JOURNAL OF BACTERIOLOGY, Aug. 1995, p. 4801–4804 Vol. 177, No. 16
0021-9193/95/$04.00+0
Copyright © 1995, American Society for Microbiology

NOTES

Identification of *Pseudomonas aeruginosa glpM*, Whose Gene Product Is Required for Efficient Alginate Biosynthesis from Various Carbon Sources

HERBERT P. SCHWEIZER,* CECILIA PO, AND MELISSA K. BACIC

Department of Microbiology and Infectious Diseases, University of Calgary Health Sciences Center, Calgary, Alberta, T2N 4N1, Canada

Received 10 May 1995/Accepted 5 June 1995

In a *mucB* (*algN*) genetic background, insertion of an Ω element ~200 bp downstream of *glpD*, encoding *sn*-glycerol-3-phosphate dehydrogenase from *Pseudomonas aeruginosa*, had an adverse effect on alginate biosynthesis from various carbon sources. The insertion inactivated *glpM*, a gene encoding a 12,040-M_r hydrophobic protein containing 109 amino acids. This protein, which was expressed in a T7 RNA polymerase expression system, appears to be a cytoplasmic membrane protein.

Mucoid strains of the opportunistic pathogen *Pseudomonas aeruginosa* isolated from cystic fibrosis patients with chronic pulmonary infections secrete copious amounts of the extracellular polysaccharide alginate (for comprehensive reviews, see references 16 and 17). Whereas over the past few years significant progress has been made toward (i) understanding the complex regulation of the expression of the alginate biosynthetic genes and (ii) characterization of the gene products involved in the biosynthetic process (16, 17), little is known about the origin(s) of the carbon moieties found in the alginate molecule. The desire to understand these origins is also driven by our lack of understanding of the availability of carbon in the cystic fibrosis lung, the environment in which mucoid derivatives of *P. aeruginosa* are most often found and in which they afflict the most damage. Of particular interest to our studies are the previously reported findings that triose phosphates are obligate intermediates in the biosynthesis of alginate (1) and that fructose 1,6-bisphosphate aldolase is essential for this to occur (2). We are therefore focussing our efforts on one peripheral carbon metabolic pathway which is capable of directly providing these triose phosphate intermediates (2), namely the glycerol metabolic pathway.

Although some of the basic events involved in glycerol metabolism were studied some time ago (4, 18, 29, 31), we have only recently begun to unravel at the molecular level the pathway involved in glycerol metabolism in *P. aeruginosa*. As a first step in elucidating the molecular organization and mode(s) of regulation of the *glp* genes of *P. aeruginosa*, we have recently cloned the *glpD* gene, encoding *sn*-glycerol-3-phosphate dehydrogenase, a key enzyme of the glycerol metabolic pathway (29), and subsequently the genes encoding the glycerol transporter (*glpF*), glycerol kinase (*glpK*), and the regulatory gene (*glpR*) (23) (Fig. 1A). In this communication, we report the observation that insertions in the *glpD* region have an adverse effect on alginate biosynthesis and that this may be due to inactivation of a closely linked gene, *glpM.*

Insertions in the *glpD* region have an adverse effect on alginate biosynthesis. Construction of insertion mutants in the *glpD* region of wild-type strain PAO1 (8) was done by a previously described *sacB*-based gene replacement procedure (26). The *glpD* mutant strain PAO151 was obtained by insertion of the tetracycline resistance (Tc^r)-encoding Ω element from pHP45ΩTc (5) into the single *Not*I site (Fig. 1A) within *glpD* on pEB22ΔE1 (29). Similarly, strain PAO206 containing a chromosomal mutation of the *glpD* downstream region was obtained by insertion of a gentamicin resistance (Gm^r)-encoding Ω element (27) at a *Nae*I site (marked in Fig. 1B). Mucoid *P. aeruginosa* derivatives were isolated as described by Martin et al. (15), utilizing plasmids on which *mucB* (*algN*) was inactivated with the appropriate selectable marker, *mucB*::ΩGm^r in the *glpD*::ΩTc^r mutant PAO151 and *mucB*::ΩTc^r (15) in the *glpM*::ΩGm^r mutant strain PAO206 and wild-type PAO1. The resulting *mucB* (*algN*) strains were designated PAO151M, PAO206M, and PAO1M, respectively.

