Membrane Topology of the Xenobiotic-exporting Subunit, MexB, of the MexA,B-OprM Extrusion Pump in *Pseudomonas aeruginosa*

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The MexA,B-OprM efflux pump assembly of *Pseudomonas aeruginosa* consists of two inner membrane proteins and one outer membrane protein. The cytoplasmic membrane protein, MexB, appears to function as the xenobiotic-exporting subunit, whereas the MexA and OprM proteins are supposed to function as the membrane fusion protein and the outer membrane channel protein, respectively. Computer-aided hydropathy analyses of MexB predicted the presence of up to 17 potential transmembrane segments. To verify the prediction, we analyzed the membrane topology of MexB using the alkaline phosphatase gene fusion method. We obtained the following unique characteristics. MexB bears 12 membrane spanning segments leaving both the amino and carboxyl termini in the cytoplasmic side of the inner membrane. Both the first and fourth periplasmic loops had very long hydrophilic domains containing 311 and 314 amino acid residues, respectively. This fact suggests that these loops may interact with other pump subunits, such as the membrane fusion protein MexA and the outer membrane protein OprM. Alignment of the amino- and the carboxyl-terminal halves of MexB showed a 30% homology and transmembrane segments 1, 2, 3, 4, 5, and 6 could be overlaid with the segments 7, 8, 9, 10, 11, and 12, respectively. This result suggested that the MexB has a 2-fold repeat that strengthen the experimentally determined topology model. This paper reports the structure of the pump subunit, MexB, of the MexA,B-OprM efflux pump assembly. This is the first time to verify the topology of the resistant-nodulation-division efflux pump protein.

Nosocomial patients with cancer, transplantation, burn, cystic fibrosis, etc. are easily infected by bacteria with low virulence. Among those opportunistic pathogens, *Pseudomonas aeruginosa* is particularly problematic, since the bacteria show resistance to many structurally and functionally diverse antibiotics (1). Recent studies have revealed that this type of resistance is attributable to a synergy of low outer membrane permeability and active drug extrusion (2). An increasing number of multidrug extrusion systems are being reported in both prokaryotes and eukaryotes (2–8). It is likely, therefore, that active extrusion systems play a crucial role in the cellular defense mechanism against incoming noxious compounds in many living organisms. It is of great interest and importance, therefore, to analyze the mechanism by which such universally occurring extrusion pump function.

The wild-type *P. aeruginosa* expresses a low level of the MexA,B-OprM drug extrusion machinery (9, 10). Mutations in *nalB* gene cause overexpression of the mexA,B-oprM operon rendering the bacterium more resistant than the wild-type strain to a broad spectrum of antibiotics (3). Deletion of the coding region of the wild-type mexA, mexB, or oprM renders the mutant more susceptible than the wild-type strain to many antibiotics (9, 11). Thus, it is apparent that the MexA,B-OprM machinery is involved in the both basal and elevated levels of intrinsic antibiotic resistance in *P. aeruginosa*.

The MexA,B-OprM pump consists of three subunits, MexA, MexB, and OprM, located at the inner and outer membrane, respectively (9, 10). MexB consists of 1046 amino acid residues and is assumed to extrude the xenobiotics utilizing the proton motive force as the energy source (4, 12). This protein belongs to the resistant/nodulation/division (RND) family (13, 14). MexA is an inner membrane-associated lipoprotein belonging to the membrane fusion protein family. OprM is an outer membrane protein probably forming the xenobiotics exit channel. A complex formation by these three subunit proteins has been suggested for many efflux pump assemblies in Gram-negative bacteria (13, 15–17). In fact, the functional coupling of the RND protein and the membrane fusion protein was recently demonstrated by the subunit exchange experiments using three efflux pump systems in *P. aeruginosa* (18–21), while the outer membrane components could be substituted with other proteins having a similar function. However, the precise molecular mechanism of substrate recognition and efflux through these pumps remained to be clarified. For a better understanding of how this pump extrudes the xenobiotics, it is essential to elucidate the structure and membrane topology of the individual pump subunit.

