Molecular Microbiology (1990) 4(7), 1069–1075

MicroReview

# Outer membrane proteins of *Pseudomonas*

**R. E. W. Hancock,* R. Siehnel and N. Martin**
*Department of Microbiology, University of British Columbia, 300–6174 University Boulevard, Vancouver, British Columbia, Canada V6T 1W5.*

## Summary

**In this review, we describe the outer membrane proteins of *Pseudomonas aeruginosa* and related strains from the *Pseudomonas fluorescens* rRNA homology group of the Pseudomonadaceae, with emphasis on the physiological function and biochemical characteristics of these proteins. The use of *opr* (for outer membrane protein) is proposed as the genetic designation for the *P. aeruginosa* outer membrane proteins and letters are assigned, in conjunction with this designation, to known outer membrane proteins. Proteins whose primary functions involve pore formation, transport of specific substrates, cell structure determination and membrane stabilization are discussed. The conservation of selected proteins in the above *Pseudomonas* species is also examined.**

## Introduction

The outer membrane plays an important role in excluding potentially harmful molecules such as detergents, disinfectants, and enzymes while acting as a selective permeability barrier for other solutes. Molecules cross the outer membrane while entering the cell by several different routes, including specific and non-specific pores (Nikaido and Vaara, 1985), specific receptor complexes (Nikaido and Vaara, 1985) and, under special conditions, by a hydrophobic pathway (Nikaido and Vaara, 1985). It is assumed that the outer membrane is also involved in the secretion of molecules (Hirst and Welch, 1988) since this membrane must be crossed to gain access to the exterior of the cell. In addition, pili, flagella, lipopolysaccharides (LPS), and capsules are also anchored to the cell via the outer membrane.

This review briefly outlines current information on the outer membrane proteins in *Pseudomonas* spp. The emphasis has been placed on recent data which have been obtained since the last major review on this subject (Nikaido and Hancock, 1986). It is not the authors' intent to provide a detailed commentary, but to emphasize advances in this area of research. For more detailed discussion beyond the scope of this review, the reader should refer to reviews by Nikaido and Hancock (1986), Nikaido (1989) and Siehnel *et al.* (1990a).

Since most of the detailed studies have been performed with the opportunistic human pathogen, *Pseudomonas aeruginosa*, and since rRNA homology studies seem to indicate enormous diversity in the Family Pseudomonoaceae, here we will consider only *P. aeruginosa* and taxonomically related organisms from the *Pseudomonas fluorescens* rRNA homology group of this family (Palleroni *et al.*, 1973; DeVos and DeLey, 1983). In Table 1 we have listed some of the properties of the known outer membrane proteins of *P. aeruginosa*, while Table 2 describes the extent of conservation of selected proteins in related species. Some of these proteins are shown in Fig. 1. We have recently proposed the use of *opr* (for outer membrane protein) as the genetic designation for the protein P, F, and H1 genes (Siehnel *et al.*, 1988b; Woodruff and Hancock, 1988; Bell and Hancock, 1989) to prevent confusion with *omp*, the designation used in *Escherichia coli*. In Table 1, genetic designations for many of the *P. aeruginosa* outer membrane proteins are also proposed and these will be utilized throughout this review.

## Iron-repressible outer membrane protein (IROMP)

Under conditions of iron deprivation most Gram-negative bacteria produce three to nine extra outer membrane proteins (de Weger *et al.*, 1986; Cody and Gross, 1987; Page and Huyer, 1984; Meyer *et al.*, 1979). Studies with mutants lacking individual IROMPs have shown that they function as receptors for the binding of complexes of iron with specific chemicals called siderophores (e.g. Hancock *et al.*, 1976; Braun, 1985). These siderophores are either produced by the same bacterium under iron-starvation conditions, or can be supplied exogenously by other sources. *Pseudomonas* spp. can produce at least two classes of siderophores, called pyochelins and pyoverdins (Cox, 1985), as well as two or more IROMPs (Meyer *et al.*, 1979; Ohkawa *et al.*, 1980; Brown *et al.*, 1984). Studies on *P. aeruginosa* strains isolated from human or animal model infections (Brown *et al.*, 1984) provided data in

