end. The nucleotide change of T to A at the +4 position in the PR mutant changed one mismatched T-T pair to a matched A-T pair, resulting in a perfectly matched 11-bp stem (Fig. 4). It is well known that the 5′-terminal stem-loop is a determinant of mRNA stabilization (9, 10). Therefore, we compared the stabilities of pbmr3 mRNA for strain 168 and the PR mutant. To determine the stability of mRNA, rifampin was added to the early-log-phase culture growing in LB medium at a final concentration of 500 μg/ml, and samples were removed at set time intervals as described previously (3). In strain 168 cells, the half-life of pbmr3 mRNA was 3.0 min, whereas in the PR mutant, it was more than 12 min (Fig. 5). As a control, we also measured the half-life of the bcrC mRNA, which encodes a membrane protein involved in bacitracin resistance (20). The half-life of bcrC mRNA (1.8 min) in the PR mutant was found to be the same as that of strain 168 (data not shown).

The T-to-A mutation at the +4 position resulted in increased mRNA stability of the pbmr3-lacZ fusion gene. In order to examine whether the 5′ UTR of pbmr3 mRNA functioned as a determinant of mRNA stability when it was fused to a heterologous gene, we also determined the mRNA stability of pbmr3-lacZ fusion genes. Relative values for steady-state mRNA levels of fusion genes were calculated from the intensities of the bands at time zero (Fig. 6) and were found to be 1.20:2.10 for 168 amyE::pbmr3(wild type)-lacZ, 168 amyE::pbmr3(PR mutant), 168 amyE::pbmr3(C→T), and 168 amyE::pbmr3 (T→A)-lacZ, respectively. The relative values of β-galactosidase activity in these strains, shown in Table 2, reflect the mRNA levels of the fusion genes.

The half-lives of the pbmr3(wild type)-lacZ and pbmr3(C→T)-lacZ fusion gene mRNAs were about 4 min, whereas those of the pbmr3(PR mutant)-lacZ and pbmr3(T→A)-lacZ mRNAs were more than 15 min (Fig. 6). These results indicate that the 5′ UTR sequence of the pbmr3 gene is a determinant of mRNA stability and that the base change of T to A at the +4 position resulted in increased stability of pbmr3 mRNA. The base change of C to T at the −18 position had no effect on mRNA stability. Therefore, the twofold increase of pbmr3(C→T)-lacZ fusion gene mRNA compared to that of wild-type fusion gene mRNA may be due to an increase in the rate of mRNA synthesis.

**Endonucleases RNase III and RNase M5 are not involved in the degradation of pbmr3 mRNA.** A predicted ρ-independent transcriptional terminator, which includes 17-bp perfectly
matched inverted repeats, is located immediately downstream of the stop codon of the \textit{bmr3} gene. Therefore, \textit{bmr3} mRNA is predicted to have stem-loop structures at both the 3' and 5' ends. We attempted to determine whether endonucleases are involved in degradation of \textit{bmr3} mRNA. It is expected that the deletion of an RNase, which is involved in the critical step of \textit{bmr3} mRNA degradation in strain 168, may result in increased drug resistance as well as an increase in the expression of the \textit{bmr3} gene. Therefore, \textit{bmr3} mRNA stability has not been excluded. Further work is required to elucidate the precise mechanism of the \textit{bmr3} mRNA degradation process.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig_6}
\caption{The 5' UTR of the \textit{bmr3} transcript is a determinant of mRNA stability. Strain 168 clones containing various \textit{pbmr3-lacZ} fusion genes at the \textit{amyE} locus were grown in LB medium to early log phase (OD$_{600}$, 0.5), and 500 µg of rifampin/ml was added to inhibit transcription. Portions of the culture were removed at the indicated times (in minutes) after the addition of rifampin (shown at the top of each lane). Five micrograms of total RNA was loaded per lane. m, molecular size standard (Invitrogen RNA ladder, 0.24 to 9.5 kb and 0.16 to 1.77 kb). A $^{32}$P-labeled \textit{lacZ} probe was used for hybridization. An arrow indicates the mRNA of the \textit{pbmr3-lacZ} fusion gene. (A) 168 \textit{amyE}:\textit{pbmr3}(wild type)-\textit{lacZ}; (B) 168 \textit{amyE}:\textit{pbmr3}(C$\rightarrow$T)-\textit{lacZ}; (C) 168 \textit{amyE}:\textit{pbmr3}(T$\rightarrow$A)-\textit{lacZ}; (D) 168 \textit{amyE}:\textit{pbmr3}(PR)-\textit{lacZ}. (E) The relative amounts of mRNA remaining after the addition of rifampin were calculated from the results shown in panels A through D and plotted against time. Open circles, 168 \textit{amyE}:\textit{pbmr3}(wild type)-\textit{lacZ}; closed circles, 168 \textit{amyE}:\textit{pbmr3}(C$\rightarrow$T)-\textit{lacZ}; open squares, 168 \textit{amyE}:\textit{pbmr3}(T$\rightarrow$A)-\textit{lacZ}; closed squares, 168 \textit{amyE}:\textit{pbmr3}(PR mutant)-\textit{lacZ}.}
\end{figure}