The membrane topology of several RND family proteins was suggested to have 12 transmembrane domains and two large hydrophilic domains (13, 22). Hydropathy analysis of MexB by the TOP-PRED II 1.1 software package (23) suggested that it might have 12 certain and 5 putative transmembrane segments (TMS). Some other software predicted the presence of 11 TMS.

The topology of cytoplasmic membrane proteins in Gram-negative bacteria was often studied by the phoA gene fusion method. Alkaline phosphatase (AP) is enzymatically active after translocation to the periplasm, but is inactive when local-
ized cytoplasmically (24, 25). Several tools, including TnphoA, TnTAP, pPHO7, and pBADphoA (24, 26, 27), have been developed to construct the phoA fusion to the target protein. Although transposon-mediated generation of gene fusion is simple, insertion of the reporter gene at a specific target site can be tedious. This difficulty is even more pronounced if a target protein has short extramembranous loops. An alternative method is cloning of PCR products to the 5′-end of the signal sequenceless phoA gene (28). Using these two methods, we analyzed the topology of MexB. This paper reports the two-dimensional transmembrane structure of MexB.

**Experimental Procedures**

**Bacteria and Plasmids**—The *Escherichia coli* strains used were LMG194 [F− Δara714 leu−:Tn10 ΔlacX74 ΔphoA (PouII) gale gak thi rpsL] (29) and CC118 (araD139 Δara, leu7697 ΔlacX74 ΔphoA20 gale gak thi rpsE rpoB argE39, recA1) (24). The plasmid pBADphoA is a cloning vector containing a signal sequenceless phoA with a KpnI cloning site just in front of phoA. The construction of pBADphoA from pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere. The transposon delivery plasmid pMMI carries a mini-transposon TnTAP and pSWFI (30) and pBAD22 (29) will be described elsewhere.

**Construction of mexB-phoA Fusions by TnTAP**—To obtain in-frame
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Table I

| Strain          | Fusion plasmid*      | Method of construction |
|-----------------|----------------------|------------------------|
| TNE040          | pBADphoA             |                        |
| TNE041          | pBAD-P9              | Oligo<sup>a</sup>      |
| TNE042          | pBAD-Q34             | PCR                    |
| TNE043          | pBAD-E246            | PCR                    |
| TNE044          | pBAD-L688            | PCR                    |
| TNE045          | pBAD-T932            | PCR                    |
| TNE046          | pBAD-V411            | PCR                    |
| TNE047          | pBAD-G440            | PCR                    |
| TNE048          | pBAD-T473            | PCR                    |
| TNE049          | pBAD-R538            | PCR                    |
| TNE050          | pBAD-Q871            | PCR                    |
| TNE051          | pBAD-P897            | PCR                    |
| TNE052          | pBAD-S921            | PCR                    |
| TNE053          | pBAD-P972            | PCR                    |
| TNE054          | pBAD-Q1007           | PCR                    |
| TNE055          | pBAD-Q1046           | PCR                    |
| TNE056          | pMEXB1               |                        |
| TNE057          | pMEXB-D597TAP        |                        |
| TNE058          | pMEXB-T90TAP         |                        |
| TNE059          | pMEXB-V105TAP        |                        |
| TNE060          | pMEXB-R124TAP        |                        |
| TNE061          | pMEXB-I214TAP        |                        |
| TNE062          | pMEXB-G271TAP        |                        |
| TNE063          | pMEXB-T309TAP        |                        |
| TNE064          | pMEXB-L349TAP        |                        |
| TNE065          | pMEXB-F450TAP        |                        |
| TNE066          | pMEXB-N616TAP        |                        |
| TNE067          | pMEXB-L696TAP        |                        |

* AP was fused to the numbered amino acid residues.
<sup>a</sup> Oligonucleotide annealing method.