Received 15 November, 1989. *For correspondence. Tel. (604) 228 3308; Fax (604) 228 6041.

agreement with earlier conclusions (Griffiths and Humphreys, 1980) that bacteria grow in mammalian hosts under conditions of iron deprivation. However, for *P. aeruginosa* strains there is no formal proof that the IROMPs have a role in siderophore-mediated iron transport. Mutants lacking specific IROMPs have been isolated in *P. aeruginosa* by pyocin selection (Ohkawa *et al.*, 1980), as they were earlier in *E. coli* using colicin selection (Hancock *et al.*, 1976), and it is hoped that such mutants will soon provide the formal proof of such a role. In contrast, mutants of *Pseudomonas* spp. strain B10 (Magazin *et al.*, 1986), *Pseudomonas putida* (cited in Marugg *et al.*, 1989) and *Pseudomonas syringae* pv. *syringae* (Cody and Gross, 1987) lacking specific IROMPs with molecular weights of 74–80000 have been isolated and shown to be deficient in uptake of ferripyoverdin. The genes for the *Pseudomonas* B10 protein and an analogous protein from *P. putida* have been cloned (Magazin *et al.*, 1986; Marugg *et al.*, 1989). The pyoverdins of individual *Pseudomonas* spp. vary somewhat in structure. Thus, growth stimulation of iron-starved cells can usually only be accomplished by a subset of pyoverdins complexed to iron (Hohnadel and Meyer, 1988), a specificity that is apparently mediated by specific IROMPs.

Sokol and Woods (1983; 1986) have provided evidence that ferripyochelin uptake in *P. aeruginosa* involves a unique low molecular-weight iron-regulated outer membrane ferripyochelin binding protein (FBP). Although preliminary reports have suggested that the FBP gene has been cloned, and mutants deficient in surface expression of FBP have been described, proof of the function of this protein awaits the isolation of mutants lacking this protein.

## OprC and OprE

Proteins C and E (OprC and OprE) of *P. aeruginosa* have

**Table 1.** Outer membrane proteins of *Pseudomonas aeruginosa*.[a]

| Protein[b] | Proposed genetic name[c] | Apparent monomer $M_r$ on SDS-PAGE | Heat modifiability on SDS-PAGE | Conditions favouring production | Function | Gene cloned | Mutant available | Refs[i] |
|---|---|---|---|---|---|---|---|---|
| IROMP | – | 78–87000 | ND[d] | Low iron | $Fe^{3+}$-siderophore-binding/transport | – | Yes | 1, 2, 3 |
| C | OprC | 70000 | ND | ND | Porin | – | – | 4 |
| Esterase | – | 55000 | ND | Constitutive | Growth on acyl esters | – | Yes | 5 |
| P | OprP | 48000[e] | OmpF-like[f] | Low phosphate | Phosphate transport | Yes | Yes | 6, 7, 8, 9 |
| D1 | OprB | 46000 | OmpA-like[g] | Glucose as C source | Glucose porin | – | – | 10, 11, 12 |
| D2 | OprD | 45500 | OmpA-like | Certain C sources | Imipenem/basic amino acid selective porin | Yes | Yes | 4, 10, 13, 14, 15 |
| E | OprE | 44000 | None | ND | Porin | – | – | 4, 10 |
| F | OprF | 38000[e] | OmpA-like | Constitutive | Porin/structural | Yes | Yes | 10, 16, 17, 18, 19 |
| G | OprG | 25000 | OmpA-like | High iron, high $Mg^{2+}$, certain C sources | Unknown | – | – | 10 |
| H1 | OprH | 21000[e] | OmpA-like | Low $Mg^{2+}$, $Ca^{2+}$, $Sr^{2+}$, $Mn^{2+}$ | Stabilizing $Mg^{2+}$-deprived cells | Yes | – | 10, 19, 20, 21 |
| H2 | OprL | 20500 | None | Constitutive | Structural/lipoprotein | – | Yes[h] | 10, 22 |
| FBP | Fbp | 9–14000 | ND | Low iron | Ferripyochelin binding and transport | – | – | 23 |
| I | OprI | 9000[e] | None | Constitutive | Structural/lipoprotein | Yes | – | 24, 25, 26 |

**a.** See text for specific references.

**b.** Name as used by Hancock and Carey (1979) or others as described in the text.

**c.** Opr P, F and H have been used in the literature (Woodruff and Hancock, 1988; Siehnel *et al.*, 1988; Bell and Hancock, 1989). All other designations are proposed for the first time here.