Several mRNAs with extreme stability have been reported for \textit{B. subtilis}. Individual factors contributing to the stabilization of these mRNAs have been discussed in each case. The half-life of an \textit{ermC} transcript increases about 20-fold upon exposure to erythromycin. The erythromycin-bound ribosome stalls while translating a leader peptide preceding the coding region of \textit{ermC}. The stalled ribosome protects the transcript from degradation (4, 14). The \textit{gsiB} mRNA, which encodes a $\sigma^B$-dependent general stress protein, has a remarkably long half-life (~20 min) (15). It was found that a strong ribosome binding site was crucial for the increased stability of the \textit{gsiB} mRNA. The mRNA of the \textit{aprE} gene, which encodes subtilisin, is stable, with a half-life exceeding 20 min (9). \textit{aprE}-5' UTR-\textit{lacZ} fusion mRNA has a similar half-life, indicating that the determinants for \textit{aprE} mRNA stability are located in the 5' UTR, which is predicted to fold into a stem-loop structure at the 5' end. Our results indicate that endonucleases RNase III and RNase M5 are not involved in the degradation of \textit{bmr3} mRNA. Based on a gene array analysis, Condon et al. reported that RNase M5 has few, if any, mRNA substrates in \textit{B. subtilis} (6).
The results obtained in the present study indicate the possibility that MDR clinical isolates have a mutation which results in the stabilization of MDT mRNA. This finding provides an additional target for potential drugs designed to overcome MDR pathogens.

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REFERENCES

1. Ahmed, M., C. M. Borsch, S. S. Taylor, N. Vázquez-Laslop, and A. A. Neyfakh. 1994. A protein that activates expression of a multidrug efflux transporter upon binding the transporter substrates. J. Biol. Chem. 269: 28006–28013.

2. Ahmed, M., L. Lyass, P. N. Markham, S. S. Taylor, N. Vázquez-Laslop, and A. A. Neyfakh. 1995. Two highly similar multidrug transporters of *Bacillus subtilis* whose expression is differentially regulated. J. Bacteriol. 177:3904–3910.

3. Aiso, T., and R. Ohki. 2003. Instability of sensory histidine kinase mRNAs in *Escherichia coli*. Genes Cells 8:179–187.

4. Bechhofer, D. H., and K. H. Zen. 1989. Mechanism of erythromycin-induced *ermC* mRNA stability in *Bacillus subtilis*. J. Bacteriol. 171:5803–5811.

5. Condon, C., D. Brechemier-Baey, B. Beltchev, M. Grunenman-Logano, and H. Putzer. 2001. Identification of the gene encoding the 5S ribosomal RNA maturase in *Bacillus subtilis*: mature 5S rRNA is dispensable for ribosome function. RNA 7:242–253.

6. Condon, C., J. Rourera, D. Brechemier-Baey, and H. Putzer. 2002. Ribonuclease M5 has few, if any, mRNA substrates in *Bacillus subtilis*. J. Bacteriol. 184:2845–2849.

7. Eguchi, Y., T. Oshima, H. Mori, R. Aono, K. Yamamoto, A. Ishihama, and R. Utsumi. 2003. Transcriptional regulation of drug efflux genes by EvgA, a two-component system in *Escherichia coli*. Microbiology 149:2819–2828.

8. Fukuchi, K., Y. Kasahara, K. Asai, K. Kobayashi, S. Moriya, and N. Ogasawara. 2000. The essential two-component regulatory system encoded by *yvfC* and *yvfG* modulates expression of the *ftsAZ* operon in *Bacillus subtilis*. Microbiology 146:1573–1583.

