Characterization of the *fomA* and *fomB* Gene Products from *Streptomyces wedmorensis*, Which Confer Fosfomycin Resistance on *Escherichia coli*

SEIJI KOBAYASHI, TOMOHISA KUZUYAMA, AND HARUO SETO*

Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

Together, the *fomA* and *fomB* genes in the fosfomycin biosynthetic gene cluster of *Streptomyces wedmorensis* confer high-level fosfomycin resistance on *Escherichia coli*. To elucidate their functions, the *fomA* and *fomB* genes were overexpressed in *E. coli* and the gene products were characterized. The recombinant FomA protein converted fosfomycin to fosfomycin monophosphate, which was inactive on *E. coli*, in the presence of a magnesium ion and ATP. On the other hand, the recombinant FomB protein did not inactivate fosfomycin. However, a reaction mixture containing FomA and FomB proteins converted fosfomycin to fosfomycin monophosphate and fosfomycin diphosphate in the presence of ATP and a magnesium ion, indicating that FomA and FomB catalyzed phosphorylations of fosfomycin and fosfomycin monophosphate, respectively. These results suggest that the self-resistance mechanism of the fosfomycin-producing organism *S. wedmorensis* is mono- and diphosphorylation of the phosphonate function of fosfomycin catalyzed by FomA and FomB.

Fosfomycin is a medically important antibiotic produced by various species of *Streptomyces* (4), by *Pseudomonas syringae* (14), and by *Pseudomonas viridiflava* (9). It possesses unique structural features, including a carbon-phosphorus bond and an epoxide. As an analog of phosphoenolpyruvate (PEP), this compound irreversibly inhibits PEP UDP-*N*-acetylglucosamine-3-*O*-enolpyruvyltransferase (enolpyruvyltransferase), which catalyzes the first step of peptidoglycan biosynthesis (8). Fosfomycin does not inhibit, however, other enzymes using PEP and shows almost no toxicity in humans (1, 7).

Several clinical isolates resistant to fosfomycin had been found to have fosfomycin resistance genes on plasmids, such as *fosA* from *Serratia marcescens* (12) and *fosB* from *Staphylococcus epidermidis* (15). Both gene products catalyzed the irreversible addition of glutathione to fosfomycin (12). The deduced amino acid sequences of these gene products exhibit 36.6% identity to each other. On the other hand, to the best of our knowledge, the only precedent for self-resistance genes in the fosfomycin-producing organisms was *fosC*, found in *P. syringae* PB-5123 by Garcia et al. (2). They showed that its product inactivated fosfomycin in the presence of ATP. The inactivated products, however, were not well characterized. They also demonstrated that this inactivation was reversed by subsequent treatment with alkaline phosphatase.

Members of our group have cloned the fosfomycin biosynthetic gene cluster of *Streptomyces wedmorensis* (11) and identified four fosfomycin biosynthetic genes, *fom1* to *fom4* (6). In addition to these genes, encoding the enzymes necessary for fosfomycin biosynthesis, six open reading frames (ORFs), *orfA* to *orfF*, with unknown functions were found on a sequenced fragment including the fosfomycin gene cluster. Among these ORFs, *orfA* and *orfB*, contained together in a 2.9-kb fragment, gave *Escherichia coli* a high-level of resistance to fosfomycin, and fosfomycin was converted to inactivated forms in the presence of ATP by a crude extract from this transformant (10).

Subsequently we isolated the inactivated compounds and determined their structures to be fosfomycin monophosphate and fosfomycin diphosphate. The genes *orfA* and *orfB* were thus renamed *fomA* and *fomB*, respectively. However, individual functions of the *fomA* and *fomB* gene products remained to be studied.

We show here that the *fomA* gene confers fosfomycin resistance on *E. coli* and that the *fomA* and *fomB* gene products catalyze phosphorylations of fosfomycin and fosfomycin monophosphate, respectively. We also describe detailed characterization of the purified recombinant *fomA* and *fomB* gene products overexpressed in *E. coli*.

MATERIALS AND METHODS

Growth media. Luria-Bertani (LB) medium containing 50 μg of ampicillin per ml was used for pregrowth and growth of *E. coli* HB101 harboring pFBG1204, pFBG2204, pFBG1216, pFBG1221, or pUC118. The pregrowth medium for *E. coli* K-12 strain HW8235 was nutrient broth (Eiken, Tokyo, Japan). All *E. coli* strains were grown at 37°C. Strains and plasmids used are listed in Table 1.