As expected, the *glpD* mutant strain PAO151 no longer grew on glycerol but grew normally on the other carbon sources tested (glucose, succinate, and mannitol). In contrast, PAO206 grew normally on glycerol and expressed *sn*-glycerol-3-phosphate dehydrogenase and glycerol transport in the same manner as PAO1. In contrast to their isogenic parental strain PAO1M, after an overnight incubation on *Pseudomonas* isolation agar (PIA) plates (DIFCO, Detroit, Mich.), PAO151M and PAO206M appeared nonmucoid and even after prolonged incubation on this medium did not appear as mucoid as PAO1M. For quantitative measurements, alginates were collected from supernatants of cultures of the various mutants grown for 48 h in alginate-promoting (AP) medium (20) supplemented with the carbon sources (10 mM) indicated in Fig. 2 in addition to the 100 mM L-gluconate and 100 mM D-monosodium glutamate already contained in AP medium. Alginate was assayed utilizing the carbazole method (10), as described previously (6). Surprisingly, both PAO151M and PAO206M were pleiotropically defective in alginate biosynthesis from all of the tested carbon sources (Fig. 2) (since the data

* Corresponding author. Present address: Department of Microbiology, College of Veterinary Medicine and Biomedical Science, Colorado State University, Fort Collins, CO 80523. Phone: (970) 491-6136. Fax: (970) 491-1815.

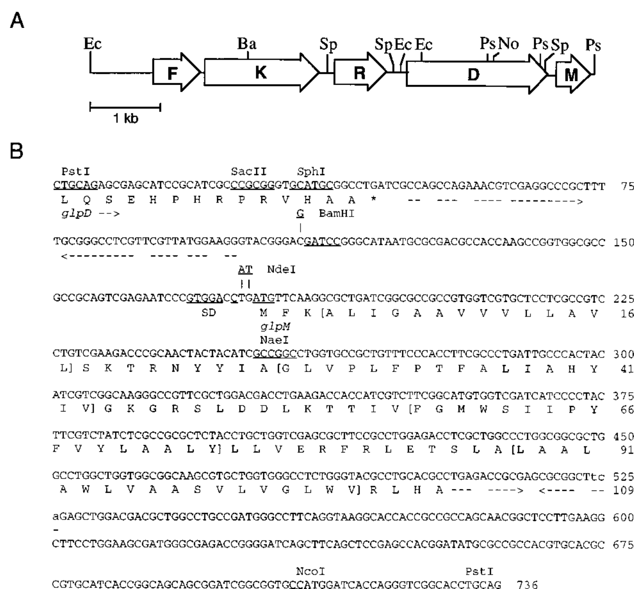

FIG. 1. Organization of *glpD* region of *P. aeruginosa* and nucleotide sequence of *glpM* region. (A) The map and genomic organization of the *glpD* region of the *P. aeruginosa* chromosome was derived from our previously published work (29) as well as from unpublished data (23). Abbreviations: D, *glpD* (glycerol-3-phosphate dehydrogenase); F, *glpF* (glycerol facilitator); K, *glpK* (glycerol kinase); M, *glpM* (this study); R, *glpR* (*glp* regulatory gene). Only the relevant restriction enzyme cleavage sites are shown (Ba, *Bam*HI; Ec, *Eco*RI; No, *Not*I; Ps, *Pst*I; and Sp, *Sph*I). (B) The nucleotide sequence shown was determined from double-stranded plasmid templates by the dideoxy-chain termination method either by using ^{35}S-labeled nucleotides as previously described (29) or by using the Applied Biosystems *Taq* DyeDeoxy Terminator cycle sequencing kit and the protocols provided therein. The predicted amino acid sequence of the *glpM* reading frame is indicated below the appropriate codons. Its putative ribosome-binding (Shine-Dalgarno [SD]) site is underlined. Amino acids constituting hydrophobic regions are bracketed. The dashed underlines (between nt 49 to 100 and nt 505 to 526) mark bases capable of forming putative stem-loop structures. Nucleotides indicated above the continuing nucleotide sequence are changes introduced by site-directed mutagenesis (12) to generate the indicated restriction sites.

