JOURNAL OF BACTERIOLOGY, Jan. 1991, p. 768–775
0021-9193/91/020768-08$02.00/0

Vol. 173, No. 2

Conservation of the Gene for Outer Membrane Protein OprF in the Family *Pseudomonadaceae*: Sequence of the *Pseudomonas syringae oprF* Gene

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Received 27 June 1990/Accepted 26 October 1990

The conservation of the *oprF* gene for the major outer membrane protein OprF was determined by restriction mapping and Southern blot hybridization with the *Pseudomonas aeruginosa oprF* gene as a probe. The restriction map was highly conserved among 16 of the 17 serotype type strains and 42 clinical isolates of *P. aeruginosa*. Only the serotype 12 isolate and one clinical isolate showed small differences in restriction pattern. Southern probing of *PstI* chromosomal digests of 14 species from the family *Pseudomonadaceae* revealed that only the nine members of rRNA homology group I hybridized with the *oprF* gene. To reveal the actual extent of homology, the *oprF* gene and its product were characterized in *Pseudomonas syringae*. Nine strains of *P. syringae* from seven different pathovars hybridized with the *P. aeruginosa* gene to produce five different but related restriction maps. All produced an OprF protein in their outer membranes with the same apparent molecular weight as that of *P. aeruginosa* OprF. In each case the protein reacted with monoclonal antibody MA4-10 and was similarly heat and 2-mercaptoethanol modifiable. The purified OprF protein of the type strain *P. syringae* pv. syringae ATCC 19310 reconstituted small channels in lipid bilayer membranes. The *oprF* gene from this latter strain was cloned and sequenced. Despite the low level of DNA hybridization between *P. aeruginosa* and *P. syringae* DNA, the OprF gene was highly conserved between the species with 72% DNA sequence identity and 68% amino acid sequence identity overall. The carboxy terminus-encoding region of *P. syringae oprF* showed 85 and 33% identity, respectively, with the same regions of the *P. aeruginosa oprF* and *Escherichia coli ompA* genes.

The family *Pseudomonadaceae* is an extremely diverse collation of bacteria, although rRNA homology studies have revealed five more closely related groupings (9, 20, 28–30). One such grouping, group I according to Palleroni (28), is commonly called the fluorescent pseudomonads, since some members of this group secrete fluorescent pigments (28). Two important species belonging to this group are the human opportunistic pathogen *Pseudomonas aeruginosa* and the plant pathogenic species *Pseudomonas syringae*. Despite sharing rRNA homology, these two species have many differences including cell shape, optimal growth temperature, number of flagella, nutritional and metabolic properties, and guanosine plus cytosine (G+C) content of their DNA (67% for *P. aeruginosa* and 59 to 61% for *P. syringae*). Limited comparative studies on their outer membranes, however, have indicated some similarities (17, 19), although the outer membrane proteins of *P. syringae*, with the exception of the iron-regulated proteins (5), have received far less attention than have those of *P. aeruginosa* (17, 26). In this study we examined in detail the well-characterized protein OprF and its structural gene to determine the extent of its conservation in the fluorescent pseudomonads and particularly in *P. syringae*.

The outer membrane protein OprF of *P. aeruginosa* is a bifunctional protein. It has been shown to have porin activity, forming small water-filled channels (38). In addition, it has been proposed to form a limited number of large channels in the *P. aeruginosa* outer membrane (3, 26), although this proposal has been recently disputed in the literature (40). OprF also has a structural role in determining cell shape and ability to grow in low-osmolarity medium (14, 38). The structural role of OprF has been shown to be homologous to that of the *Escherichia coli* OmpA protein in an *E. coli lpp* mutant background (38). Indeed, it has been shown that OprF produced from the cloned gene can substitute for OmpA in determining cell shape in such a background (38). Furthermore, OprF and OmpA are immunologically cross-reactive and demonstrate 33% identity of amino acid sequences in their carboxy-terminal halves. Other studies have indicated that both *Neisseria* sp. (15) and *Haemophilus influenzae* (35) contain an outer membrane protein related to OmpA and OprF. Since we previously demonstrated that one class of monoclonal antibodies directed against OprF of *P. aeruginosa* also recognized an outer membrane protein of *P. syringae* (23), we cloned and sequenced the gene for this latter protein to determine the extent of conservation of OprF in these two species.