9. Hambraeus, G., M. Persson, and B. Rutberg. 2000. The *aprE* leader is determinant of extreme mRNA stability in *Bacillus subtilis*. Microbiology 146:3051–3059.

10. Hansen, M. J., L.-H. Chen, M. L. S. Fejzo, and J. G. Belasco. 1994. The *ompA* 5′ untranslated region impedes a major pathway for mRNA degradation in *Escherichia coli*. Mol. Microbiol. 12:707–716.

11. Herskovitz, M. A., and D. H. Bechhofer. 2000. Endoribonuclease RNase III is essential in *Bacillus subtilis*. Mol. Microbiol. 38:1027–1033.

12. Hirakawa, H., K. Nishino, J. Yamada, T. Hirata, and A. Yamaguchi. 2003. β-Lactam resistance modulated by the overexpression of response regulators of two-component signal transduction systems in *Escherichia coli*. J. Antimicrob. Chemother. 52:576–582.

13. Hirakawa, H., K. Nishino, T. Hirata, and A. Yamaguchi. 2003. Comprehensive studies of drug resistance mediated by overexpression of response regulators of two-component signal transduction systems in *Escherichia coli*. J. Bacteriol. 185:1851–1856.

14. Hue, K. K., and D. H. Bechhofer. 1991. Effect of *ermC* leader region mutations on induced mRNA stability. J. Bacteriol. 173:3732–3740.

15. Jürgen, B., T. Schweder, and M. Hecker. 1998. The stability of mRNA from the *gisB* gene of *Bacillus subtilis* is dependent on the presence of a strong ribosome binding site. Mol. Gen. Genet. 258:538–545.

16. Murata, M., S. Ohno, M. Kuman, K. Yamane, and R. Ohki. 2003. Multi-drug resistant phenotype of *Bacillus subtilis* spontaneous mutants isolated in the presence of puromycin and lincomycin. Can. J. Microbiol. 49:71–77.

17. Neyfakh, A. A., V. E. Bidnenko, and L. B. Chen. 1994. Efflux-mediated multidrug resistance in *Bacillus subtilis*: similarities and dissimilarities with the mammalian system. Proc. Natl. Acad. Sci. USA 91:4781–4785.

18. Oguro, A., H. Kakeshita, K. Nakamura, K. Yamane, W. Wang, and D. H. Bechhofer. 1998. *Bacillus subtilis* RNase III cleaves both 5′- and 3′-sites of the small cytoplasmic RNA precursor. J. Biol. Chem. 273:19542–19547.

19. Ohki, R., C. Yany, K. Tateno, W. Masuya, S. Moriya, K. Kobayashi, and N. Ogasawara. 2003. The BceRS two-component regulatory system induces expression of the bacitracin transporter, BceAB, in *Bacillus subtilis*. Mol. Microbiol. 49:1325–1344.

20. Ohki, R., K. Tateno, Y. Okada, H. Okajima, K. Asai, Y. Sadaie, M. Murata, and T. Aiso. 2003. A bacitracin-resistant *Bacillus subtilis* gene encodes a homologue of the membrane-spanning subunit of the *Bacillus licheniformis* ABC transporter. J. Bacteriol. 185:51–59.

21. Ohki, R., and M. Murata. 1997. *bmrA*, a third multidrug transporter gene of *Bacillus subtilis*. J. Bacteriol. 179:1423–1427.

22. Panganiban, A. T., and H. R. Whiteley. 1983. *Bacillus subtilis* RNase III cleavage sites in phage SP62 early mRNA. Cell 32:907–913.

23. Petit, M.-A., C. Bruand, L. Jannière, and S. D. Ehrlich. 1990. Tn10-derived transposons active in *Bacillus subtilis*. J. Bacteriol. 172:6736–6740.

24. Yamane, K., M. Kuman, and K. Kurita. 1996. The 25′-36′ region of the *Bacillus subtilis* chromosome: determination of the sequence of a 146 kb segment and identification of 113 genes. Microbiology 142:3047–3056.

25. Yoshioka, K.-L., Y.-H. Ohki, M. Murata, M. Kinahara, H. Matsuoka, H. Yamaguchi, R. Ohki, M. Kuman, K. Yamane, and Y. Fujita. 2004. *Bacillus subtilis* LmrA is a repressor for the *bmrAB* and *yusGH* operons: identification of its binding sites and functional analysis of *yusR* and *yusGH*. J. Bacteriol. 186:5640–5648.