**d.** ND – not determined.

**e.** Genes have been sequenced and consequently the actual molecular weights (in all cases lower than the given values) are known.

**f.** OmpF-like heat modifiability means that the polypeptide runs on SDS-PAGE at a higher apparent molecular weight (due to retention of the trimer configuration) when solubilized in SDS at low temperature, and at the monomer molecular weight given in the adjacent column after solubilization at higher temperatures (e.g. Hancock *et al.*, 1982).

**g.** OmpA-like heat modifiability means that the polypeptide runs on SDS-PAGE at a lower molecular weight when solubilized in SDS at low temperature (due to retention of tertiary configuration) and at the monomer molecular weight when solubilized at higher temperature (Hancock and Carey, 1979).

**h.** Natural isolates lacking OprL are available (Mutharia and Hancock, 1985a).

**i.** Reference numbers refer to the following papers: 1, Meyer *et al.* (1979); 2, Ohkawa *et al.* (1980); 3, Brown *et al.* (1984); 4, Yoshihara and Nakae (1989); 5, Ohkawa *et al.* (1979); 6, Hancock *et al.* (1982); 7, Worobec *et al.* (1988); 8, Poole and Hancock (1986a); 9, Siehnel *et al.* (1988b); 10, Hancock and Carey (1979); 11, Hancock and Carey (1980); 12, Trias *et al.* (1988); 13, Quinn *et al.* (1986); 14, Trias *et al.* (1989); 15, *Gene Cloning*: R. Siehnel *et al.*, manuscript in preparation; 16, Woodruff *et al.* (1986); 17, Benz and Hancock (1980); Gotoh *et al.* (1989); 18, Woodruff and Hancock (1989); 19, Nicas and Hancock (1980); 20, Nicas and Hancock (1983a); 21, Bell and Hancock (1989); 22, Mizuno (1979); 23, Sokol and Woods (1983); 24, Mizuno and Kageyama (1979); 25, Cornelis *et al.* (1989); 26, Duchêne *et al.* (1989).

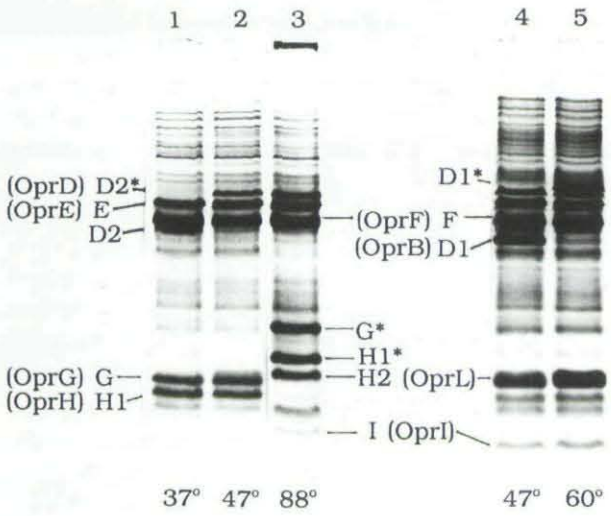


**Fig. 1.** Lanes 1–3, outer membrane proteins of *P. aeruginosa* grown in BM2-succinate to late log-phase; lanes 4 and 5, outer membrane proteins of *P. aeruginosa* grown in BM2-glucose to late log-phase. Samples were solubilized at the indicated temperatures for 10 min prior to loading. An asterisk indicates that the protein is located in the heat-modified position on the gel.

been purified from outer membranes by Yoshihara and Nakae (1989) and demonstrated by liposome-swelling techniques to be general porins. By applying the Renkin (1954) equation to the data of Yoshihara and Nakae (1989) on influx of sugars of different sizes, these channels should allow passage of trisaccharides (Siehnel *et al.*, 1990a), although the authors suggest a smaller exclusion limit. OprC is a minor outer membrane protein in our hands and the information presented to date makes definitive identification difficult. OprE has been described in more detail, but the presence of two or more polypeptides with similar molecular masses but different isoelectric points (B. L. Angus and R. E. W. Hancock, unpublished) means that this protein also requires better definition.