Table II

| Nucleotide sequence of the mexB-phoA fusion junction derived from pBADphoA |
|---------------------------|---------------------------|---------------------------|
| plasmid                  | 5' end                   | 3' end                   |
| pBAD-P9                  | ATG gca c-g-c TCG AAC GAG TTT | C                         |
|                          | M V P S K F              | R P P P D S              |
| pBAD-Q34                 | ATG gca c-g-c TCG AGG TTT | Aac CAG g-a-c TGC GAC TCT |
|                          | M V P S K F              | N Q V V P D S              |
| pBAD-E246                | ATG gca c-g-c TCG AGG TTT | GGC GAG g-c-t TGC GAC TCT |
|                          | M V P S K F              | G E V V P D S              |
| pBAD-L688                | ATG gca c-g-c TCG AGG TTT | AAG CTG g-c-t TGC GAC TCT |
|                          | M V P S K F              | T L V P D S              |
| pBAD-Q1007               | ATG gca c-g-c TCG AGG TTT | AAG CTG g-c-t TGC GAC TCT |
|                          | M V P S K F              | T L V P D S              |
| pBAD-Q1046               | ATG gca c-g-c TCG AGG TTT | Aac CAG g-a-c TGC GAC TCT |
|                          | M V P S K F              | N Q V V P D S              |
| pBAD-V411                | ATG - TCG AAC GGT       | GGC ATC GTC GTC GAC TCT |
|                          | M S K F                  | A I V A D S              |
| pBAD-Q1049               | ATG gca c-g-c TCG AGG TTT | CAG GCC g-c-t TGC GAC TCT |
|                          | M V P S K F              | Q V V P D S              |
| pBAD-T473                | ATG gca c-g-c TCG AGG TTT | ATC CAC g-a-c TGC GAC TCT |
|                          | M V P S K F              | I T V P D S              |
| pBAD-R538                | ATG gca c-g-c TCG AGG TTT | CAT CAG g-a-c TGC GAC TCT |
|                          | M V P S K F              | H R V P D S              |
| pBAD-Q871                | ATG gca c-g-c TCG AGG TTT | TGC CAG g-c-t TGC GAC TCT |
|                          | M V P S K F              | S Q V P D S              |
| pBAD-P897                | ATG gca c-g-c TCG AGG TTT | ATT CCG g-c-t TGC GAC TCT |
|                          | M V P S K F              | I P V P D S              |
| pBAD-S921                | ATG gca c-g-c TCG AGG TTT | CTG CTC g-c-t TGC GAC TCT |
|                          | M V P S K F              | L S V P D S              |
| pBAD-Q972                | ATG gca c-g-c TCG AGG TTT | CGG CCG g-c-t TGC GAC TCT |
|                          | M V P S K F              | R P V P D S              |
| pBAD-G1007               | ATG - TCG AAC GGT       | ATC GCC g-c-t TGC GAC TCT |
|                          | M S K F                  | L G A D S              |
| pBAD-Q1046               | ATG - TCG AAC GGT       | GGC AAA C TGC GAC TCT |

<sup>1</sup> This isoleucine residue should be valine. This was accidentally replaced by an error in the oligonucleotide synthesis. However, this replacement did not affect the result.

RESULTS

Construction of the mexB-phoA Fusions—To analyze the membrane topology of the MexB protein, we took the 12-TMS fusions of TnTAP to mexB, the pMM1 containing TnTAP was transformed into the strain E. coli CC118 carrying pMEXB1, which encodes the wild-type mexB. After transposition during overnight growth, a pool of plasmid DNA was isolated and digested with NheI to destroy pMM1, but not pMEXB1 and TnTAP. The restriction digestions were transformed again into strain CC118. Blue colonies on agar plates containing 40 μg/ml 5-bromo-4-chloro-3-indolyl phosphate, 200 μg/ml ampicillin (for pMEXB1), and 100 μg/ml kanamycin were purified. Insertions into mexB were identified by PCR.

DNA Sequencing—The nucleotide sequence was determined using the ABI PRISM<sup>™</sup> Dye Terminator Cycle Sequencing Core Kit with ampliTaq® DNA polymerase, FS. The sequencing primer used was GCAGTAATATCGCCCTGAGCAGC, reading out of the phoA gene toward the mexB gene. In addition, a primer GCCGTACACTTTGCTATT-GCC reading out of the pBADphoA vector toward the mexB gene was also used. Assay of AP Activity—AP activity was assayed by measuring the rate of hydrolysis of p-nitrophenyl phosphate in permeabilized cells as described elsewhere (31). One unit of AP activity corresponds to the rate of p-nitrophenyl phosphate hydrolysis, 1 μmol of p-nitrophenyl phosphate/minute/mg of protein at 23 °C.