obtained for PAO151 and PAO151M were similar to those shown for strains PAO206 and PAO206M, they were omitted from the figure for the sake of clarity). Similar results were obtained for PIA medium whose formulation calls for the addition of glycerol to a final concentration of 27 mM. It

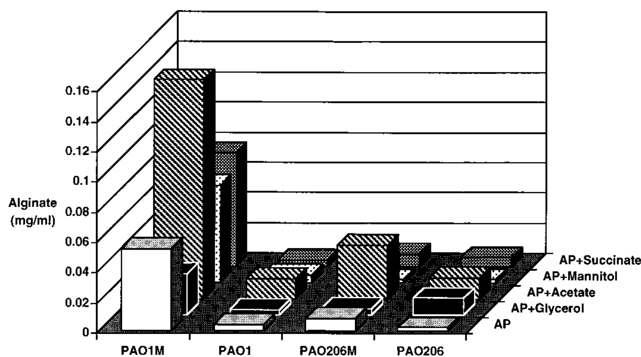

FIG. 2. Alginate biosynthesis from carbohydrates in *glpM*$^+$ and *glpM* strains. Strains PAO1 (*glpM*$^+$ *mucB*$^+$), PAO1M (*glpM*$^+$ *mucB*::ΩTc^r), PAO206 (*glpM*::ΩGm^r *mucB*$^+$), and PAO206M (*glpM*::ΩGm^r *mucB*::ΩTc^r) were grown for 48 h at 37°C in AP medium supplemented with the indicated carbon sources. The alginate content in the culture supernatants was determined as described in the text.

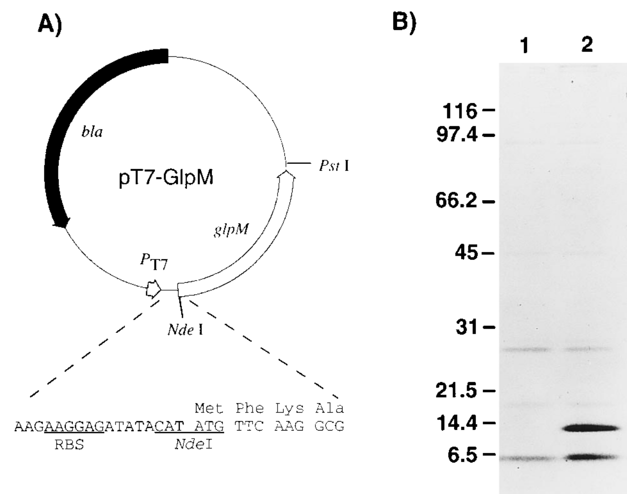

FIG. 3. Identification of GlpM polypeptide. (A) After introduction of an *Nde*I site at the ATG start codon of *glpM*, a 559-bp *Nde*I-*Pst*I fragment was ligated between the same sites of pT7-7 (32). P_{T7} and RBS indicate the Φ10 T7 promoter and ribosome-binding site, respectively. (B) Proteins were selectively labeled with [^{35}S]methionine, separated by sodium dodecyl sulfate-polyacrylamide electrophoresis, and visualized by autoradiography as described in the text. Lanes: 1, pT7-7; 2, pT7-GlpM. The positions of the molecular weight (in thousands) markers (BioRad) for (top to bottom) β-Gal, phosphorylase *b*, bovine serum albumin, carbonic anhydrase, trypsin inhibitor, lysozyme, and aprotinin are indicated on the left.

should be emphasized that in our hands *mucB* derivatives always produce much smaller amounts of alginate, compared with some mucoid cystic fibrosis isolates which can produce alginate at ~1 mg/ml (20), although they appear mucoid on solid medium. It is evident that PAO206M cannot efficiently utilize mannitol for alginate biosynthesis (Fig. 2). This is in contrast to a fructose-1,6-bisphosphate aldolase (*ald*) mutant which was unable to synthesize alginate from the same carbon sources tested, with the exception of mannitol, which was normally incorporated into alginate (2). Thus, the mutations isolated in the present study seem to not merely affect carbon flux through its metabolic pathways but rather seem to affect alginate biosynthesis more directly.

