Specificity of the *Pseudomonas aeruginosa* PAO1 lipoprotein I gene as a DNA probe and PCR target region within the *Pseudomonadaceae*

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The lipoprotein I gene (*oprI*) of *Pseudomonas aeruginosa* PAO1 was cloned and sequenced. A high degree of homology was found between our cloned PAO1 gene sequence and two published *oprI* sequences. Specific oligonucleotides were designed to amplify the *oprI* gene by the polymerase chain reaction (PCR). The potential of either the complete gene sequence or the specific oligonucleotide primers as a tool for rapid strain identification was directly assessed against bacterial colonies by PCR or against purified genomic DNA by Southern blot analysis, using a number of representative strains within the *Pseudomonadaceae*. The *oprI* gene was found to be well conserved within RNA group I.

Introduction

There is an increasing interest in techniques for the rapid identification of *Pseudomonas* species because of their importance in the clinical environment. *Pseudomonas* species have been identified as the causal agent of many opportunistic infections (Sanford, 1990) and also as the causative agent of corneal ulcers (Reid & Wood, 1979; Wilson *et al.*, 1981). The currently used identification methods require a variety of biochemical tests, which are sometimes insufficient for the identification of certain species of *Pseudomonas*, such as various glucose-oxidizing strains, mucoid strains and apyocyanogenic *P. aeruginosa* (Gilardi, 1985). It would therefore be of interest to supplement existing methods with DNA probe and PCR techniques, provided that these are sufficiently accurate and specific.

The first applications of molecular genetic techniques for bacterial taxonomy studies used the techniques of

whole genome hybridization (for DNA) and of cataloguing oligonucleotides produced by degradation of rRNA species. These methods form the basis upon which *Pseudomonadaceae* are classified into five RNA groups containing DNA subgroups (Palleroni, 1984). Although the results obtained are still very useful for taxonomic purposes, the techniques are generally too time-consuming and difficult for clinical application. Also, a given RNA group may bring together species which differ greatly in their pathogenicity toward humans, and human pathogens may be dispersed between several different RNA groups within a family.

The newer DNA hybridization techniques with specific DNA probes have been widely used for the identification of many pathogens (Tenover, 1988); however, these techniques still involve a considerable expense of time and reagents and commonly use radioactive materials. New approaches based on the amplification of a specific genomic DNA target by a thermostable polymerase, the polymerase chain reaction (PCR) (Mullis & Faloona, 1987), promise to extend the scope of DNA-based methods in terms of rapidity and simplicity. The characteristic amplified DNA fragment can serve for direct identification on gel electrophoresis without the need for radiolabelling.

In this study, we describe the use of a gene coding for an outer-membrane protein (*OprI*, Hancock *et al.*, 1990)

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of *P. aeruginosa* PAO1 (Holloway, 1969) to serve both as a DNA probe and as a PCR target for the rapid identification of RNA group I of the *Pseudomonadaceae*.

Methods

Bacterial strains, phages and growth conditions. The reference and clinical bacterial strains used in this study are listed in Tables 1 and 2 according to the most recent classification (Gilardi, 1990). The clinical isolates used were obtained from patients of the Sainte-Justine Hospital and identified using standard microbiology laboratory techniques (Gilardi, 1985). *Escherichia coli* HB101 (*supE44 hsdS20* (r_B m_B) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*) was used for propagation of the cosmid pLAFR1 (Friedman *et al.*, 1982) and *E. coli* TG1 (*supE hsdΔ5 thi Δ(lac-proAB) F[traD36 proAB⁺ lacI^a lacDM15]*) as a host for M13 phages (Yanisch-Perron *et al.*, 1985).

Pseudomonas, *Flavimonas*, *Chryseomonas*, *Xanthomonas* and *E. coli* strains were grown in 2YT broth (Sambrook *et al.*, 1989). The *Streptococcus*, *Staphylococcus* and *Branhamella* strains were grown in trypticase soy broth (30 g l⁻¹) (BBL 11768). The *Haemophilus* strain