MATERIALS AND METHODS

Bacterial strains and plasmids. The main strains of *P. aeruginosa*, *P. syringae*, and *E. coli* and their plasmids used in this study are listed in Table 1. The *P. syringae* pathovars were a generous gift from Richard Moore, Agriculture Canada, London, Ontario. In addition, we utilized the 17 *P. aeruginosa* type strains of the International Antigen Typing scheme, 43 clinical isolates of *P. aeruginosa* (including 35

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TABLE 1. Main bacterial strains and plasmids utilized

Strain or plasmid	Characteristics	Reference or source
P. aeruginosa H103	PA01 Cm^r prototroph, wild-type reference strain	16
P. syringae		
ATCC 19310	pv. syringae (type strain, weakly pathogenic for lilac)	ATCC 19310
3679	pv. papulans (pathogenic for apple)	R. Moore
B3	pv. glycenea (pathogenic for soybean)	R. Moore
5D19	pv. syringae (pathogenic for lilac)	R. Moore
HB6	pv. phaseolicola (pathogenic for bean)	R. Moore
3000	pv. tomato (pathogenic for tomato)	R. Moore
1108	pv. tomato (pathogenic for tomato)	R. Moore
2738	pv. antirrhini (pathogenic for snapdragon)	R. Moore
GB1	pv. tabaci (pathogenic for tobacco)	R. Moore
E. coli		
DH5αF′	F′ *endA1 hsdR17*(r_K^-, m_K^+) *supE44 thi-1 recA1 gyrA96 relA1* λ^- φ80d*lacZ*ΔM15 Δ(*lacZYA argF*)U169	Bethesda Research Laboratories
C483	DH5α(pGC31)	This study
Plasmids		
pTZ19U	GeneScribe cloning vector	U.S. Biochemical
pTZ19R	GeneScribe cloning vector	U.S. Biochemical
pRK404	Broad-host-range, low-copy-number vector	10
pGC31	pRK404 plus a 2.5-kb *Pst*I-*Bam*HI fragment encoding *P. syringae oprF* gene	This study

from patients with cystic fibrosis and 8 blood isolates from patients with sepsis), and several individual representative strains from *Pseudomonas* sp. and *Azotobacter vinelandii* as previously described (23, 24).

Media and growth conditions. The culture media were Luria broth (1% tryptone, 0.5% yeast extract, 1% NaCl) for *E. coli* strains, proteose peptone no. 2 (1%) for *P. aeruginosa*, and 0.8% nutrient both–0.5% yeast extract–0.5% glucose for *P. syringae* strains. For preparation of single-stranded DNA, the medium used was 1.6% tryptic peptone–1% yeast extract–0.5% NaCl. Solid media contained 2% agar. All medium components were from Difco Laboratories, Detroit, Mich. *E. coli* and *P. aeruginosa* strains were grown at 37°C overnight. *P. syringae* strains were grown at 25 to 27°C overnight or longer if growth was slow. Most strains of *P. syringae* would not grow at 30°C. All cultures were agitated during growth. Antibiotic concentrations for cultures of *E. coli* were 20 μg/ml for tetracycline and 50 μg/ml for ampicillin. Short-term storage of strains was on plates at 4°C, and long-term storage was in 7% dimethyl sulfoxide at −70°C.

DNA techniques. Bacterial chromosomal DNA was isolated by the hexadecyltrimethylammonium bromide method (1). For Southern hybridization, restriction enzyme-digested DNA was separated on 0.8% agarose gels and transferred to Zeta-probe nylon membranes (Bio-Rad Laboratories, Richmond, Calif.) by the method of Reed and Mann (33). Hybridizations were done by the standard protocol recommended by Bio-Rad Laboratories at 50°C with washes at 42°C. All other general DNA techniques were carried out as described in two methodology manuals (1, 22).

Cloning of the *P. syringae oprF* gene. Cloning of the *P. syringae oprF* gene followed the general strategy described for the *oprH* gene (2). Briefly, restriction digests of *P. syringae* chromosomal DNA were screened by Southern hybridization with plasmid pWW2200 containing a 2.4-kb *Pst*I insert specifying the entire *P. aeruginosa oprF* gene. This identified a single 9-kb *Bam*HI-*Hin*dIII fragment, which was cloned from restriction enzyme-digested, size-fractionated *P. syringae* DNA into the low-copy-number vector pRK404. The resultant clone, identified by colony Southern hybridization with plasmid pWW2200 and by colony Western immunoblotting with monoclonal antibody MA4-10, contained the entire *P. syringae oprF* gene as determined by Western immunoblotting with MA4-10. The region containing the *oprF* gene was then subcloned on a 2.5-kb *Bam*HI-*Pst*I fragment into the vector pRK404 to create plasmid pGC31.