### Esterase

Ohkawa *et al.* (1979) identified and purified a minor outer membrane protein with esterase activity. Mutants lacking this esterase were unable to grow on Tween 80 as a carbon source. The esterase was extremely stable and had specificity for long-chain acyl oxy- or thio-esters with hydrophilic head groups.

### OprP

Protein P (OprP) is not present in outer membranes of cells unless they are grown on low (0.15 mM or less) phosphate medium (Hancock *et al.*, 1982). Mutational studies have demonstrated that OprP is an important component of the high-affinity, phosphate-starvation-inducible, phosphate-specific transport (Pst) system of *P. aeruginosa* (Poole and Hancock, *1986a*). The regulatory system for OprP and other Pst system components has been well conserved during evolution, since elements analogous to those of the *E. coli* Pst regulatory system, including a Pho-box in the putative *oprP* gene promoter (Siehnel *et al.*, 1988a) and *phoB* and *phoR* activator genes (Filloux *et al.*, 1988), have been demonstrated. Also expression of the cloned *oprP* gene in *E. coli* is regulated by the phosphate content of the medium. In contrast, despite several similarities in gross biochemical properties between OprP and the equivalent *E. coli* phosphate-starvation-inducible outer membrane protein PhoE (Worobec *et al.*, 1988) there are no major similarities in either function (Hancock *et al.*, 1986) or amino acid sequence (Siehnel *et al.*, 1990b). PhoE contains a weakly anion-selective general pore (Benz *et al.*, 1984) while the channel contained within the OprP porin is anion specific with a 100-fold preference for phosphate over other anions (Hancock and Benz, 1986) because of a phosphate-binding site in the OprP channel ($K_d$ = 0.3 mM). Chemical modification studies have demonstrated that charged lysine ε-amino groups comprise the

**Table 2.** Immunological (I), biochemical (B) or molecular genetic (M) relatedness of outer membrane proteins in species related to *P. aeruginosa*.

| Equivalent *P. aeruginosa* protein | Identification of Related Outer Membrane Protein[a,b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *P. putida* | *P. syringae* | *P. fluorescens* | *P. chloraphis* | *P. aureofaciens* | *P. stutzeri* | *P. anguilliseptica* | *A. vinelandii* |
| IROMP | B | B | B | B | – | –[c] | – | B |
| OprP | I, B, M | NP[d] | I, B, M | I, B, M | I, B, M | NP | – | – |
| OprF | I, B, M | I, B, M | B, M | M | – | M | M | B, M |
| OprH | B | NP | B[e] | I, B | – | B | – | – |
| OprL | I, B | I, B | I, B | I, B | I, B | I, B | I, B | I, B |
| Fbp | I | – | I | – | – | I | – | – |

**a.** I = immunological cross-reactivity, B = biochemical similarities, M = cross-hybridization with *oprP* and *oprF* gene probes.
**b.** Data is obtained in part from Meyer *et al.* (1979); de Weger *et al.* (1986); Mizuno (1981); Sokol and Woods (1986); Mutharia and Hancock. Unpublished data from the authors' laboratory includes the cross-hybridization of *oprP* and *opr*$^+$ genes, the existence of biochemically related proteins to OprP and the data for OprH.
**c.** No information available.
**d.** NP = no protein observed but hybridizing DNA was present.

phosphate-binding site. A model has been presented in which three amino groups (each provided by one monomer in the OprP trimer) coordinate the two symmetrical negative charges and one partial negative charge on $HPO_4^{2-}$ (Hancock and Benz, 1986).

Proteins homologous to OprP have been purified from four *Pseudomonas* spp. and shown to have nearly identical channel properties (Poole *et al.*, 1987) and to cross-react immunologically (Poole and Hancock, 1986b), while the *P. syringae* and *Pseudomonas stutzeri* type strains lacked such a protein and did not grow on phosphate-deficient medium. However, all six species contained a gene which hybridized with the *oprP* gene (Siehnel *et al.*, 1990b). In addition, an open reading frame was identified upstream of the OprP gene. Gene fusions, Southern hybridization (Siehnel *et al.*, 1988b) and preliminary sequence information (R. Siehnel, unpublished data) have suggested that this reading frame encodes a protein with substantial homology to OprP. However, we do not yet know if this gene is expressed under any growth condition.