Identification of *glpM*. To ascertain the nature of the mutation caused by insertion of the ΩGm^r determinant in strain PAO206M, the nucleotide sequence of the *glpD* downstream region was determined and is presented in Fig. 1B. The region contains an open reading frame (ORF) of 326 nucleotides (nt) which could encode a protein composed of 109 amino acids with a combined calculated molecular weight of 12,039. This ORF could be translated from two possible start codons in close proximity to one another, a GTG at nt 169 or an ATG at nt 178. Although both potential start codons are preceded by reasonable Shine-Dalgarno sequences (30), we favor the ATG as the start codon because of its much higher abundancy as an initiation codon, especially in close proximity to a GTG. However, in the absence of N-terminal amino acid sequencing data the correct start cannot be ascertained. Several lines of evidence indicate that this ORF represents a gene. First, a computer-assisted analysis utilizing the codon preference plot function of the MacVector program (IBI, New Haven, Conn.) and the *P. aeruginosa* codon bias file showed a high probability for the indicated ORF. The same analyses indicated no probable ORFs on the opposite strand in this region. Second, a protein of the expected size is expressed in an *Escherichia coli* T7 expression system containing the sequences encompassing nt

TABLE 1. Subcellular distribution of Φ(*glpM′*-′*lacZ*)*hyb*-encoded β-Gal Activity

Fraction[a]	β-Gal		NADH oxidase		Glucose-6-phosphate dehydrogenase	
	nmol/min/mg	% Total activity	nmol/min/mg	% Total activity	nmol/min/mg	% Total activity
Soluble	1.7	3	0.14	2	0.59	100
Membrane	56.9	97	7.92	98	ND[b]	

[a] Strain PAO1 harboring pPS202 expressing a Φ(*glpM′*-′*lacZ*)*hyb* from P*lac* was grown overnight in Luria broth medium supplemented with 500 μg of carbenicillin per ml. Subcellular fractions were prepared, and enzymatic activities were measured spectrophotometrically as described in the text.

[b] ND, not detectable.

178 to 736 (Fig. 3B) (see below). Third, cleavage with *Nae*I between the alanine and glycine codons at nt 255 and ligation to the *Sma*I sites of the ORF probe vectors of the pPZ series (24) lead to formation of an active β-galactosidase (β-Gal) in-frame fusion protein only in the predicted vector (see below). The intergenic region between *glpD* and the gene encoding this ORF does not contain any sequences with similarities to known promoter sequences, suggesting that *glpD* and the gene encoding this ORF may form a transcriptional unit. Therefore, this gene may be a member of the *glp* regulon and is being designated *glpM* as an acronym for membrane-associated *glp* gene product. This notion is corroborated by several observations. First, we previously described that the potentially strong stem-loop structure that could be formed by the palindromic sequences of the *glpD*-*glpM* intergenic region (Fig. 1B) does not function as an efficient transcriptional terminator (29). Second, a cistronic organization of *glpD* and *glpM* may explain the polar effects of the ΩTc insertion in strain PAO151M (see above). Third, analysis of the deduced amino acid sequence and other evidence indicate that its gene product encodes a membrane-associated protein (see below). The nucleotide sequences immediately downstream of *glpM* and including the TGA stop codon could form a relatively stable secondary structure ($\Delta G = -13.3$ kcal/mol [-55.5 kJ/mol]; underlined in Fig. 1B). On the same strand, there is no obvious ORF with the same transcriptional orientation downstream of *glpM*. However, a BLAST search of GenBank revealed a potential ORF on the opposite strand extending from nt 736 to 524 (its TGA stop codon is indicated in lowercase letters in Fig. 1B) with significant similarity (35% identity and 62% similarity over a span of 63 amino acids) to RdmC, a putative esterase from *Streptomyces purpurascens* (GenBank accession no. U10405). This ORF is also predicted by a codon preference plot analysis. Thus, the adverse effects on alginate metabolism obtained by insertional inactivation of this ORF at the *Nae*I site within *glpM* are unlikely due to polar effects on another downstream gene.