Sequencing. The 2.5-kb *Bam*HI-*Pst*I fragment containing the *P. syringae oprF* gene was digested with *Sal*I to produce 0.9-kb *Bam*HI-*Sal*I and 1.6-kb *Sal*I-*Pst*I fragments. These fragments were isolated by using an NA-45 DEAE membrane (Schleicher and Schuell, Inc., Keene, N.H.) and the band interception method of Winberg and Hammerskjold (37) and then ligated into the Gene Scribe-Z vectors pTZ19R and pTZ19U (U.S. Biochemical Corp., Cleveland, Ohio). These clones were then subjected to Erase-A-Base (Promega, Madison, Wis.) exonuclease III digestion (18) to give progressive unidirectional deletions. However, since limited heterogeneity of deletion endpoints was obtained, seven different oligonucleotides were synthesized as sequencing primers. Single-stranded DNA was isolated as described previously (2) and sequenced by the Sanger et al. method (34) with a combination of the Sequenase (U.S. Biochemical) and Taq Track (Promega) kits as described in the manufacturers' protocols, since in our hands neither kit was capable of unambiguously determining the entire DNA sequence of *oprF*. In summary, we sequenced both DNA strands of a region of 1,137 bp covering the *P. syringae oprF* gene.

Outer membrane and black lipid bilayer techniques. Outer membranes were prepared by the one-step sucrose gradient method of Hancock and Carey (16). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and differential solubilizations to determine heat and 2-mercaptoethanol modifiability were done as described previously (16). Western immunoblotting with monoclonal antibody MA4-10 was performed as described previously (23). *P. syringae* OprF protein was purified by differential solubilization, SDS-

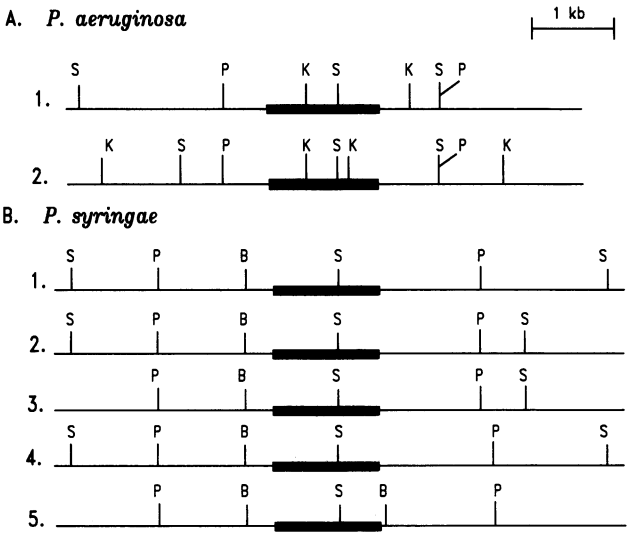

FIG. 1. (A) Restriction endonuclease map of chromosomal DNA from *P. aeruginosa*: Lines: 1, serotypes 1 through 11 and 13 through 17; 2, serotype 12. (B) Restriction endonuclease map of chromosomal DNA from *P. syringae* pathovar strains (Table 1). Lines: 1, ATCC 19310 and B3 (no data for *Pst*I); 2, 5D19; 3, 3679; 4, HB6 and GB1; 5, 3000, 2738, and 1108 (no data for *Sal*I). Abbreviations: B, *Bam*HI; K, *Kpn*I; P, *Pst*I; S, *Sal*I. The thick bar indicates the location of the *oprF* gene (transcribed from left to right). All maps aligned at a conserved *Sal*I site found within the *oprF* gene.

polyacrylamide gel electrophoresis, and elution from gel slices as described previously for *P. aeruginosa* OprF (39), except that passive elution instead of electroelution from gel slices was utilized.

Nucleotide sequence accession number. The nucleotide sequence data presented in this report have been submitted to GenBank under accession number M55408.

RESULTS

Conservation of the *oprF* gene in *P. aeruginosa* and other fluorescent pseudomonads. The *oprF* gene was mapped in several *P. aeruginosa* strains by Southern hybridization of a 2.5-kb *Pst*I fragment containing the *oprF* gene with agarose-gel-separated chromosomal DNA cleaved by restriction endonuclease digestion. Comparisons of our laboratory wild-type strain H103 (the source of the cloned gene) with the 17 type strains of the International Antigen Typing Scheme revealed extensive conservation of the restriction map within 1 kb of the *oprF* gene; only a single strain, the serotype 12 type strain, had an altered map for *Kpn*I (Fig. 1). Interestingly, the *oprF* sequence published by Duchene et al. (12) was obtained from a serotype 12 isolate and also featured two *Kpn*I sites within the *oprF* genes at the positions shown in Fig. 1. We also found one clinical isolate from a patient with sepsis, isolate 50, which had *Kpn*I sites in the same place as the serotype 12 isolate, but we did not serotype this isolate. A further 42 clinical isolates of *P. aeruginosa* were characterized with respect to their *Kpn*I and *Pst*I sites. Only a *Kpn*I site found 3.3 kb upstream of the *oprF* gene varied in location in two of these isolates; it was 0.5 kb further upstream of *oprF*.