Identification of the resistance gene(s). In order to identify the resistance gene(s), DNA fragments derived from the 12.1-kb fragment containing fosfomycin biosynthetic genes were inserted downstream of the *lacZ* promoter in pUC118 or pUC119 and then introduced into *E. coli* HB101 as described by Hanahan (3). Each transformant mixture was spread on LB agar plates containing 50 μg of ampicillin per ml and 400 μg of fosfomycin per ml and then incubated at 37°C for 24 h.

Plasmid constructions. Deletion plasmids, pFBG1216 and pFBG1221, derived from pFBG1204 (which includes the *fomA* and *fomB* genes) (Fig. 1) were constructed by using exonuclease III and mung bean nucleases as described by Henikoff (5). These deletion plasmids were introduced into *E. coli* HB101 as described by Hanahan (3) for further experiments.

Determination of MICs of fosfomycin. *E. coli* HB101 harboring one of the plasmids mentioned above (pFBG1204, pFBG2204, pFBG1216, pFBG1221, and pUC118) was grown in LB medium containing 50 μg of ampicillin per ml with or without 6.25 to 800 μg/ml of fosfomycin at 37°C. Growth of each strain was measured by optical density at 660 nm.

DNA sequencing. DNA sequencing was carried out using a DNA sequencer (model 4000L; Licor, Lincoln, Nebr.). Sequencing reactions were made with a Thermo Sequenase cycle sequencing kit (U.S. Biochemical Corporation, Cleveland, Ohio) using infrared-dye-labeled M13 forward (−29) primer (Nisshinbo, Tokyo, Japan) or infrared-dye-labeled M13 reverse primer (Nisshinbo).

Protein expression and purification of recombinant FomA and FomB. On the basis of the entire nucleotide sequences of *fomA* and *fomB* genes from *S. wedmorensis*, two pairs of oligonucleotide primers, 5′-GGGGGGATCCACGCCCGATTTCTTGGCC-3′ (5′ of the *fomA* gene) plus 5′-GGGGGGATCCCGCAGAAGCAGTCGTGGTG-3′ (3′ of the *fomA* gene) and 5′-GGGGGGATCCCTG

* Corresponding author. Mailing address: Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan. Phone: 81-3-5841-7839. Fax: 81-3-5841-8485. E-mail: haseto@imcbns.iam.u-tokyo.ac.jp.

TABLE 1. Properties of bacterial strains and plasmids

Strain or plasmid	Relevant characteristics[a]	Source or reference
E. coli strains		
HB101	*supE44 hsdS20* (r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1 leuB6 thi-1*	Takara Shuzo
JM109	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 Δ(lac-proAB)/F′ (traD36 proAB⁺ lacI^q ZΔM15)*	Takara Shuzo
M15	*lac ara gal mtl* F⁻ *recA⁺ uvr⁺*	Qiagen
Plasmids		
pFBG1204	pUC118Ω*Bam*HI-*Bgl*II 2.9 kb; Amp^r	This study (Fig. 1)
pFBG2204	pUC119Ω*Bam*HI-*Bgl*II 2.9 kb; Amp^r	This study (Fig. 1)
pFBG1216	Deleted plasmid derived from pFBG1204; Amp^r	This study (Fig. 1)
pFBG1221	Deleted plasmid derived from pFBG1204; Amp^r	This study (Fig. 1)
pUC118	Vector; Amp^r	Takara Shuzo
pUC119	Vector; Amp^r	Takara Shuzo
pREP4	*neo lac1*; Km^r	Qiagen
pQE30	Vector; Amp^r	Qiagen
pQEFA	pQE30Ω*fomA*; Amp^r	This study (see text)
pQEFB	pQE30Ω*fomB*; Amp^r	This study (see text)

[a] Amp^r, ampicillin resistant; Km^r, kanamycin resistant.