Expression of the Hybrid Proteins—For the Western blot analysis of the hybrid proteins, the crude envelope fraction and whole cell lysate were prepared as described elsewhere (10). SDS-polyacrylamide gel electrophoresis (10%) and Western blotting were carried out as described previously (32). The monoclonal antibody raised against AP was used to probe the hybrid proteins. Boiling the MexB protein in SDS caused disappearance of the protein band from the gel.

Alkaline Phosphatase Activities of the Cells Harboring the Fusion Plasmids—We quantified the AP activities of the cells harboring the mexB-phoA fusions (Fig. 1). The AP activities in
these cells harboring the fusions derived from pBADphoA were divided into two major classes. One class of cells showed about 0.2–0.4 unit of AP activity, which is close to the activity in the control cell (pBADphoA, 0.32 unit) and another showed 1.7–11 units. Fusions at Pro9, Leu366, Val411, Gln440, Arg538, Pro897, Pro972, and Gln1046 belonged to the former class. Therefore, these fusion sites are most likely to be located at the periplasmic side (Fig. 1). The remaining fusions, including the Gln34, Gly440, Thr309, Leu349, Phe459, Asn616, and Leu696 locations were at the periplasmic side (Fig. 1).

All the cells harboring the mexB-phoA fusions derived from TnTAP showed an AP activity of about 1–3 units (Fig. 1), whereas the AP activity of cells containing mexB without phoA (pMEXB1) was only 0.33 unit. Based on these results, we concluded that all the fusion sites, including Asp59, Thr89, Val105, Arg124, Ile214, Gly271, Thr309, Leu349, Phe459, Asn616, and Leu696 were located at the periplasmic side (Fig. 1).

Expression of the Hybrid Proteins—The expression of hybrid proteins derived from TnTAP and pBADphoA is under the control of lac and araBAD promoters, respectively, and therefore the cells harboring the fusions were induced in the presence of 100 μM isopropyl-β-D-thiogalactopyranoside and 100 μM L-arabinose, respectively. The hybrid proteins with high and low AP activities are shown in Fig. 2, lanes 3–20 and lanes 21–27, respectively. The size of the hybrid proteins was within the range of the expected molecular mass. The protein band of the fusion at Pro9 was undetectable, since the hybrid protein is expected to be soluble in the cytoplasm. All the bands for cytoplasmic hybrid proteins showed weaker signals than the periplasmic hybrid proteins, which probably was attributable to the proteolytic degradation of the hybrid protein as reported earlier (32, 33, 34). In addition, protein bands with a higher molecular mass than expected were seen as reported elsewhere (35). This might be explained by suggesting that the chimeric proteins maintaining the native conformation bind less SDS than fully denatured proteins in the electrophoresis buffer, because the samples were subjected to electrophoresis without heating. Fusion Gly440 appeared only in a higher molecular weight range than expected and was barely seen.

**TABLE III**

| plasmid      | Sequence of the mexB-phoA fusion junction                  |
|--------------|------------------------------------------------------------|
| pMEXB-c297TA | AGC GGG CAG GAC -CTG ACT CTG ATA                          |
| pMEXB-c979TA | GCC AGG AGC ACC -CTG ACT CTG ATA                          |
| pMEXB-c105TA | ATC GGC GAC GCC -CTG ACT CTG ATA                          |
| pMEXB-c124TA | GAAGG CTG CGC -CTG ACT CTG ATA                           |
| pMEXB-c121TA | AAC CTG CGA ATT -CTG ACT CTG ATA                         |
| pMEXB-c110TA | GGG GGC CCC GCC -CTG ACT CTG ATA                         |
| pMEXB-c149TA | GAG GGC GCC GCC -CTG ACT CTG ATA                         |
| pMEXB-c459TA | ACC GCC TTC ACC -CTG ACT CTG ATA                         |
| pMEXB-c166TA | GAA GTC CGC -CTG ACT CTG ATA                             |