Analysis and expression of *glpM*. Several attempts to express GlpM in *Escherichia coli* and *P. aeruginosa* by using various expressions systems utilizing as templates (cf. Fig. 1B) (i) a 2,266-bp *Bsi*CI-*Pst*I fragment containing *glpD* and *glpM* (29), (ii) the 736-bp *Pst*I fragment, or (iii) a 627-bp *Bam*HI-*Pst*I fragment, which did not contain the palindromic sequences located in the *glpD*-*glpM* intergenic region, failed. Although the exact reasons for this failure are unclear, it appears that expression of *glpM* may be tightly regulated at the RNA level and that overexpression may be lethal to the cell (28). This notion is supported by the observations that plasmids containing *glpD* and *glpM* are unstable in both *E. coli* and *P. aeruginosa*, whereas plasmids containing *glpD* alone are stable in both organisms (28, 29). To facilitate subcloning and expression of the *glpM* gene in the tightly regulated pT7-7 system (32), an *Nde*I site was introduced at the putative ATG start codon. This was achieved by site-directed mutagenesis (12), employing a mutagenic primer which introduced two nucleotide changes (indicated in Fig. 1B) and generated a unique *Nde*I site. Digestion with *Nde*I plus *Pst*I and ligation between the same sites of pT7-7 placed the *glpM* ATG start codon at the appropriate distance from the strong ribosome-binding site of the Φ10 gene on pT7-7 (detailed in Fig. 3A). T7 RNA polymerase-directed protein synthesis was performed in *E. coli* BL21(DE3) as previously described (25). Labeled samples were analyzed on a 0.1% sodium dodecyl sulfate–5 to 20% polyacrylamide gradient gel, using the discontinuous buffer system of Laemmli (11). As shown in Fig. 3B, utilizing this technology we were able to express GlpM.

Is GlpM a membrane protein? A Kyte-Doolittle analysis showed that GlpM is a hydrophobic protein with several distinct stretches of hydrophobic residues (bracketed in Fig. 1B) which are separated by shorter stretches of hydrophilic residues. This analysis indicated that GlpM may be a membrane protein, and a limited gene fusion analysis supported this notion. A GlpM′-′LacZ in-frame protein fusion was constructed by ligating a 480-bp *Nae*I-*Pvu*II fragment, composed of the first 255 bp of the sequence shown in Fig. 1B plus 225 bp from pUC19 (34) containing the *lac* promoter (P*lac*), to *Sma*I-cleaved pPZ10, pPZ20, and pPZ30 ORF probe vector DNAs (24). These broad-host-range vectors allow the isolation of β-Gal protein fusions in all three translational reading frames. As predicted, only clones derived from the pPZ10 ligation formed active an Φ(*glpM′*-′*lacZ*)*hyb* transcribed from P*lac* as a result of in-frame fusion of the GCC alanine codon (nt 253 to 255) to a GGG glycine codon from the polylinker region of pPZ10 (24). One such clone, pPS202, was transformed into PAO1, and the cells were cultured overnight at 37°C in 200 ml of Luria broth medium supplemented with 500 μg carbenicillin per ml. A cell extract was obtained after sonication (six times for 15 s each time, with cooling, in a Braunsonic 1510 disrupter [Braun AG, Melsungen, Germany] equipped with a microtip and set at 100 W), and total membranes were obtained by subjecting the cell extract to centrifugation at $200{,}000 \times g$ for 1 h at 4°C in a Beckman tabletop ultracentrifuge TLA100.2 rotor. Enzyme activities present in subcellular fractions were determined by previously described methods and include β-Gal (19), NADH oxidase (21), and glucose-6-phosphate dehydrogenase (33). Protein concentrations were determined by the method of Bradford (3), with bovine serum albumin as the standard. The results presented in Table 1 show that the GlpM′-′LacZ hybrid protein is tightly membrane associated. When the total membrane fraction was further subfractionated into inner and outer membranes by sucrose gradient centrifugation (9), β-Gal activity was found in the inner membrane (data not shown). It is hypothesized that this membrane association of the GlpM′-′LacZ hybrid protein is mediated by the hydrophobic N-terminal GlpM sequences (first bracketed stretch in Fig. 1B) and thus, that GlpM itself may be a membrane-associated protein.