Table 1. Reference bacterial strains used in this study

Strain	Origin*
<i>Pseudomonadaceae</i>	
<i>Pseudomonas aeruginosa</i> PAO1	LSPQ
<i>Pseudomonas aeruginosa</i> serotype 1 to 17	ATCC 33338 to 33364
<i>Pseudomonas alcaligenes</i>	ATCC 14909
<i>Pseudomonas cepacia</i>	LSPQ and ATCC 25416
<i>Pseudomonas chlororaphis</i>	ATCC 9446
<i>Pseudomonas diminuta</i>	LSPQ and ATCC 11568
<i>Pseudomonas fluorescens</i>	LSPQ and ATCC 13525
<i>Pseudomonas mendocina</i>	ATCC 25411
<i>Pseudomonas paucimobilis</i> †	LSPQ and ATCC 29837
<i>Pseudomonas pickettii</i>	LSPQ and ATCC 27511
<i>Pseudomonas pseudoalcaligenes</i>	strain KF707‡ and ATCC 17440
<i>Pseudomonas putida</i> biotype A	LSPQ and ATCC 12633
<i>Pseudomonas stutzeri</i>	LSPQ and ATCC 17588
<i>Pseudomonas vesicularis</i>	LSPQ and ATCC 11426
<i>Chryseomonas luteola</i> §	ATCC 43273
<i>Comamonas acidovorans</i>	LSPQ and ATCC 15668
<i>Comamonas testosteroni</i>	LSPQ and ATCC 11996
<i>Flavimonas oryzihabitans</i> §	LSPQ and ATCC 43272
<i>Xanthomonas maltophilia</i> ¶	LSPQ and ATCC 13637
Other species	
<i>Branhamella catarrhalis</i>	ATCC 25238
<i>Escherichia coli</i> HB101	Bolivar & Backman (1979)
<i>Haemophilus influenzae</i>	ATCC 33391
<i>Staphylococcus aureus</i>	ATCC 29213
<i>Streptococcus mitis</i>	ATCC 33399

* LSPQ, Laboratoire de Santé Publique du Québec, Sainte-Anne des Plaines, Quebec, Canada; ATCC, American Type Culture Collection, Rockville, MD, USA.

† The transfer of *P. paucimobilis* to a new genus, *Sphingomonas*, has been proposed (Yabuuchi *et al.*, 1990).

‡ Furukawa & Miyazaki (1986).

|| Tamaoka *et al.* (1987).

§ Holmes *et al.* (1987).

¶ Swings *et al.* (1983).

Table 2. Clinical isolates used in this study

Isolate*	Origin†	Isolate*	Origin†
<i>Pseudomonas aeruginosa</i> ‡		<i>Pseudomonas cepacia</i>	
M/S 16 Expectoration (CFP)		M/S 567 Expectoration (CFP)	
M/S 32 Expectoration		<i>Pseudomonas putida</i>	
M/S 41 Expectoration		M/S 430 Throat	
M/S 47 Expectoration (CFP)		<i>Pseudomonas stutzeri</i>	
M/S 86 Expectoration (CFP)		M/S 441 Expectoration (CFP)	
M/S 96 Neck wound		<i>Xanthomonas maltophilia</i>	
M/S 99 Expectoration (CFP)		M/S 426 Endocarditis	
M/S 179 Peritoneum		M/S 428 Septicaemia	
M/S 190 Pus			
M/S 193 Urine			
M/S 225 Urine			
M/S 240 Expectoration			
M/S 246 Faeces			
M/S 273 CFP			
M/S 280 Urine			
M/S 313 Bronchial secretions (CFP)			
M/S 316 Expectoration (CFP)			

* Identification number of clinical isolates from Sainte-Justine hospital.

† CFP, sample obtained from cystic fibrosis patients.

‡ All *P. aeruginosa* isolates had a non-mucoid phenotype.

was grown in brain heart infusion broth with 10 mg haemin l⁻¹. *E. coli* HB101 containing the cosmid pLAFR1 was grown in the presence of tetracycline at 15 mg l⁻¹ in 2YT broth.