GAAAACCTCACGATCCGC-3′ (5′ of the *fomB* gene) plus 5′-GGG<u>GGATC</u><u>C</u>TTCGGCAAGCTGCTTGAGCGTC-3′ (3′ of the *fomB* gene), including *Bam*HI restriction sites (underlined), were synthesized (Amersham Pharmacia, Uppsala, Sweden). Then, these pairs of primers were used with pFBG1204 to amplify the *fomA* or *fomB* gene. By using *Taq* DNA polymerase (Boehringer, Mannheim, Germany) and a standard PCR protocol, an 813-bp fragment for the *fomA* gene or a 1,009-bp fragment for the *fomB* gene was amplified. These PCR fragments were cleaved with *Bam*HI and cloned into pUC118 (Takara Shuzo, Kyoto, Japan). The sequences of the cloned DNA fragments were confirmed with a Licor DNA sequencer. The coding region of the *fomA* or *fomB* gene was cloned into the expression vector pQE30 (Qiagen, Hilden, Germany) to yield pQEFA or pQEFB, respectively.

E. coli M15 containing pREP4 (Qiagen) was used as a host for expression of FomA. *E. coli* (pREP4) harboring pQEFA was cultured at 37°C in 100 ml of LB medium containing 25 μg of kanamycin per ml and 200 μg of ampicillin per ml for 5 h with 2 mM isopropyl-β-D-thiogalactopyranoside. Upon reaching an optical density at 660 nm of 0.8, cells were harvested by centrifugation and resuspended in 100 mM Tris-HCl (pH 7.5). After 5 min of sonication, the lysate was centrifuged at 10,000 × g for 20 min and the supernatant was collected. The supernatant was applied to a nickel-nitrilotriacetic acid (Ni-NTA)-agarose resin (Qiagen) column (7.5 by 10 mm). After a washing with 100 mM Tris-HCl buffer (pH 7.5) containing 50 mM imidazole, the protein which had bound to the Ni-NTA agarose resin was eluted with 100 mM Tris-HCl buffer (pH 7.5) containing 100 mM imidazole. The eluate was used as purified recombinant FomA in subsequent experiments. Expression and purification of recombinant FomB were done in a way similar to that for recombinant FomA, with replacement of pQEFA by pQEFB.

Identification of reaction products synthesized by the *fomA* or *fomB* gene product. A reaction mixture containing the recombinant FomA protein, 5.4 mM ATP, 10 mM MgCl₂, 6 mM KCl, and 5.5 mM fosfomycin was made up to 5 ml and incubated at 30°C for 3 h. One equivalent of methanol was added to the

reaction mixture to stop the reaction, followed by centrifugation (15,000 × g, 10 min) to remove insoluble materials. The amount of fosfomycin remaining in the supernatant was quantitatively determined by bioassay against *E. coli* K-12 strain HW8235 (Meiji Seika Kaisha Ltd., Tokyo, Japan).

The supernatant (10 ml) was passed through an activated carbon column (15 by 27 mm) and, after dilution to 20 ml with H₂O, subjected to Dowex 1-X8 (Cl⁻ type, 15 by 55 mm) chromatography with elution with 2.0% NaCl (50 ml). The eluate was diluted twofold with water and lyophilized. After being dissolved in H₂O, the sample was analyzed by ³¹P nuclear magnetic resonance (NMR) (JEOL [Tokyo, Japan] A-500 instrument).

Phosphorylation of fosfomycin monophosphate by FomB was done in a way similar to that for phosphorylation by FomA, with replacement of fosfomycin by 5 mM fosfomycin monophosphate.

Assay for FomA and FomB activities. FomA and FomB activities were measured by a spectrophotometric method. The reaction mixture for the fosfomycin phosphorylation by FomA contained 100 mM sodium phosphate buffer (pH 7.0), 10 mM MgCl₂, 10 mM dithiothreitol, 0.5 mM NADH, 1 mM PEP, 4.2 U of pyruvate kinase, 6.6 U of lactate dehydrogenase, and several concentrations of fosfomycin and ATP. The final volume of the reaction mixture was 200 μl. The FomA enzyme assay was conducted at 37°C on a Benchmark microplate reader (Bio-Rad). The background rate of NADH oxidation was measured for the reaction mixture without fosfomycin or ATP. The reaction rate was measured for 10 min. One unit of FomA protein was defined as the amount of protein required to inactivate 1 μmol of fosfomycin per min.