### OprB

Protein D1 (called OprB here) is co-regulated with the glucose-uptake system of *P. aeruginosa* and with a periplasmic glucose-binding protein (Hancock and Carey, 1980). It has been shown to function as a porin (Hancock and Carey, 1980) and liposome-swelling studies have suggested a preference of the channel for glucose and xylose over other sugars of similar size (Trias *et al.*, 1988). A similar glucose-inducible protein was identified in *Pseudomonas fluorescens* (de Weger *et al.*, 1986) and *Pseudomonas putida* (B. L. Angus and R. E. W. Hancock, unpublished). The latter was heat-modifiable and immunologically cross-reactive with OprB of *P. aeruginosa*.

### OprD

*P. aeruginosa* mutants resistant to the broad-spectrum carbapenem β-lactam imipenem have been isolated both from clinical sources and the laboratory. They lack an outer membrane protein of molecular weight 45000 to 46000 (Quinn *et al.*, 1986; Buscher *et al.*, 1987). This protein has been shown to be analogous to protein D2 (OprD) (Trias *et al.*, 1989). While the purified protein is apparently capable of allowing size-dependent uptake of monosaccharides in liposome-swelling assays (Yoshihara and Nakae, 1989), it appears to demonstrate selectivity for imipenem over other β-lactams based on the lack of cross-resistance of *oprD* mutants to other β-lactams (Quinn *et al.*, 1986) and on whole-cell outer membrane permeability and enzyme-encapsulated liposome data (Trias *et al.*, 1989) comparing *oprD* mutants and the wild type. Thus OprD has the characteristics of a substrate-selective porin, i.e. one with a specific binding site for imipenem. Presumably the physiologically important substrate is not imipenem but a natural analogue (e.g. basic peptides or dipeptides; J. Trias, personal communication).

### OprF

The function of OprF as a *P. aeruginosa* porin is currently a source of controversy (see the discussion in Siehnel *et al.*, 1990a). Some researchers, utilizing the liposome exclusion (Hancock *et al.*, 1979), black lipid bilayer (Benz and Hancock, 1981) and liposome-swelling (Yoshimura *et al.*, 1983; Godfrey and Bryan, 1987; Trias *et al.*, 1988) methods have shown OprF to function as a porin. These studies have suggested that a small proportion of the 200000 OprF molecules per cell, perhaps as few as 400, form large channels (estimated to average 2nm in diameter and to allow passage of saccharides with molecular weights of 3000; Nikaido and Hancock, 1986). The remaining OprF channels appear to form small channels that are predicted to be antibiotic impermeant (Woodruff *et al.*, 1986). The presence of a relatively small number of large OprF channels per cell would result in a low total area for antibiotic diffusion, and this has been proposed as the basis for the measured low outer membrane permeability of *P. aeruginosa* (cf. *E. coli*, Angus *et al.*, 1982; Yoshimura and Nikaido, 1982; Nicas and Hancock, 1983b). This, in turn, was proposed to be the major basis for the medically problematic, high intrinsic resistance of *P. aeruginosa* to antibiotics (Hancock, 1986b; Nikaido and Hancock, 1986).

In contrast to these findings, Nakae and collaborators have suggested that the *P. aeruginosa* outer membrane has a low exclusion limit (resulting in impermeability to disaccharides) and that OprF has no function as a porin (Yoneyama *et al.*, 1986; Yoshihara *et al.*, 1988; Gotoh *et al.*, 1989; Yoshihara and Nakae, 1989). While some of these data are based on methodologies that have been criticized (Hancock, 1986a; Nikaido, 1989), it is puzzling that many of these studies involve liposome-swelling assays (Yoneyama *et al.*, 1986; Gotoh *et al.*, 1989; Yoshihara and Nakae, 1989) and are in direct contradiction to earlier liposome-swelling assays (Yoshimura *et al.*, 1983; Trias *et al.*, 1988). The basis for these discrepancies has not been investigated to date, but may involve slight methodological differences in outer membrane and OprF isolation techniques (e.g. utilizing EDTA in the more recent studies). However, it is difficult to see how an outer membrane that is impermeable to disccharides (mol. wt 340), as suggested by Nakae and collaborators (Yoneyama *et al.*, 1986; Gotoh *et al.*, 1989), allows uptake of and killing by β-lactams of molecular weights greater than 600 (Siehnel *et al.*, 1990a). We feel that only studies