Hybridization of the *oprF* gene with *Pst*I-digested chromosomal DNA from a range of species revealed that only species belonging to the same rRNA homology group as *P. aeruginosa*, including eight *Pseudomonas* sp. and *A. vinelandii*, were capable of hybridizing to our *oprF* gene probe (Table 2). This pattern of hybridization correlated well with the antigenic conservation of another outer membrane protein, OprL (protein H2), as revealed by reactivity with monoclonal antibody MA1-6 (24) (Table 2).

TABLE 2. Hybridization of *Pst*I-digested *Pseudomonas* DNA with plasmid pWW2200 containing the *oprF* gene of *P. aeruginosa*

Source of *Pst*I-digested chromosomal DNA	rRNA homology group	Size of hybridizing fragment (kb)	Reaction with MA1-6[a]
P. aeruginosa PAO1	I	2.4	+
P. syringae ATCC 19310[T][b]	I	3.4	+
P. fluorescens ATCC 13525[T]	I	4.0	+
P. putida ATCC 12633[T]	I	3.4	+
P. stutzeri ATCC 17588[T]	I	3.0	+
P. aureofaciens ATCC 13985[T]	I	2.8	+
P. chlororaphis ATCC 9446[T]	I	2.8	+
P. anguilliseptica ET2	I	3.0	+
A. vinelandii OP	I	5.0	+
P. pseudomallei ATCC 23343[T]	II	–[c]	–
P. solanacearum ATCC 11696[T]	II	–	–
P. cepacia ATCC 25416[T]	II	–	–
P. acidovorans ATCC 15668	III	–	–
P. maltophilia ATCC 13637[T][d]	V	–	–
E. coli DH5αF′		–	–

[a] Data of Mutharia and Hancock (24). Monoclonal antibody MAI-6 is specific for outer membrane protein H2 (OprL). +, Reaction observed; –, no reaction observed.
[b] T, Type strain.
[c] –, No hybridizing fragment observed.
[d] Three other clinical isolates also gave negative results.

Conservation of the *oprF* gene and OprF protein in *P. syringae*. The above data were consistent with indications (38) that the *oprF* gene product was evolutionarily conserved and thus might be of taxonomic interest. To further evaluate this, we examined its conservation in a selection of *P. syringae* isolates. *P. syringae* strains are generally phytopathogenic and are subdivided, according to host range, into over 40 pathovars. They were of great interest in this study due to the known diversity of the different pathovars and their clear differences from the opportunistic human pathogen *P. aeruginosa* (28). Despite this, it has been shown that a class of OprF-specific monoclonal antibodies recognizes the *P. syringae* type strain ATCC 19310 (23). This was confirmed here and extended to another eight *P. syringae* isolates representing seven pathovars (Fig. 2, lanes 4 through 8). Outer membranes were isolated from all nine *P. syringae* isolates listed in Table 1. All strains had an OprF equivalent (as revealed by Western immunoblotting with monoclonal antibody MA4-10) that was of apparently similar molecular weight in all pathovars and in *P. aeruginosa* (Fig. 2). Altering solubilization conditions before SDS-polyacrylamide gel electrophoresis permitted the observations that heating in SDS and treatment with 2-mercaptoethanol caused shifts in electrophoretic mobility (data not shown). Thus OprF proteins from all pathovars were, like *P. aeruginosa* OprF (16), both heat and 2-mercaptoethanol modifiable.

OprF was purified from the *P. syringae* type strain ATCC 19310 and examined for its ability to reconstitute water-filled channels in black lipid bilayers. Small channels were observed, with an average single-channel conductance of 0.28 nS from 100 recorded events (compared with an average single-channel conductance of 0.35 nS for the small OprF

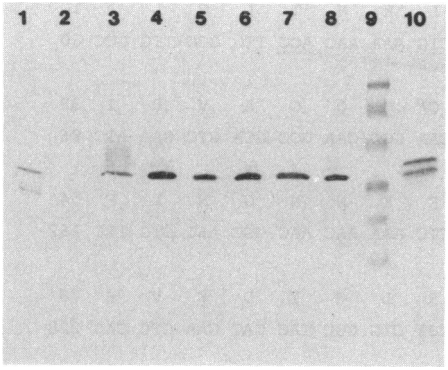

FIG. 2. Antigenic similarities in OprF proteins from different species as demonstrated by Western immunoblot transfer of SDS-polyacrylamide gel-separated outer membrane proteins to a nylon membrane and interaction with *P. aeruginosa* protein OprF-specific monoclonal antibody MA4-10. Lanes: 1, *E. coli* DH5αF'(pGC31); 2, *E. coli* DH5αF'; 3, *P. aeruginosa* H103; 4, *P. syringae* ATCC 19310; 5, *P. syringae* 3679; 6, *P. syringae* 5D19; 7, *P. syringae* HB6; 8, *P. syringae* GB1; 9, molecular weight markers; 10, purified *P. aeruginosa* protein OprF.

channel [39]). Larger channels were also observed, but no extensive attempts were made to characterize these.