The effect of temperature on the enzymatic activity was investigated over the range of 20 to 50°C. The effect of pH on the enzymatic activity was investigated over the ranges of pH 5.5 to 7.0 with morpholineethanesulfonic acid (MES)-NaOH buffer, pH 6.8 to 7.8 with sodium phosphate buffer, and pH 7.0 to 9.0 with Tris-HCl buffer.

The K_m for fosfomycin was determined using an enzyme assay system containing 4 mM Na₂ATP²⁻, fosfomycin concentrations between 20 and 200 μM,
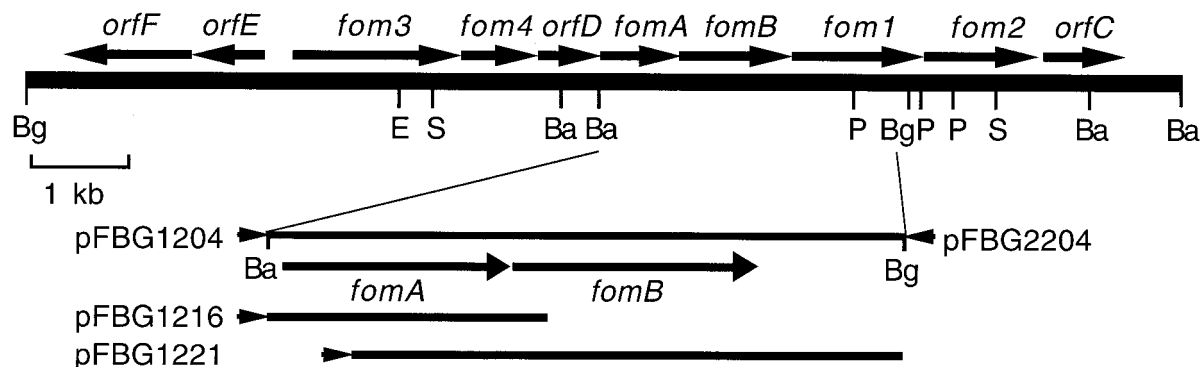

FIG. 1. Fosfomycin biosynthetic gene cluster and deletion plasmids used to determine the ORFs necessary to confer fosfomycin resistance on *E. coli* HB101. Abbreviations for restriction sites: Ba, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; P, *Pst*I; S, *Sph*I. Arrows indicate the direction of transcription from the *lac* promoter of pUC118 or pUC119 with respect to the inserted fragments.

and 10 μg of purified recombinant FomA. Data were analyzed on a Lineweaver-Burk plot, and the slope and *x* intercept were determined using a linear-regression computer program.

The K_m for Na$_2$ATP^{2-} was determined using an enzyme assay system containing 4 mM fosfomycin, Na$_2$ATP^{2-} concentrations between 20 and 200 μM, and 10 μg of purified recombinant FomA. Data were analyzed as stated above for fosfomycin.

The reaction mixture for the FomB activity assay contained 100 mM sodium phosphate buffer (pH 7.0), 10 mM MgCl$_2$, 10 mM dithiothreitol, 0.5 mM NADH, 1 mM PEP, 4.2 U of pyruvate kinase, 6.6 U of lactate dehydrogenase, and several concentrations of fosfomycin monophosphate (40 to 1,000 μM) and ATP (40 to 1,000 μM). The final volume of the reaction mixture was 200 μl. Enzyme assays were conducted in the same way as for FomA. The background rate of NADH oxidation was measured for the reaction mixture without fosfomycin monophosphate or ATP. The reaction rate was measured for 10 min. One unit of FomB protein was defined as the amount of protein required to produce 1 μmol of fosfomycin diphosphate per min.

The K_m for fosfomycin monophosphate was determined using an enzyme assay system containing 4 mM Na$_2$ATP^{2-}, fosfomycin monophosphate concentrations between 40 and 1,000 μM, and 5 μg of purified recombinant FomB. Data were analyzed as stated above for FomA.

The K_m for Na$_2$ATP^{2-} was determined using an enzyme assay system containing 2 mM fosfomycin, Na$_2$ATP^{2-} concentrations between 40 and 1,000 μM, and 5 μg of purified recombinant FomB. Data were analyzed as for fosfomycin monophosphate.