Conclusions. Besides GlpM, the only other known examples of membrane proteins which are similar in size and architecture are the proteins of the family of staphylococcal multiple-drug-resistant (*smr*) proteins (7). These proteins are members of the family of efflux membrane transporter proteins which were first implicated in antibiotic resistance (14) but more recently have been shown to be also involved in other processes necessitating membrane translocation events, e.g., capsular polysialic acid biosynthesis in *E. coli* K1 (22). Although in recent years significant progress has been made toward understanding the biochemistry of alginate biosynthesis (for reviews, see references 16 and 17), little is known about how its precursors are transported across the cytoplasmic membrane, although a very small (10-kDa) membrane-associated protein has previously been implicated in the binding of GDP-mannuronate for its translocation through the membrane (17). Although it is tempting to speculate about the possible role of GlpM in this process based on its overall hydrophobicity and putative membrane location, the relationship of the previously observed 10-kDa protein and the 12-kDa GlpM protein, if any, is presently unclear.

Clearly, before answers to these questions can be sought, the difficulties of expression of GlpM alone in *P. aeruginosa* must be resolved. In the absence of a functional complementation system the exact nature of the contribution of *glpM* and its gene product to alginate biosynthesis cannot be completely ascertained. The data in this report point to a role of the GlpM protein in biosynthesis of this important *P. aeruginosa* virulence factor. However, because we have not succeeded in complementing the *glpM* mutation in PAO206M, the possibility that the loss of mucoidy in this strain is not due to loss of GlpM function cannot be dismissed. In this context it should also be noted that the *glpD* region has recently been mapped to the same *Dpn*I and *Spe*I chromosomal macrorestriction fragments in the 30-min region of the *P. aeruginosa* chromosome as *algD*, encoding a key enzyme (GDP-mannose dehydrogenase) of the alginate biosynthetic pathway (13).

Nucleotide sequence accession number. The nucleotide sequence of the DNA segment discussed in this report has been deposited in GenBank and assigned accession no. L06231.

We thank V. Deretic for the gift of plasmid pDMB100 and M. Vasil for assistance with the codon preference plot analysis.

This research was supported by grant MT-11245 from the Medical Research Council of Canada. H.P.S. is an MRC Medical Scholar.