Restriction mapping of the *oprF* gene in the different *P. syringae* isolates revealed strong conservation of sites immediately adjacent to the gene with all isolates having a triad of sites (*Pst*I-*Bam*HI-*Sal*I) in common (Fig. 1). Some heterogeneity was observed beyond the carboxy terminus-encoding end of the *oprF* gene (Fig. 1B). Three of the strains had an extra *Bam*HI site, whereas two alternative locations for a *Pst*I site were observed. In all, five different patterns were observed. To more fully characterize the conservation of the *oprF* gene in *P. syringae*, we decided to clone and sequence *oprF* from the type strain ATCC 19310.

Cloning and sequence of the *oprF* gene. As described in Materials and Methods, plasmid pGC31, containing a 2.5-kb *Bam*HI-*Pst*I insert, was isolated. *E. coli* strains harboring plasmid pGC31 expressed the *P. syringae* OprF protein in their outer membranes (Fig. 2, cf. lanes 1 and 2). Although some of the OprF protein was proteolyzed to a lower-molecular-weight form, the majority of the expressed OprF proteins had molecular weights indistinguishable from that of OprF in the *P. syringae* outer membrane, reacted with the OprF-specific monoclonal antibody MA4-10 (Fig. 2, lane 4), and, like the native protein, were heat and 2-mercaptoethanol modifiable on SDS-polyacrylamide gels (data not shown).

The sequence of DNA encoding OprF was determined (Fig. 3). The gene had one continuous reading frame of 1,032 bp coding for a sequence of 344 amino acids. The first 24 amino acids were predicted to be a typical procaryotic signal sequence found for all precursors of outer membrane proteins studied to date (36). The deduced molecular weight of the mature protein was 34,225.

Comparison of the *P. syringae* and *P. aeruginosa oprF* genes. The *P. aeruginosa oprF* gene sequence was previously determined by Duchene et al. (12). Its size (1,050 bp) was similar to that of the above-described *P. syringae oprF* gene. Comparison of the sequences revealed that a *Sal*I site was similarly placed in both genes in that there was conservation of the special features of the amino acid sequence flanking this region (notably four closely spaced cysteine residues that form two disulfide bonds (16) and an alanine-proline-rich region [12]).

The overall G+C content of the *P. syringae oprF* gene was 55.3%, which is lower than the 60.2% value for the *P. aeruginosa oprF* gene (12), reflecting the respective G+C contents of these species. The difference was apparently due to a lower bias for G+C in the third position of codons of *P. syringae oprF* (61.7%) compared with that of *P. aeruginosa oprF* (78.2%). Despite this, the DNA sequences, when aligned to maximize amino acid identities (see below), were found to be 72.3% identical. In contrast, the DNA sequences flanking the *oprF* gene showed only 34% identity.

Introduction of five small gaps permitted substantial alignment of the predicted amino acid sequences of OprF from *P. syringae* and *P. aeruginosa* (Fig. 4, Table 3). In particular, the carboxy-terminal half of *P. aeruginosa* OprF, which had been previously shown to have homology to the equivalent portion of the *E. coli* OmpA protein (4, 12, 38), was highly similar to the carboxy terminus of *P. syringae* OprF with 85% identity and 10% conservative substitutions.

The high normalized alignment scores of 647 for the entire sequence, 459 for the amino-terminal halves, and 875 for the carboxy-terminal halves indicated certain matches between the two OprF amino acid sequences (11). Comparison of the sequences by the Needleman and Wunsch method (25), with a genetic code matrix with a bias of 0, a gap penalty of 4, and 100 random runs gave alignment scores of 61.9 for the entire sequence, 31.2 for the amino-terminal halves, and 49.2 for the carboxy-terminal halves. All of these values were significant (above 3 standard deviation units) matches.

As shown previously for the carboxy terminus of the *P. aeruginosa* OprF sequence (38), the same portion of the *P. syringae* sequence could be matched to the *E. coli* OmpA carboxy-terminal amino acid sequence (data not shown). The normalized alignment score of 264 and alignment score of 7.9 (Table 3) indicated probable and significant (above 3 standard deviation units) matches, respectively. In contrast, the amino-terminal halves of *P. syringae* OprF and *E. coli* OmpA proteins could not be well aligned, giving improbable or not significant results.