Nucleotide sequence accession number. The DNA sequence of the *fomA* gene has been corrected (one nucleotide). The updated version is available in DDBJ/EMBL/GenBank under both the original accession number (D38561) and a new one (AB016934).

RESULTS

Identification of the fosfomycin resistance gene in the biosynthetic gene cluster. Among tested plasmids, only pFBG1204 conferred fosfomycin resistance (>800 μg/ml) on *E. coli*. On the other hand, pFBG2204, with the same 2.9-kb *Bam*HI-*Bgl*II fragment as pFBG1204 inserted in the opposite orientation relative to the direction of transcription of the *lacZ* promoter, did not provide fosfomycin resistance. Thus, *fomA* and/or *fomB* was judged to confer fosfomycin resistance on the host under the control of the *lacZ* promoter.

To determine the minimum region essential for fosfomycin resistance, deletion plasmids derived from pFBG1204 were constructed and then introduced into *E. coli* HB101. The resulting transformants were spread on LB agar plates containing various concentrations of fosfomycin. The MICs of fosfomycin for *E. coli* carrying pFBG1204, pFBG2204, pFBG1216, pFBG1221, and pUC118 were >800, <6.25, >800, <6.25, and <6.25 μg/ml, respectively. As can be seen from these results, a slight loss of *fomA* (pFBG1221) caused the complete disappearance of resistance, showing that *fomA* is essential for resistance. On the other hand, the loss of *fomB* (pFBG1216) did not influence resistance.

Individual function of the *fomA* and *fomB* gene products. To elucidate the *fomA* and *fomB* gene functions, we constructed plasmids for the overexpression of the gene products followed by a one-step purification of the recombinant enzyme by Ni-NTA-agarose resin. Based on the total sequence of the *fomA* gene deposited in DDBJ/EMBL/GenBank under accession number D38561, we first constructed a FomA overexpression system. The overexpressed protein, however, did not inactivate fosfomycin under the conditions described previously (10). We then carried out resequencing of the *fomA* gene around the region corresponding to the N terminus and found an error, an omission of one base (see Materials and Methods). We constructed the FomA overexpression system again using primers described in Materials and Methods. The purified recombinant FomA and FomB proteins gave homogeneous protein bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with subunit sizes of 29 and 50 kDa, respectively (Fig. 2).

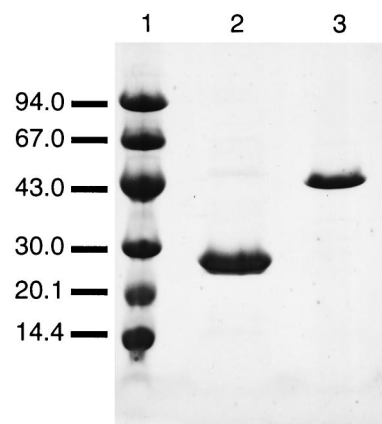We had previously reported isolation and structural deter-

FIG. 2. SDS-PAGE of purified FomA and FomB. Purified enzymes were analyzed by SDS-PAGE with 8 to 25% polyacrylamide gels. Lanes: 1, molecular mass standard; 2, SDS-treated FomA (1.7 μg); 3, SDS-treated FomB (0.5 μg). Proteins were stained with Coomassie brilliant blue R-250.

mination of the inactivated fosfomycin derivatives fosfomycin monophosphate and fosfomycin diphosphate, formed by a crude extract of *E. coli* carrying pFBG1204 containing both the *fomA* and *fomB* genes in the presence of ATP (10). To elucidate the function of FomA, we used a purified recombinant FomA protein instead of the crude extract of *E. coli* carrying pFBG1204. The reaction was carried out under the same condition as for pFBG1204. ^{31}P NMR analysis revealed formation of fosfomycin monophosphate in the reaction mixture. Since fosfomycin diphosphate was not produced by the *fomA* gene product, the *fomB* product must convert fosfomycin monophosphate into fosfomycin diphosphate.

To confirm this hypothesis, purified recombinant FomA and FomB were mixed for fosfomycin inactivation experiments. Both fosfomycin monophosphate and fosfomycin diphosphate were detected in the reaction mixture after incubation. In addition, fosfomycin was not inactivated by the recombinant FomB protein alone under the same conditions. Attempts to characterize the reaction product of fosfomycin monophosphate and the FomB protein, however, were hampered by the unstable nature of fosfomycin monophosphate, which prevented preparation of the sample in a quantity large enough for NMR analysis.