**Fig. 2. Western blot analysis of the MexB-AP hybrid proteins.** Crude envelope fraction was prepared as described under “Experimental Procedures,” subjected to polyacrylamide (10%) gel electrophoresis in SDS without heating, electrophotographed and visualized with monoclonal antibody raised against alkaline phosphatase (lanes 2-20). Whole cell lysate was subjected to the electrophoresis and stained as above (lanes 21-27). The sample was coded by the fusion site. Lane 1, molecular weight markers (Western doctor); lane 2, Pro9; lane 3, Gln34; lane 4, Asp59; lane 5, Thr89; lane 6, Val105; lane 7, Arg124; lane 8, Ile214; lane 9, Gln34; lane 10, Thr309; lane 11, Gln440; lane 12, Leu366; lane 13, Thr309; lane 14, Phe459; lane 15, Thr473; lane 16, Asn616; lane 17, Leu696; lane 18, Gln71; lane 19, Ser921; lane 20, Gly1007; lane 21, Leu696; lane 22, Val411; lane 23, Gln440; lane 24, Arg538; lane 25, Pro897; lane 26, Pro972; lane 27, Gln1046; lane 28, pBADphoA; lane 29, pMEXB1; lane 30, bacterial alkaline phosphatase.
MexB polypeptide. Our results experimentally ruled out this possibility.

We carried out computer-aided alignment analysis of the amino-terminal and carboxyl-terminal halves of the polypeptide from Met1 to Arg 529 and Gly 530 to Gln 1046, respectively. Fig. 3 shows 30% homology between the first and second halves. TMS 1, 2, 3, 4, 5, and 6 of the amino-terminal half could be overlaid by TMS 7, 8, 9, 10, 11, and 12 in the carboxyl-terminal half, respectively. This 2-fold repeat suggested that mexB is evolved from an ancestral gene encoding a protein of six TMS by an intragenic duplication as predicted earlier (13, 22). This is to doubly support the transmembrane nature of the segments experimentally assigned to be the membrane-spanning domain. In addition, the distribution of positive charges in the cytoplasmic and periplasmic domains were 22 and 2, respectively, excepting the first and fourth large periplasmic domains with more than 60 amino acid residues (Fig. 1). The result was in accord with the positive inside rule (38).

This is the first experimental verification of the transmembrane topology of the RND family extrusion pump protein. This experimentally verified model has the following features. (i) The MexB protein spans the membrane 12 times leaving amino and carboxyl termini at cytoplasmic side of the inner membrane as suggested for the RND family proteins (13, 22). (ii) MexB has two large hydrophilic segments with 311 and 314 amino acid residues from 29 to 338 (between TMS 1 and 2) and from 558 and 871 (between TMS 7 and 8), respectively. (iii) The membrane topology of MexB appeared to have a 2-fold repeat. These big loops might interact with the periplasmic subunit, MexA, and the outer membrane subunit, OprM. It is conceivable that these large loops transmit cellular energy to the OprM channel gate. In addition, we found 5 charged amino acid residues in the transmembrane domains. These charged residues were highly conserved in the RND family efflux proteins as aligned by the Clustal W multi-alignment software (data not shown). Specific localization of the highly conserved charged residues in the TMS suggested that they might play an important role in substrate binding and proton transport. Further studies are needed to elucidate the role of these amino acid residues in the mechanism of xenobiotics extrusion.

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REFERENCES

1. Brown, M. R. W. (1975) in Resistance of Pseudomonas aeruginosa (Brown, M. R. W., ed) pp. 71–107, John Wiley and Sons, London, 116–124

2. Nakae, T., Yoshihara, E., and Yoneyama, H. (1997) J. Infect. Chemother. 3, 173–183

3. Rella, M., and Haas, D. (1982) Antimicrob. Agents Chemother. 22, 242–249

4. Lei, Y., Sato, K., and Nakae, T. (1991) Biochem. Biophys. Res. Commun. 178, 3323–3329

5. Fukuda, H., Hosaka, M., Hirai, K., and Iyobe, S. (1990) Antimicrob. Agents Chemother. 34, 2552–2556

6. Goldstein, L. J., Galski, H., Fojo, A., Willingham, M., Lai, S. L., Gazdar, A., Pricker, R., Green, A., Crist, W., and Brodeur, G. M. (1989) J. Natl. Cancer Inst. 81, 116–124