on the association, or lack thereof, of OprF deficiency with β-lactam resistance will resolve this issue. However, due to the dramatic structural alterations of mutants lacking OprF (Gotoh *et al.*, 1989; Woodruff and Hancock, 1989), it has been impossible to make definitive conclusions about this relationship. Nevertheless we feel that other mutant evidence is consistent with a role for OprF in antibiotic uptake since an OprF-deficient, antibiotic-resistant post-therapy clinical derivative (Piddock *et al.*, 1987) as well as an apparently OprF-altered antibiotic-resistant laboratory isolate (Godfrey and Bryan, 1987) have been reported.

OprF-lacking mutants in *P. aeruginosa* PA01 grow poorly in certain media unless osmotic stabilizers are added (Nicas and Hancock, 1983b), demonstrate nearly spherical, rather than normal, elongated morphology, increased leakage of periplasmic β-lactamase (Gotoh *et al.*, 1989; Woodruff and Hancock, 1989), and enhanced outer membrane permeability to the hydrophobic probe, NPN (Woodruff and Hancock, 1988). Thus OprF has an important function in outer membrane stability and in cell shape determination. Some of the above properties of *oprF* mutants were analogous to those of *E. coli ompA* mutants in an *lpp* mutant background (N.B. *lpp* encodes the major outer membrane lipoprotein, the peptidoglycan-associated form of which is lacking in *P. aeruginosa* PA01 strains; see Oprl protein described below). Woodruff and Hancock (1989) compared the OmpA and OprF proteins. These proteins showed substantial sequence similarity in their carboxy-terminal halves and cross-reacted immunologically. Furthermore, OprF expressed from the cloned *oprF* gene in *E. coli ompA lpp* mutants, restored normal rod-shaped morphology to these rounded mutants. Thus it was concluded that OprF serves an analogous function to OmpA (in the appropriate genetic background).

Many other fluorescent *Pseudomonas* spp. have a major OprF-like protein based on biochemical characteristics and Southern and Western probing with the gene and OprF-specific monoclonal antibodies, respectively (Table 2). We have recently sequenced the *oprF* gene of *P. syringae* pv. *syringae* (C. Ullstrom, R. Siehnel and R. E. W. Hancock, manuscript in preparation) and observed 68% amino acid identity overall and 85% identity within the OmpA-related carboxy termini of the OprF proteins of *P. syringae* and *P. aeruginosa* (Duchêne *et al.*, 1988).

### OprG

The appearance of OprG in the outer membrane of *P. aeruginosa* is strongly dependent on the growth conditions. For example, iron-sufficient conditions, growth into the stationary phase, higher growth temperatures, $Mg^{2+}$-deficiency, certain LPS alterations and the presence of specific carbon sources all result in altered levels of OprG in the outer membrane (Hancock and Carey, 1979; 1980; Nicas and Hancock, 1980; Ohkawa *et al.*, 1980; Kropinski *et al.*, 1987). These considerations make assignment of a function to OprG difficult at present, but roles in fluoroquinolone uptake (Chamberland *et al.*, 1989) or in low-affinity iron uptake (Yates *et al.*, 1989) have been suggested.