DISCUSSION

The *P. aeruginosa* and *P. syringae* OprF protein sequences showed a high degree of similarity, especially at their carboxy-terminal ends, which had an amino acid identity of 85%. The overall amino acid identity of the two proteins was 68%; when conservative substitutions were included, the identity was 86%. This reflected a nucleotide sequence identity of 72.3%. This similarity is remarkable, since the DNA homology between the two *Pseudomonas* species is much lower, and, as discussed above, the two species are different in many ways. Although *P. syringae* and *P. aeruginosa* have an rRNA similarity of 88 to 92% (30), they have DNA similarity levels of 0 to 14% as determined by hybridization experiments (20, 29, 31). Consistent with this observed high degree of similarity between the two OprF amino acid sequences, these proteins were immunologically cross-reactive, of similar molecular weights, heat and 2-mercaptoethanol modifiable, and capable of reconstituting channels in lipid bilayer membranes. The extent of conservation of the *oprF* gene and its product between these two species, in contrast to the apparent divergence of the chromosomal DNA, suggests an important role for protein OprF in the cell, with the carboxy-terminal end of the protein being most important. This was further

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opr F

                                                    M   K   L   K   N   T   L   G   L   A  10
TCCCCATGTGTGGGACTGCTTAATAATCATCAGATGGGGATTTAACGG ATG AAA CTG AAA AAC ACC TTG GGC TTG GCC  30
                                                  ----

 I   G   T   I   V   A   A   T   S   F   G   A   L   A | Q   G   Q   G   A   V   E   I  32
ATT GGT ACT ATT GTT GCC GCA ACT TCG TTC GGC GCG CTG GCT CAA GGC CAA GGC GCA GTC GAA ATC  96

 E   G   F   A   K   K   E   M   Y   D   S   A   R   D   F   K   N   N   G   N   L   F  54
GAA GGC TTC GCC AAG AAA GAA ATG TAC GAC AGC GCC CGT GAT TTC AAA AAC AAC GGC AAC CTG TTC 162

 G   G   S   I   G   Y   F   L   T   D   D   V   E   L   R   L   G   Y   D   E   V   H  76
GGC GGC TCG ATT GGC TAC TTC CTG ACC GAC GAC GTT GAA TTG CGT CTG GGC TAC GAC GAA GTC CAC 228

 N   V   R   S   D   D   G   K   N   I   K   G   A   D   T   A   L   D   A   L   Y   H  98
AAC GTT CGT AGC GAT GAT GGC AAG AAC ATC AAG GGC GCA GAC ACT GCC CTG GAC GCT CTC TAC CAC 294

 F   N   N   P   G   D   M   L   R   P   Y   V   S   A   G   F   S   D   Q   S   I   G 120
TTC AAC AAC CCA GGC GAC ATG CTG CGT CCA TAC GTT TCT GCC GGT TTC TCC GAC CAG AGC ATT GGC 360

 Q   N   G   R   N   G   R   N   G   S   T   F   A   N   I   G   G   G   P   K   L   Y 142
CAG AAC GGT CGT AAC GGT CGT AAC GGT TCT ACC TTC GCC AAC ATC GGC GGC GGC CCC AAG CTC TAC 426

 F   T   D   N   F   Y   A   R   A   G   V   E   A   Q   Y   N   I   D   Q   G   D   T 164
TTC ACT GAC AAC TTC TAC GCC CGT GCT GGC GTT GAA GCT CAA TAC AAC ATC GAC CAA GGC GAC ACC 492

 E   W   A   P   S   V   G   I   G   V   N   F   G   G   G   S   K   K   V   E   A   A 186
GAG TGG GCT CCA AGC GTC GGT ATC GGC GTA AAC TTC GGT GGC GGC AGC AAG AAA GTT GAA GCA GCA 558

 P   A   P   V   A   E   V   C   S   D   S   D   N   D   G   V   C   D   N   V   D   K 208
CCA GCT CCA GTA GCT GAA GTG TGC TCC GAC AGC GAC AAC GAC GGC GTG TGC GAC AAC GTC GAC AAG 624

 C   P   D   T   P   A   N   V   T   V   D   A   D   G   C   P   A   V   A   E   V   V 230
TGC CCG GAC ACC CCA GCC AAC GTT ACC GTT GAC GCT GAT GGC TGC CCA GCA GTT GCC GAA GTG GTT 690