7. Poole, K., Krebes, K., McNally, C., and Neshat, S. (1993) J. Bacteriol. 175, 7563–7572

8. Moreshed, S. R. M., Lei, Y., Yoneyama, H., and Nakae, T. (1995) Biochem.
Membrane Topology of the Extrusion Pump, MexB

Biophys. Res. Commun. 210, 356–362
11. Yoneyama, H., Ocaktan, A., Tsuda, M., and Nakae, T. (1997) Biochem. Biophys. Res. Commun. 233, 611–618
12. Ocaktan, A., Yoneyama, H., and Nakae, T. (1997) J. Biol. Chem. 272, 21964–21969
13. Paulsen, I. T., Brown, M. H., and Skurray, R. A. (1996) Microbiol. Rev. 60, 575–608
14. Dong, Q., and Mergeay, M. (1994) Mol. Microbiol. 14, 185–187
15. Dinh, T., Paulsen, I. T., and Saier, M. H., Jr. (1994) J. Bacteriol. 176, 3825–3831
16. Nikaido, H. (1994) Science 264, 382–388
17. Ma, D., Cook, D. N., Hearst, J. E., and Nikaido, H. (1994) Trends Microbiol. 2, 489–493
18. Srikumar, R., Li, X., and Poole, K. (1997) J. Bacteriol. 179, 7875–7881
19. Gotoh, N., Tsujimoto, H., Nomura, A., Okamoto, K., Tsuda, M., and Nishino, T. (1998) FEMS Microbiol. Lett. 165, 21–27
20. Yoneyama, H., Ocaktan, A., Gotoh, N., Nishino, T., and Nakae, T. (1998) Biochem. Biophys. Res. Commun. 244, 898–902
21. Kohler, T., Michéa-Hamzehpour, M., Henze, U., Gotoh, N., Curty, L. K., and Peche`re, J.-C. (1997) Mol. Microbiol. 23, 345–354
22. Saier, M. H., Jr., Tam, R., Reizer, A., and Reizer, J. (1994) Mol. Microbiol. 11, 841–847
23. von Heijne, G. (1992) J. Mol. Biol. 225, 487–494
24. Manoil, C., and Beckwith, J. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8129–8133
25. Manoil, C., Mekalanos, J. J., and Beckwith, J. (1990) J. Bacteriol. 172, 515–518
26. Ehrmann, M., Bolek, P., Mondigler, M., Boyd, D., and Lange, R. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 13111–13115
27. Gutierrez, C., and Devedjian, J. (1989) Nucleic Acids Res. 17, 3999
28. Boyd, D., Traxler, B., and Beckwich, J. (1995) J. Bacteriol. 177, 553–556
29. Guzman, L.-M., Belin, D., Carson, M. J., and Beckwith, J. (1995) J. Bacteriol. 177, 4121–4130
30. Ehrmann, M., Boyd, D., and Beckwich, J. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7574–7578
31. Michaelis, S., Inouye, H., Oliver, D., and Beckwith, J. (1983) J. Bacteriol. 154, 366–374
32. Laemmli, U. K. (1970) Nature 227, 680–685
33. Gott, P., and Boos, W. (1988) Mol. Microbiol. 2, 655–663
34. Allard, J. D., and Bertrand, K. P. (1992) J. Biol. Chem. 267, 17809–17819
35. Enomoto, H., Utemoto, T., Nishibuchi, M., Padan, E., and Nakamura, T. (1998) Biochim. Biophys. Acta 1370, 77–86
36. Cole, S. P., Bhardwaj, G., Gerlach, J. H., Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M., and Deeley, R. G. (1992) Science 258, 1650–1654
37. Fujii, R., Mutoh, M., Niwa, K., Yamada, K., Aikou, T., Nakagawa, M., Kusunoki, M., and Akiyama, S. (1994) Jpn. J. Can. Res. 85, 426–433
38. von Heijne, G. C. (1986) EMBO J. 5, 3921–3927