Characterization of the *fomA* and *fomB* gene products. The enzymatic properties of purified recombinant FomA are summarized along with those of FomB in Table 2. The results of SDS-PAGE and gel filtration suggested that FomA and FomB are a tetramer and a dimer, respectively.

DISCUSSION

We have shown in this study that the *fomA* and *fomB* gene products phosphorylated fosfomycin to fosfomycin monophosphate and fosfomycin monophosphate to fosfomycin diphosphate, respectively. These results raise two questions about the self-resistance mechanism of the fosfomycin-producing organism. (i) How does the fosfomycin-producing organism convert phosphorylated fosfomycin into active fosfomycin and export it into the fermentation medium? (ii) Why does the *fomB* gene product catalyze the additional phosphorylation of biologically inactive fosfomycin monophosphate?

Several clues to answer these questions may be found in the following observations: while a large amount of fosfomycin accumulated in the fermentation medium, no phosphorylated

TABLE 2. Kinetic properties of the *fomA* and *fomB* gene products

Parameter	Value for:	
	FomA	FomB
Molecular mass (kDa) as determined by:		
Deduced amino acid sequence	29	37
SDS-PAGE	29	50
Gel filtration	110	75
K_m (μM) for:		
Fosfomycin	150	
Fosfomycin monophosphate		680
ATP	120	560
V_{max} (μmol/s)	0.050	0.12
Optimum temp (°C)	45	37
Optimum pH	7.2	7.5

fosfomycin was detected in *S. wedmorensis* cells (data not shown). In addition, phosphorylation and diphosphorylation of the phosphonate function in the fosfomycin molecule had proved to be reversible (10). A simple model for the export of fosfomycin may thus be speculated as follows. Fosfomycin biosynthesized in the cytosol is rapidly converted to biologically inactive fosfomycin monophosphate by FomA to confer self-resistance on the producing organism. Next, fosfomycin monophosphate is converted by FomB to fosfomycin diphosphate, which is an energy-rich molecule. Finally, fosfomycin diphosphate is exported into the fermentation medium as its free form, fosfomycin, by utilization of free energy liberated in the hydrolysis of the two phosphate bonds in the fosfomycin diphosphate molecule. In this model, the role of the first phosphorylation by FomA is inactivation of fosfomycin for self-resistance and that of the second phosphorylation by FomB is accumulation of free energy required to export fosfomycin diphosphate outside of the cell. This reversible self-resistance mechanism together with the efflux could be reasonable for fosfomycin-producing organisms. We are now trying to identify the phosphatase involved in the hydrolysis of fosfomycin diphosphate.

It has been reported that the *fosC* gene product of another fosfomycin-producing organism, *P. syringae* PB-5123, converted fosfomycin into an inactive compound by using ATP and that the inactive compound was reactivated by treatment with alkaline phosphatase. These results suggest that like the *fomA* gene product, the *fosC* gene product converts fosfomycin into fosfomycin monophosphate. Therefore, the *fosC* gene product, which seems to be fosfomycin phosphotransferase, should show similarity to the *fomA* gene product in amino acid sequence. However, the amino acid sequence of the *fomA* gene product, as corrected in this study, has only 25.8% identity to the *fosC* gene product. This low identity may suggest that the two fosfomycin-producing organisms, taxonomically quite different from each other, independently acquired the self-resistance mechanism against fosfomycin during evolution. On the other hand, no FomB homolog has yet been found in *P. syringae* PB-5123.

Suarez and Mendoza had proposed that the fosfomycin resistance genes, *fosA* and *fosB*, on plasmids isolated from clinical isolates were transferred horizontally from fosfomycin-producing organisms such as *Streptomyces* (13). The hypothesis was based on the following two findings. (i) The GC content ratios of the *fosA* and *fosB* genes are higher than those of the other genes on the chromosome and as high as those of actinomycetes. (ii) Most antibiotic-producing organisms have self-resistance genes.