 R   V   E   L   D   V   K   F   D   F   D   K   S   V   V   K   P   N   S   Y   G   D 252
CGT GTT GAG CTG GAC GTG AAG TTC GAT TTC GAC AAA TCC GTA GTC AAG CCT AAC AGC TAC GGC GAC 756

 I   K   N   L   A   D   F   M   Q   Q   Y   P   Q   T   T   T   T   V   E   G   H   T 274
ATC AAG AAC CTC GCT GAC TTC ATG CAG CAG TAC CCA CAG ACC ACC ACC ACT GTT GAA GGT CAC ACT 822

 D   S   V   G   P   D   A   Y   N   Q   K   L   S   E   R   R   A   N   A   V   K   Q 296
GAC TCC GTC GGT CCT GAC GCT TAC AAC CAA AAA CTG TCC GAG CGT CGT GCA AAC GCC GTT AAA CAG 888

 V   L   V   N   Q   Y   G   V   G   A   S   R   V   N   S   V   G   Y   G   E   S   K 318
GTT CTG GTT AAC CAG TAC GGT GTT GGC GCT AGC CGC GTA AAC TCG GTT GGT TAC GGC GAA AGC AAG 954

 P   V   A   D   N   A   T   E   A   G   R   A   V   N   R   R   V   E   A   E   V   E 340
CCA GTT GCT GAT AAC GCA ACT GAA GCT GGC CGC GCA GTT AAC CGT CGC GTA GAA GCA GAA GTA GAA 1020

 A   Q   A   K   *                                                                   344
GCT CAA GCT AAG TAA TTAGCCGCTTGTACTGAAAAGCCCGGCTTAGGCCGGGCTTTTCTTTGCCTGCGATTTGGCATTGCGT 1102
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CTGTTCAGGCGGGCTTGATGTCAGCCGGCATTCAGCTGTGCGTGGTCCATCAGCGTTTGATTCAGCAGCTCATTGCTGTACC      1184
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FIG. 3. DNA sequence and derived amino acid sequence of the *oprF* gene from *P. syringae* ATCC 19310. The vertical line indicates the end of the signal peptide. The putative ribosome-binding site (GGGA) and rho-independent terminator region are underlined.

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P.a. OprF  MKLKNTLGVVIGSLVAASAMNAFAQGQNSVEIEAFGKRYFTDSVRNMKN-ADLYGGSIGY 59
P.s. OprF  MKLKNTLGLAIGTIVAATSFGALAQGQGAVEIEGFAKKEMYDSARDFKNNGNLFGGSIGY 60

P.a. OprF  FLTDDVELALSYGEYHDVRGTYETGNKKVHGNLTSLDAIYHFGTPGVGLRPYVSAGLAHQ 119
P.s. OprF  FLTDDVELRLGYDEVHNVRSD---DGKNIKGADTALDALYHFNNPGDMLRPYVSAGFSDQ 117

P.a. OprF  NITNINSDSQGRQQMTMANIGAGLKYYFTENFFAKASLDGQYGLEKRDNGHQGEWMA--G 177
P.s. OprF  SIGQ--NGRNGRNGSTFANIGGGPKLYFTDNFYARAGVEAQYNIDQGDT----EWAPSVG 171

P.a. OprF  LGVGFNFGGSKAAPAPEPVADVCSDSDNDGVCDNVDKCPDTPANVTVDANGCPAVAEVVR 237
P.s. OprF  IGVNFGGGSKKVEAAPAPVAEVCSDSDNDGVCDNVDKCPDTPANVTVDADGCPAVAEVVR 231

P.a. OprF  VQLDVKFDFDKSKVKENSYADIKNLADFMKQYPSTSTTVEGHTDSVGTDAYNQKLSERRA 297
P.s. OprF  VELDVKFDFDKSVVKPNSYGDIKNLADFMQQYPQTTTTVEGHTDSVGPDAYNQKLSERRA 291

P.a. OprF  NAVRDVLVNEYGVEGGRVNAVGYGESRPVADNATAEGRAINRRVEAEVEAEAK*        350
P.s. OprF  NAVKQVLVNQYGVGASRVNSVGYGESKPVADNATEAGRAVNRRVEAEVEAQAK*        344
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FIG. 4. Comparison of the *P. syringae* OprF amino acid sequence with the *P. aeruginosa* OprF amino acid sequence. Vertical lines indicate amino acid identities, and dots indicate conserved substitutions. Dashes indicate gaps introduced to maximize identity. Conservative substitutions were determined by the Dayhoff minimum-mutation matrix using a matching score of 0.9 as a cutoff (6). The *P. aeruginosa* sequence (P.a. OprF) was from reference 12. The *P. syringae* sequence (P.s. OprF) is deduced from the nucleotide sequence in Fig. 3.

suggested by the similarity observed between *P. syringae* protein OprF and *E. coli* protein OmpA, which demonstrated 33% identical amino acids at their carboxy-terminal ends and, when conservative substitutions were included, 58.6% similarity at their carboxy-terminal ends. In contrast, the amino-terminal ends showed only 18.5% similarity. These data are consistent with previous observations indicating a significant role for OprF and OmpA in cell structure (38).