Cloning and sequencing of the *oprI* gene. A *P. aeruginosa* PAO1 genomic DNA library was constructed using the cosmid pLAFR1. Approximately 10⁴ clones were screened with sera from cystic fibrosis patients known to have high antibody titres against the outer-membrane proteins of *P. aeruginosa* (Hancock *et al.*, 1984). The recombinant clones were transferred to nitrocellulose membranes and incubated with sera which had previously been absorbed against *E. coli* HB101 containing the pLAFR1 cosmid. The bound antibodies were visualized with an alkaline phosphatase biotin-streptavidin detection system (Jackson Immunological). All antibody-positive clones contained a similar 23 kb fragment as shown by restriction enzyme analysis (data not shown). The positive clones were shown to express a 10 kDa protein by SDS-PAGE, reacting with the cystic fibrosis antibodies by Western blot analysis. This molecular mass corresponded to the OprI protein as previously reported (Mizuno & Kageyama, 1979). The 23 kb insert from one of the positive cosmids was restricted with *Pst*I and a 2.3 kb fragment was ligated to *Pst*I-digested M13mp19 vector. The ligation mixture was then used to transform *E. coli* TG1.

M13 clones were sequenced by the dideoxy chain-termination method (Sanger *et al.*, 1977). Oligonucleotides derived from the recently published sequence of the *oprI* gene from a clinical isolate of *P. aeruginosa* (Cornelis *et al.*, 1989) were used to identify the appropriate M13 clones and also to sequence the gene. The sequencing reactions were performed as described in the protocol for the T7 Sequencing Kit (Pharmacia) with [α -³²S]dATP. Approximately 350 base pairs were sequenced in both orientations.

Extraction of genomic DNA. The procedure for the extraction of total genomic DNA was based on a sodium perchlorate procedure (Marmur, 1961; Tardif, 1985). The following protocol was found to be critical for the subsequent restriction enzyme digestion of the genomic DNA. Cells were grown overnight in 15 ml of appropriate medium, then centrifuged (6000 g for 5 min) and resuspended in 10 ml GET (50 mM-

glucose, 10 mM-EDTA and 25 mM-Tris/HCl, pH 8) containing 12 g lysozyme l⁻¹. The suspension was divided into four tubes (2.5 ml) and incubated at 37 °C for 30 min with agitation (225 r.p.m.). Then 0.5 ml 20% (w/v) SDS was added and the incubation was continued for 30 min at the same temperature. A volume of 500 µl of 5 M-sodium perchlorate was added in five steps (100 µl at a time, incubating for 5 min between each addition) to a final concentration of 0.83 M. The DNA was extracted with phenol/chloroform (1:1, v/v), precipitated with an equal volume of ice-cold 2-propanol and recovered by centrifugation at 3000 g for 10 min. The DNA was redissolved in 10 mM-Tris/HCl and 1 mM-EDTA (pH 8.0) and treated with RNAase A (100 mg l⁻¹) for approximately 1 h at 37 °C. The DNA solution was then extracted with chloroform to remove impurities and stored at -20 °C.

Agarose gel electrophoresis and Southern blotting. Purified genomic DNA concentrations were estimated visually by loading samples on 0.7% agarose gel containing ethidium bromide (200 µg l⁻¹) and examination under UV light after electrophoresis. Approximately equal amounts of genomic DNA (10 µg) from each organism were digested overnight with the restriction enzyme *Bst*EII at 60 °C. In order to avoid evaporation the digests were overlaid with paraffin oil. Chromosomal digests and PCR reaction mixtures (5 µl) were separated by electrophoresis in 0.7% and 1.5% (w/v) agarose respectively, containing ethidium bromide (200 µg l⁻¹). The DNA was transferred onto nylon membranes (Zeta Probe, Bio-Rad) by vacuum blotting. The steps for the depurination, denaturation, neutralization and transfer were performed as specified by the manufacturer of the vacuum blotting equipment used (LKB 2016 VacuGene, Pharmacia).

PCR amplification. The 25-mer primers used for enzymic amplification were derived from the extremities of the *P. aeruginosa* PAO1 sequenced lipoprotein I gene. Restriction enzyme sites (underlined) were built into the primers at their 5' end to facilitate eventual cloning of the gene in an expression vector. The forward primer had the sequence 5'-ACTCGAATTCATGAACAACGTTCTG-3' and the reverse primer had the sequence 5'-CTGGAAGCTTCTATTACTTGCGGCT-3'. All manipulations were performed using disposable, sterile equipment and wearing gloves to avoid contamination. A medium to large bacterial colony was transferred with a sterile tip or needle into 50 µl of sterile distilled water. The samples were boiled for 5 min and centrifuged (13000 g) for another 5 min. Thirty-five microlitres of supernatant was added to 5 µl 10 × PCR buffer (500 mM-KCl, 100 mM-Tris/HCl pH 8.3, 15 mM-MgCl₂), 200 µM each of the deoxynucleotides dGTP, TTP, dATP and dCTP (Pharmacia), and 25 pmol each of forward and reverse primer. The samples were boiled for 5 min, quickly centrifuged (10 s), placed on ice, and 2 units of *Thermus aquaticus* DNA polymerase (Pharmacia) was added. Amplification was performed in a DNA thermal cycler (Perkin Elmer-Cetus). The amplification programme used was as follows: 30 s at 94 °C, 25 s at 50 °C and 30 s at 72 °C for 25 cycles, followed by a final 10 min extension time at 72 °C and chilling at 4 °C.