Our finding that the self-resistance mechanism of the fosfomycin-producing *S. wedmorensis* is quite different from those of the clinical isolates, however, strongly indicates that clinical isolates independently acquired the fosfomycin resistance mechanism. In addition, irreversible inactivation of fosfomycin by addition of glutathione is not reasonable as the self-resistance mechanism, even for fosfomycin-producing organisms with high GC content ratios other than *S. wedmorensis*. The resistance mechanism of the fosfomycin-producing organisms may be found in clinical isolates in the future.

REFERENCES

1. **Christensen, B. G., W. J. Leanza, T. R. Beattie, A. A. Patchett, B. H. Arison, R. E. Ormond, F. A. Kuehl, Jr., G. Alders-Schonberg, and O. Jardetzky.** 1969. Phosphonomycin: structure and synthesis. Science **166:**123–125.
2. **Garcia, P., P. Arca, and J. E. Suarez.** 1995. Product of *fosC*, a gene from *Pseudomonas syringae*, mediates fosfomycin resistance by using ATP as co-substrate. Antimicrob. Agents Chemother. **39:**1569–1573.
3. **Hanahan, D.** 1983. Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. **166:**557–580.
4. **Hendlin, D., E. O. Stapley, M. Jackson, H. Wallick, A. K. Miller, F. J. Wolf, T. W. Miller, L. Chaiet, F. M. Kahan, E. L. Foltz, H. B. Woodruff, J. M. Mata, S. Hernandez, and S. Mochales.** 1969. Phosphonomycin, a new antibiotic produced by strains of *Streptomyces*. Science **166:**122–123.
5. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28:**351–359.
6. **Hidaka, T., M. Goda, T. Kuzuyama, N. Takei, M. Hidaka, and H. Seto.** 1995. Cloning and nucleotide sequence of fosfomycin biosynthetic genes of *Streptomyces wedmorensis*. Mol. Gen. Genet. **249:**274–280.
7. **Imai, S., H. Seto, H. Ogawa, A. Satoh, and N. Otake.** 1985. Studies on the biosynthesis of fosfomycin. Conversion of 2-hydroxyethylphosphonic acid and 2-aminoethylphosphonic acid to fosfomycin. Agric. Biol. Chem. **49:**873–874.
8. **Kahan, F. M., J. S. Kahan, P. J. Cassidy, and H. Kropp.** 1974. The mechanism of action of fosfomycin. Ann. N. Y. Acad. Sci. **235:**364–385.
9. **Katayama, N., S. Tsubotani, Y. Nozaki, S. Harada, and H. Ono.** 1990. Fosfadecin and fosfocytocin, new nucleotide antibiotics produced by bacteria. J. Antibiot. **43:**238–246.
10. **Kuzuyama, T., S. Kobayashi, K. O'Hara, T. Hidaka, and H. Seto.** 1996. Fosfomycin monophosphate and fosfomycin diphosphate, two inactivated fosfomycin derivatives formed by gene products of *fomA* and *fomB* from a fosfomycin producing organism *Streptomyces wedmorensis*. J. Antibiot. **49:**502–504.
11. **Kuzuyama, T., T. Hidaka, S. Imai, and H. Seto.** 1993. Studies on the biosynthesis of fosfomycin. 5. Cloning of genes for fosfomycin biosynthesis. J. Antibiot. **46:**1478–1480.
12. **Mendoza, C., J. M. Garcia, J. Llaneza, F. J. Mendez, C. Hardisson, and J. M. Ortiz.** 1980. Plasmid-determined resistance to fosfomycin in *Serratia marcescens*. Antimicrob. Agents Chemother. **18:**215–219.
13. **Saurez, J. E., and M. C. Mendoza.** 1991. Plasmid-encoded fosfomycin resistance. Antimicrob. Agents Chemother. **35:**791–795.
14. **Shoji, J., T. Kato, H. Hinoo, T. Hattori, K. Hirooka, K. Matsumoto, T. Tanimoto, and E. Kondo.** 1986. Production of fosfomycin (phosphonomycin) by *Pseudomonas syringae*. J. Antibiot. **39:**1011–1012.
15. **Zilharo, R., and P. Courvalin.** 1990. Nucleotide sequence of the *fosB* gene conferring fosfomycin resistance in *Staphylococcus epidermidis*. FEMS Microbiol. Lett. **68:**267–272.