REFERENCES

1. **Banerjee, P. C., R. I. Vanags, A. M. Chakrabarty, and P. K. Maitra.** 1983. Alginic acid synthesis in *Pseudomonas aeruginosa* mutants defective in carbohydrate metabolism. J. Bacteriol. **155:**238–245.
2. **Banerjee, P. C., R. I. Vanags, A. M. Chakrabarty, and P. K. Maitra.** 1985. Fructose-1,6-bisphosphate aldolase activity is essential for synthesis of alginate from glucose by *Pseudomonas aeruginosa*. J. Bacteriol. **161:**458–460.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
4. **Cuskey, S. M., and P. V. Phibbs.** 1985. Chromosomal mapping of mutations affecting glycerol and glucose metabolism in *Pseudomonas aeruginosa* PAO. J. Bacteriol. **162:**872–880.
5. **Fellay, R., J. Frey, and H. Krisch.** 1987. Interposon mutagenesis of soil and water bacteria: a family of DNA fragments designed for in vitro insertional mutagenesis of Gram-negative bacteria. Gene **52:**147–152.
6. **Franklin, M. J., and D. E. Ohman.** 1993. Identification of *algF* in the alginate biosynthetic gene cluster of *Pseudomonas aeruginosa* which is required for alginate acetylation. J. Bacteriol. **175:**5057–5065.
7. **Grinius, L., G. Dreguniene, E. B. Goldberg, C.-H. Liao, and S. J. Projan.** 1992. A staphylococcal multidrug resistance gene product is a member of a new protein family. Plasmid **27:**119–129.
8. **Holloway, B. W., and C. Zhang.** 1990. Pseudomonas aeruginosa PAO, p. 2.71–2.78. *In* S. J. O'Brien (ed.), Genetic maps—locus maps of complex genomes. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
9. **Ito, K., T. Sato, and T. Yura.** 1977. Synthesis and assembly of the membrane proteins of *Escherichia coli*. Cell **11:**551–559.
10. **Knutson, C. A., and A. Jeanes.** 1968. A new modification of the carbazole analysis: application to heteropolysaccharides. Anal. Biochem. **24:**470–481.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
12. **Lewis, M. K., and D. V. Thompson.** 1990. Efficient site directed in vitro mutagenesis using ampicillin selection. Nucleic Acids Res. **18:**3439–3443.
13. **Liao, X., I. Charlebois, C. Ouellet, M. J. Morency, K. Dewar, J. Lightfoot, J. Foster, R. Siehnel, H. P. Schweizer, J. Lam, R. E. W. Hancock, and R. C. Levesque.** Physical mapping of 32 genetic markers on the *Pseudomonas aeruginosa* PAO1 chromosome. Submitted for publication.
14. **Ma, D., D. N. Cook, J. E. Hearst, and H. Nikaido.** 1994. Efflux pumps and drug resistance in Gram-negative bacteria. Trends Microbiol. **2:**489–493.
15. **Martin, D. W., M. J. Schurr, M. H. Mudd, and V. Deretic.** 1993. Differentiation of *Pseudomonas aeruginosa* into the alginate-producing form: inactivation of *mucB* causes conversion to mucoidy. Mol. Microbiol. **9:**497–506.
16. **May, T. B., and A. M. Chakrabarty.** 1994. *Pseudomonas aeruginosa*: genes and enzymes of alginate synthesis. Trends Microbiol. **2:**151–157.
17. **May, T. B., D. Shinabarger, R. Maharaj, J. Kato, L. Chu, J. D. DeVault, S. Roychoudhury, N. A. Zielinski, A. Berry, R. K. Rothmel, T. Misra, and A. M. Chakrabarty.** 1991. Alginate synthesis by *Pseudomonas aeruginosa*: a key pathogenic factor in chronic pulmonary infections of cystic fibrosis patients. Clin. Microbiol. Rev. **4:**191–206.
18. **McCowen, S. M., P. V. Phibbs, and T. W. Feary.** 1981. Glycerol catabolism in wild-type and mutant strains of *Pseudomonas aeruginosa*. Curr. Microbiol. **5:**191–196.
19. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Ohman, D. E., and A. M. Chakrabarty.** 1981. Genetic mapping of chromosomal determinants for the production of the exopolysaccharide alginate in a *Pseudomonas aeruginosa* cyctic fibrosis isolate. Infect. Immun. **33:**142–148.
21. **Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson.** 1972. Mechanism of assembly of the outer membrane of *Salmonella typhimurium*. J. Biol. Chem. **247:**3962–3972.
22. **Pavelka, M. S., S. F. Hayes, and R. P. Silver.** 1994. Characterization of KpsT, the ATP-binding component of the ABC-transporter involved with the export of capsular polysialic acid in *Escherichia coli* K1. J. Biol. Chem. **269:**20149–20158.
23. **Po, C., and H. P. Schweizer.** Unpublished data.
24. **Schweizer, H. P.** 1991. Improved broad-host-range *lac*-based plasmid vectors for the isolation and characterization of protein fusions in *Pseudomonas aeruginosa*. Gene **103:**87–92.
25. **Schweizer, H. P.** 1991. The *agmR* gene, an environmentally responsive gene, complements defective *glpR*, which encodes the putative activator for glycerol metabolism in *Pseudomonas aeruginosa*. J. Bacteriol. **173:**6798–6806.
26. **Schweizer, H. P.** 1992. Allelic exchange in *Pseudomonas aeruginosa* using novel ColE1-type vectors and a family of cassettes containing a portable *oriT* and the counter-selectable *Bacillus subtilis sacB* marker. Mol. Microbiol. **6:**1195–1204.
27. **Schweizer, H. P.** Unpublished data.
28. **Schweizer, H. P., and M. K. Bacic.** Unpublished data.
29. **Schweizer, H. P., and C. Po.** 1994. Cloning and nucleotide sequence of the *glpD* gene encoding *sn*-glycerol-3-phosphate dehydrogenase from *Pseudomonas aeruginosa*. J. Bacteriol. **176:**2184–2193.
30. **Shine, J., and L. Dalgarno.** 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosome RNA; complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71:**1342–1346.
31. **Siegel, L. S., and P. V. Phibbs.** 1979. Glycerol and L-α-glycerol-3-phosphate uptake in *Pseudomonas aeruginosa*. Curr. Microbiol. **2:**251–256.
32. **Tabor, S.** 1994. Expression using the T7 RNA polymerase/promoter system, p. 16-1–16-10. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Short protocols in molecular biology. John Wiley & Sons, New York.
33. **Tisa, L. S., and B. P. Rosen.** 1990. Molecular characterization of an anion pump. J. Biol. Chem. **265:**190–194.
34. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.