The PCR fragment used for Southern blot analysis was amplified from the pLAFR1 clone under similar conditions (30 s at 94 °C, 30 s at 50 °C, 60 s at 72 °C for 30 cycles). After amplification, the reaction mixtures were analysed by electrophoresis on 1.5% (w/v) agarose gel followed by Southern blotting.

DNA hybridization. Approximately 30 ng of the 275 bp fragment obtained by the amplification reaction described above was labelled with [α -³²P]dCTP using a multiprimer DNA labelling kit (Amersham). Labelled DNA was purified by Sephadex G-25 chromatography. The final specific activity of the probe was calculated to be 3 × 10⁹ c.p.m. ml⁻¹. Zeta Probe blotting membranes (Bio-Rad) were prehybridized, hybridized and washed as described in the manufacturer's instructions. The blots were exposed to X-OMAT film (Eastman Kodak) at -80 °C with intensifying screens for 16 h.

Results

Sequence analysis of the *oprI* gene from *P. aeruginosa* PAO1

Fig. 1 shows the sequence found for the *oprI* gene of PAO1. Minor differences exist between this sequence and the published sequences of the *oprI* gene of *P. aeruginosa* serotype 6 (Duchêne *et al.*, 1989) and of a *P. aeruginosa* clinical isolate (Cornelis *et al.*, 1989). These differences are as follows: (1) in the 5' non-coding region, the GGG sequence at position -43 in PAO1 is replaced by GG in the clinical isolate and GGGG in *P. aeruginosa* serotype 6; (2) in the 3' non-coding region, between positions 293 and 299, a sequence of seven Ts is found in PAO1 and *P. aeruginosa* serotype 6 whereas a sequence of six Ts is found in the clinical isolate; (3) at position 267, an extra C is found in the clinical isolate; (4) the only difference found in the coding region between the three cloned *oprI* genes is a silent mutation at position 195, where the T in PAO1 is replaced by a C in the clinical isolate and *P. aeruginosa* serotype 6.

Distribution of the *oprI* gene within the *Pseudomonadaceae*

Enzymic amplification of the *oprI* gene. The DNA sequence established for the PAO1 *oprI* gene was used to define PCR primers, with the goal of implementing a rapid and sensitive identification of *P. aeruginosa* through the PCR technique. A pair of primers was designed to amplify a 275 bp fragment covering the entire coding region of the *oprI* gene. Through the use of DNA hybridization with an internal *oprI* oligonucleotide, it was confirmed that the 275 bp fragment amplified from pLAFR1 corresponded to the *oprI* gene of PAO1 (data not shown).

The PCR primers were then used to investigate the presence or absence of the *oprI* sequence within the *Pseudomonadaceae*. The PCR method was found to work well on DNA recovered by touching an isolated bacterial colony with a sterile toothpick or needle. Results were scored on a plus/minus basis in terms of the appearance of a band of the expected mobility on agarose gel electrophoresis. The nature of the amplified band was always confirmed by Southern hybridization under stringent conditions with a probe derived from PCR amplification of the *oprI* gene contained in the original pLAFR 1 isolate (data not shown).

Enzymic amplification was performed on LSPQ and ATCC reference strains as listed in Table 1 as well as on the freshly isolated clinical strains shown in Table 2. No differences were found between the LSPQ and ATCC strains. For each PCR test, two samples were used for

Fig. 1. Sequence of the *oprI* gene of *P. aeruginosa* PAO1 and identification of the differences from two other published sequences. PA clinical isolate: sequence of the *oprI* gene of *P. aeruginosa* clinical isolate (Cornelis *et al.*, 1989). PA serotype 6: sequence of the *oprI* gene in *P. aeruginosa* serotype 6 (Duchêne *et al.*, 1989).

negative controls, one containing *E. coli* cells and another containing only dNTP, buffer, primers and enzyme. A positive control of PAO1 was also used for each PCR experiment.