### OprH

*P. aeruginosa* protein H1 (OprH) is overexpressed when wild-type cells are grown in media deficient in $Mg^{2+}$, $Ca^{2+}$, $Mn^{2+}$ and $Sr^{2+}$, and under all conditions in specific polymyxin-resistant mutants (Nicas and Hancock, 1980; 1983a). As summarized in detail previously (Nikaido and Hancock, 1986), the properties of the overproducing strain and mutants (resistance to polymyxin B, aminoglycosides and EDTA) are consistent with the hypothesis that protein H1 replaces divalent cations in the outer membrane and blocks self-promoted uptake of the above polycationic antibiotics. This function is consistent with the sequence of OprH which shows the protein to be basic (predicted pI = 8.3–9.0) and with the association of column-purified OprH with an equimolar or greater amount of anionic lipopolysaccharide (Bell and Hancock, 1989). $Mg^{2+}$-regulated outer membrane proteins of similar molecular mass to OprH were observed in *Pseudomonas chloraphis*, *P. fluorescens* (ATCC 13525 but not ATCC 949), *P. putida* and *P. stutzeri*, but not *P. syringae*. Only the *P. chloraphis* protein was immunologically cross-reactive (A. Bell, S. Binnie and R. E. W. Hancock, unpublished data).

### OprL

*P. aeruginosa* protein H2 (OprL) is strongly associated with the peptidoglycan and contains covalently bound fatty acyl chains (Mizuno, 1979) and thus belongs to the class of outer membrane proteins called peptidoglycan-associated lipoproteins (Mizuno, 1981). The protein is strongly conserved in related pseudomonads (Table 2), since a single monoclonal antibody, MA1-6, reacts with a protein of similar molecular weight from all tested species of the *P. fluorescens* rRNA homology group as well as *Azotobacter vinelandii* (Mutharia and Hancock, 1985b).

### OprI

OprI is a highly abundant, low molecular-weight lipoprotein (Mizuno and Kageyama, 1979) that is broadly analogous to the Braun lipoprotein of *E. coli* (Halegoua *et al.*, 1974, Mizuno and Kageyama, 1979). Recent sequencing of the *oprI* gene by two groups has suggested that the amino acid sequences can be 23–30% aligned with the *E. coli* major lipoprotein sequence (Cornelis *et al.*, 1989;

Duchêne *et al.*, 1989). OprI has been shown to have a single covalently attached fatty acid residue (Mizuno and Kageyama, 1979), whereas Braun lipoprotein has three despite amino acid sequence identity at the fatty-acid-associated amino terminus. There appears to be some heterogeneity in *P. aeruginosa* strains with respect to the mode of association of OprI with the peptidoglycan. Some strains apparently contain both covalently and non-covalently peptidoglycan-associated OprI (Mizuno and Kageyama, 1979) as in *E. coli* (Hirashima *et al.*, 1973), whereas strain PA01 contains only non-covalently associated OprI (Hancock *et al.*, 1981). Several other related species have proteins that appear analogous to OprI (Nakajima *et al.*, 1983), but no systematic search has been performed.

### Additional proteins

A new 54 kiloDalton outer membrane protein has been observed in a spontaneously occurring mutant of *P. aeruginosa* selected by resistance to norfloxacin (Hirai *et al.*, 1987). This *nfxB* mutant had no apparent additional changes in lipopolysaccharide composition, suggesting that the 54 kD protein may block norfloxacin uptake by an as yet unknown mechanism (Hirai *et al.*, 1987).

A channel-forming protein, NosA, has been purified from the outer membrane of *P. stutzeri* which is involved in the uptake of copper from the external medium and the expression of which can be repressed by exogenous copper (Lee *et al.*, 1989). This channel does not appear to be specific for the uptake of copper, although it has copper tightly bound to it, but may be part of a system which involves a periplasmic copper-binding protein similar to maltose uptake via LamB and the maltose-binding protein in *E. coli* (Lee *et al.*, 1989).

During the assembly of pili, pilin must cross the outer membrane. Pilin subunit pools have been detected in both the outer (and also the inner) membrane of *P. aeruginosa* and can therefore be considered as outer membrane proteins. This observation also suggests that the pilin subunits are possibly assembled at the cell surface (Watts *et al.*, 1982). Pilin genes cloned into and expressed in *E. coli* also accumulate pilin subunits at the outer and inner membranes (Strom and Lory, 1986; Finlay *et al.*, 1986).