Restriction site mapping of the *oprF* gene from different *P. syringae* pathovars demonstrated a conserved *Sal*I site within the gene and conserved *Pst*I and *Bam*HI sites close to the amino-terminal end of the gene for all the pathovars but variability in the location of the other restriction sites surrounding the *oprF* gene. From the nine different *P. syringae* pathovars, five different maps were observed (Fig. 1). Restriction site maps of the two strains of *P. syringae* pv. tomato and the strain *P. syringae* pv. antirrhini were the most dissimilar (Fig. 1B). These three pathovars had an additional *Bam*HI site just outside the carboxy-terminal end of the gene, and the flanking *Sal*I sites were twice the distance from the middle *Sal*I site (data not shown) than were the flanking *Sal*I sites of the other pathovars, consistent in part with proposed differences between *P. syringae* pv. syringae (the type species) and *P. syringae* pv. tomato (7, 8). The genetic diversity of *P. syringae* pathovars has been explored to some extent in previous studies. Studies utilizing both large (7, 21) and small (8) DNA probes from *P. syringae* cloned DNA have served to emphasize diversity between pathovars of *P. syringae* and, in some cases, diversity among strains from a single pathovar. Such studies, while of some assistance in differentiating individual strains and/or grouping strains, offer inherent drawbacks in determining genetic and/or evolutionary relationships. An example of note concerns probing of individual *P. aeruginosa* isolates with a *toxA* gene-flanking sequence probe (27). This study has indicated that almost every single *P. aeruginosa* strain demonstrates a different-sized hybridizing restriction fragment (possibly due to the fact that the exotoxin A gene is apparently associated with an insertion sequence element

TABLE 3. Summary of the comparison of the deduced *P. syringae* OprF amino acid sequence with that of *P. aeruginosa* OprF and *E. coli* OmpA proteins

Sequences compared	Region[a]	Gaps introduced[b]	Amino acid identity (%)	Conservative substitutions (%)[c]	Total conservation (%)[d]	Doolittle normalized alignment score	Needleman/Wunsch alignment score
P. syringae OprF and *P. aeruginosa* OprF	Amino terminus encoding	5	53	25	78	459[e]	31.2[f]
	Carboxy terminus encoding	0	85	10	95	875[e]	49.2[f]
	Whole gene	5	68	18	86	653[e]	61.9[f]
P. syringae OprF and *E. coli* OmpA	Amino terminus encoding	6	20	20	40	114	2.2
	Carboxyl terminus encoding	4	33	26	59	264[g]	7.9[f]
	Whole gene	10	24	22	56	172	7.5[f]

[a] Divisions between amino- and carboxy-encoding regions were amino acids 184, 190, and 202 for *P. syringae* OprF, *P. aeruginosa* OprF, and *E. coli* OmpA, respectively.
[b] Number of gaps necessary to maximize identity.
[c] Conservative substitutions were assessed by the Dayhoff (6) minimum mutation matrix with a cutoff score of 0.9.
[d] Identity plus conservative substitutions.
[e] Certain match (11).
[f] Significant match (above 3 standard deviation units) (see reference 25 as implemented by Dayhoff [6] and Feng et al. [13] with the genetic code matrix with a gap penalty of 4 and a bias parameter of 0). A value of ≥3.0 suggests significant similarity.
[g] Probable match (11).

[32]). In contrast numerous other studies have indicated that, rather than the kind of extreme genetic diversity indicated by the above probe, *P. aeruginosa* represents a rather close grouping of strains (28). Consistent with this, the *oprF* gene restriction pattern was highly conserved in 61 *P. aeruginosa* strains (Fig. 1). Similarly *P. syringae* strains share many biochemical properties despite differences in plant host range (20, 28). Thus, we feel that it is more instructive to start from a well-conserved core sequence (e.g., the *Pst*I-*Bam*HI-*Sal*I sequence overlapping the *oprF* gene) and then utilize restriction fragment length polymorphisms in the flanking sequences to group *P. syringae* strains.

ACKNOWLEDGMENTS

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Canadian Cystic Fibrosis Foundation to R.E.W.H. W.W. was the recipient of a scholarship from the Canadian Cystic Fibrosis Foundation. S.S. was supported by a grant from the Cystic Fibrosis Foundation.

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