As shown in Table 3, positive results by PCR were found almost entirely within the RNA group I as defined by previous workers (Palleroni, 1984; Gilardi, 1985, 1990). However, under our amplification conditions, strains of two species, *P. stutzeri* and *F. oryzae*, consistently gave weak 275 bp bands, thus they were scored as +/- in the table.

In order to confirm the identity of the amplified fragments, the gels containing the amplified DNA were blotted onto nylon membranes and the latter were hybridized with the entire radiolabelled *oprI* gene. Positive hybridization was obtained in each case where the PCR result was positive, although *P. stutzeri* and *F. oryzihabitans* showed a weaker signal (data not shown).

Characterization by Southern blotting. Independent validation of the PCR results was obtained through Southern hybridization performed on a genomic DNA prep-

Table 3. Comparison between PCR and Southern blot results obtained from *Pseudomonadaceae* and five other species

Genus	RNA group	Species	PCR results confirmed by hybridization	Genomic DNA Southern blot results
<i>Pseudomonadaceae</i>				
<i>Pseudomonas</i>	I	<i>Pseudomonas aeruginosa</i>	+	+
		<i>Pseudomonas alcaligenes</i>	+	+
		<i>Pseudomonas chlororaphis</i>	+	+
		<i>Pseudomonas fluorescens</i>	+	+
		<i>Pseudomonas mendocina</i>	+	+
		<i>Pseudomonas pseudoalcaligenes</i>	+	+
		<i>Pseudomonas putida</i> biotype A	+	+
		<i>Pseudomonas stutzeri</i>	+/-	+
	II	<i>Pseudomonas cepacia</i>	-	-
		<i>Pseudomonas pickettii</i>	-	-
	IV	<i>Pseudomonas diminuta</i>	-	-
		<i>Pseudomonas vesicularis</i>	-	-
<i>Comamonas</i>	III	<i>Comamonas acidovorans</i>	-	-
		<i>Comamonas testosteroni</i>	-	-
<i>Xanthomonas</i>	V	<i>Pseudomonas paucimobilis</i>	-	-
		<i>Xanthomonas maltophilia</i>	-	-
<i>Chryseomonas</i>	-	<i>Chryseomonas luteola</i>	-	+
<i>Flavimonas</i>	-	<i>Flavimonas oryzae</i>	+/-	+
<i>Other species</i>				
		<i>Branhamella catarrhalis</i>	-	-
		<i>Haemophilus influenzae</i>	-	-
		<i>Streptococcus mitis</i>	-	-
		<i>Staphylococcus aureus</i>	-	-
		<i>Escherichia coli</i> HB101	-	-

ation for each of the strains under study. Genomic DNA was digested with the restriction enzyme *Bst*EII, which allows digestion to be performed at elevated temperature (60 °C); this was found useful for overcoming the poor digestibility of the DNA obtained from some of the strains. The results of this work are shown in Fig. 2 and summarized in Table 3.

Genomic DNA was extracted from LSPQ strains, except when only the ATCC strains were available. The digested genomic DNA was then hybridized with the radiolabelled PCR fragments. Hybridizations were performed with 18 strains of the *Pseudomonadaceae* and five negative controls. The latter were chosen from pathogenic groups of bacteria representing three Gram-negative species (*Branhamella catarrhalis*, *Haemophilus influenzae* and *Escherichia coli*) and two Gram-positive species (*Streptococcus mitis* and *Staphylococcus aureus*). In this study, *P. aeruginosa* PAO1 and *E. coli* HB101 were used as positive and negative controls, respectively.

As shown in Fig. 2, positive hybridizations were found for *P. aeruginosa*, *P. alcaligenes*, *P. chlororaphis*, *P. fluorescens*, *P. mendocina*, *P. pseudoalcaligenes*, *P. putida*, *P. stutzeri*, *C. luteola* and *F. oryzae*. All bands had similar intensity, except *F. oryzae* (marked with an arrow in Fig. 2c), which had a lower genomic DNA

concentration on the gel. Only one band of the chromosomal *Bst*EII digestion was found to hybridize with the radiolabelled PCR fragments. No fragments corresponding to the *oprI* gene were found in *P. cepacia*, *P. pickettii*, *P. diminuta*, *P. vesicularis*, *P. paucimobilis*, *X. maltophilia*, *C. testosteroni*, *C. acidovorans* and in the five other strains not belonging to the *Pseudomonadaceae*.

Analysis of *P. aeruginosa* serotypes and clinical strains

PCR amplification was also performed on representative strains of the 17 existing serotype groups, based on the major heat-stable somatic O antigens found in the International Antigenic Typing System (Liu *et al.*, 1983); the results are shown in Fig. 3. All serotypes showed a distinct band of 275 bp by agarose gel electrophoresis, indicating the presence of the *oprI* gene.

PCR results were also obtained on 22 clinical isolates from Sainte-Justine Hospital; these are summarized in Table 4. Agreement between conventional microbiological techniques and PCR was found for 19 of these strains (Table 4). Two *P. aeruginosa* isolates and a *P. putida* isolate repeatedly failed to show the predicted result by PCR. These three clinical isolates were non-mucoid, therefore the hypothesis that the DNA is not sufficiently

Fig. 2. Southern blot analysis of *BstEII*-digested chromosomal DNA using a probe comprising amplified PCR fragments corresponding to the *oprI* gene of *P. aeruginosa* PAO1. (a) Lanes: (1) *P. aeruginosa* PAO1; (2) *C. acidovorans*; (3) *P. alcaligenes*; (4) *P. cepacia*; (5) *P. chlororaphis*; (6) *P. diminuta*; (7) *P. fluorescens*; (8) *P. mendocina*; (9) *E. coli*. (b) Lanes: (1) *P. aeruginosa* PAO1; (2) *P. paucimobilis*; (3) *P. pickettii*; (4) *P. putida*; (5) *P. pseudocaligenes*; (6) *P. stutzeri*; (7) *C. testosteroni*; (8) *P. vesicularis*; (9) *E. coli*. (c) Lanes: (1) *P. aeruginosa* PAO1; (2) *C. luteola*; (3) *X. maltophilia*; (4) *B. catarrhalis*; (5) *H. influenzae*; (6) *Staph. aureus*; (7) *Strep. mitis*; (8) *E. coli*; (9) *F. oryzyhabitus*. Numbers on the left indicate sizes of reference DNA fragments. The names of organisms giving a positive signal are in boldface type.

Fig. 3. Amplification of *P. aeruginosa* chromosomal DNA target sequence from 17 serotypes of the International Antigenic Typing System. The PCR method was performed with DNA recovered from isolated colonies resuspended in water and boiled for 5 min. Lane 1, (a) serotype 1, (b) serotype 10; lane 2, (a) serotype 2, (b) serotype 11; lane 3, (a) serotype 3, (b) serotype 12; lane 4, (a) serotype 4, (b) serotype 13; lane 5, (a) serotype 5, (b) serotype 14; lane 6, (a) serotype 6, (b) serotype 15; lane 7, (a) serotype 7, (b) serotype 16; lane 8, (a) serotype 8, (b) serotype 17; lane 9, (a) serotype 9, (b) *E. coli*; lane 10, (b) no target. L, 1 kb ladder. The number on the left indicates the length of the standard closest to the fragment amplified.

Table 4. PCR results obtained on different clinical isolates

Isolate	Presence of <i>oprI</i> gene	Isolate	Presence of <i>oprI</i> gene
<i>Pseudomonas aeruginosa</i>		<i>Pseudomonas cepacia</i>	
M/S 16	+	M/S 567	—
M/S 32	—	<i>Pseudomonas putida</i>	
M/S 41	+	M/S 430	—
M/S 47	+	<i>Pseudomonas stutzeri</i>	
M/S 86	+	M/S 441	+
M/S 96	+	<i>Xanthomonas maltophilia</i>	
M/S 99	+	M/S 426	—
M/S 179	—	M/S 428	—
M/S 190	+		
M/S 193	+		
M/S 225	+		
M/S 240	+		
M/S 246	+		
M/S 273	+		
M/S 280	+		
M/S 313	+		
M/S 316	+		

released by the boiling treatment is not thought to be pertinent in this case. Repeating the microbiological identification of these three isolates confirmed the original assignment. The microbiological techniques

used were verified using the LSPQ reference strains; all strains were correctly identified. Therefore the hypothesis that the *oprI* gene can be absent from an otherwise phenotypically normal strain, as determined by culture methods, will need to be explored in subsequent work.

Discussion

The Opr proteins of *Pseudomonas* (reviewed by Hancock *et al.*, 1990) offer interesting characteristics for the development of DNA probes. Previous work at the protein and immunological levels has shown that these proteins are strongly conserved within the 17 serotypes of *P. aeruginosa* as well as within different clinical and environmental isolates (Hancock *et al.*, 1982; Mutharia & Hancock, 1985; Hancock & Chan, 1988). More recent publications (reviewed in Hancock *et al.*, 1990) confirm this conserved character through molecular genetic techniques.

The work presented here agrees with the previously published protein, immunological and genetic results. The DNA sequence determined for *oprI* of *P. aeruginosa* PAO1 is very close, though not identical, to those previously published for a clinical isolate (Cornelis *et al.*, 1989) and for *P. aeruginosa* serotype 6 (Duchêne *et al.*, 1989). The predicted protein sequence is identical, as might be expected from the protein electrophoresis and immunological data (Mutharia *et al.*, 1982). On the other hand, the fact that the DNA sequence differs, even if very slightly, indicates that caution must be used in assuming that a PCR primer derived from the sequence of a given isolate will exactly match other isolates.

Taken together, the Southern blot results and PCR results demonstrate that the *oprI* gene is found primarily within organisms of RNA group I. This result is consistent with previous studies. Fialho *et al.* (1990) reported that the four *alg* (alginate-producing) genes were conserved within RNA group I. Recent work (Siehnel *et al.*, 1990) on the *oprP* gene found this gene well conserved within RNA group I but absent in representatives of the other RNA groups. These results, when combined with the present findings that the *oprI* genes are conserved within RNA group I but not commonly found outside it, strengthen the view of group I as a homogeneous entity at the DNA, rRNA and protein levels. It will be of interest, in the light of the results found in this work for *C. luteola* and *F. oryzihabitans*, whether other indications will be found to reclassify these organisms as part of RNA group I.

The PCR results are also satisfactory within the *P. aeruginosa* serotypes inasmuch as all serotypes gave a positive signal. This was to be expected in view of previous work (Mutharia *et al.*, 1982) showing a high

degree of conservation of the outer-membrane proteins amongst the *P. aeruginosa* serotypes. Our PCR results on clinical isolates, where we found the *oprI* gene in most but not all (19/22) of the isolates examined, are also in line with previously published results. For instance, using a monoclonal antibody against OprH2, 50 out of 52 strains of *P. aeruginosa* tested were positive (Mutharia & Hancock, 1985).

The easily and rapidly obtained PCR results correlated strongly with the more labour-intensive Southern-blotting method. In the one case where the result was different, the contrast between the positive Southern hybridization result with *C. luteola* and its consistently negative PCR result is probably explained by sequence divergence at one of the sites for the PCR primers. The same hypothesis may also explain the weaker PCR signals consistently obtained for *P. stutzeri* and *F. oryzihabitans*. All other species tested gave the same result whether by PCR or Southern blot.

Sample preparation for the PCR method (harvesting a single colony followed by boiling and centrifugation) is sufficiently simple and easy that it can be readily performed in clinical or environmental laboratories. The avoidance of radiolabelling is also a significant advantage for such laboratories.

The present study has established *oprI* as a generally applicable PCR target region and DNA probe within RNA group I of the *Pseudomonadaceae*. It has also provided evidence suggesting that *C. luteola* and *F. oryzihabitans* may be related to RNA group I. Probes based on *oprI* may therefore prove to be of value as taxonomic tools within the *Pseudomonadaceae*. The use of *oprI* as a PCR target site may find applications in other areas such as the testing of pharmaceutical preparations, in particular, ophthalmic solutions and eye cosmetics (Reid & Wood, 1979). In this type of work, where tolerance for bacterial contamination is essentially zero, the rapidity and the great sensitivity of the method would provide added insurance against low-level contamination by any member of RNA group I of the *Pseudomonadaceae*.

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