Various secretion mutants named *xcp* (extracellular protein deficient) have been found in *P. aeruginosa* (Wretlind and Pavlovskis, 1984). A gene that complements one of these mutants, *xcp*-1, possibly encodes a 26 kD protein when expressed in *E. coli* which has been tentatively identified as analogous to a 30 kD outer membrane protein in *P. aeruginosa* (Bally *et al.*, 1989). This protein may play a direct role in secretion but further characterization will be necessary. Fecycz and Campbell (1985) have studied a mutant strain of *P. aeruginosa* (PAKS 18) which accumulates precursor to exocellular protease 1 in the periplasmic space and releases no active protease. Examination of the outer membrane profile of this strain on sodium dodecyl sulphate-polyacrylamide gels showed that it was lacking a protein of approximately 25 kD and was deficient in a protein of approximately 13.7 kD. Since this strain is deficient in the secretion of other exoenzymes, the outer membrane proteins identified may have a role in protein secretion (Fecycz and Campbell, 1985).

### Acknowledgements

Funding from the Canadian Cystic Fibrosis Foundation and the Natural Sciences and Engineering Research Council of Canada for the work of the authors is acknowledged.

### References

Angus, B.L. *et al.* (1982) *Antimicrob Agents Chemother* **21:** 299–309.
Bally, M. *et al.* (1989) *J Bacteriol* **171:** 4342–4348.
Bell, A., and Hancock, R.E.W. (1989) *J Bacteriol* **171:** 3211–3217.
Benz, R., Darveau, R.P., and Hancock, R.E.W. (1984) *Eur J Biochem* **140:** 319–324.
Benz, R., and Hancock, R.E.W. (1981) *Biochim Biophys Acta* **646:** 298–308.
Braun, V. (1985) *Trends Biochem Sci* **10:** 75–78.
Brown, M.R.W. *et al.* (1984) *FEMS Microbiol Lett* **21:** 113–117.
Buscher, K.H. *et al.* (1987) *J Inf Dis* **156:** 700–701.
Chamberland, S. *et al.* (1989) *Antimicrob Agents Chemother* **33:** 624–634.
Cody, Y.S., and Gross, D.C. (1987) *J Bacteriol* **169:** 2207–2214.
Cornelis, P. *et al.* (1989) *Mol Microbiol* **3:** 421–428.
Cox, C.D. (1985) *Antibiot Chemother* **36:** 1–12.
de Weger, L.A. *et al.* (1986) *J Bacteriol* **165:** 585–594.
DeVos, P., and DeLey, J. (1983) *Int J Syst Bacteriol* **33:** 487–509.
Duchêne, M. *et al.* (1988) *J Bacteriol* **170:** 155–162.
Duchêne, M. *et al.* (1989) *J Bacteriol Syst* **171:** 4130–4137.
Fecycz, I.T., and Campbell, J.N. (1985) *Eur J Biochem* **146:** 35–42.
Filloux, A. *et al.* (1988) *Mol Gen Genet* **212:** 510–513.
Finlay, B.B. *et al.* (1986) *J Bacteriol* **165:** 625–630.
Godfrey, A.J., and Bryan, L.E. (1987) *Antimicrob Agents Chemother* **31:** 1216–1221.
Gotoh, N. *et al.* (1989) *J Bacteriol* **171:** 983–990.
Griffiths, E., and Humphreys, J. (1980) *Infect Immun* **28:** 286–289.
Halegoua, S. *et al.* (1974) *J Bacteriol* **120:** 1204–1208.
Hancock, R.E.W. (1986a) In *Bacterial Outer Membranes as Model Systems.* Inouye, M. (ed.). New York: John Wiley and Sons, pp. 187–225.
Hancock, R.E.W. (1986b) *J Antimicrob Chemother* **18:** 653–659.
Hancock, R.E.W., and Benz, R. (1986) *Biochim Biophys Acta* **860:** 699–707.
Hancock, R.E.W., and Carey, A.M. (1979) *J Bacteriol* **140:** 902–910.
Hancock, R.E.W., and Carey, A.M. (1980) *FEMS Microbiol Lett* **8:** 105–109.
Hancock, R.E.W. *et al.* (1979) *Biochim Biophys Acta* **554:** 323–329.
Hancock, R.E.W. *et al.* (1981) *J Bacteriol* **145:** 628–631.

Hancock, R.E.W. *et al.* (1982) *J Bacteriol* **150:** 730–738.
Hancock, R.E.W. *et al.* (1986) *Biochim Biophys Acta* **860:** 263–267.
Hirai, K. *et al.* (1987) *Antimicrob Agents Chemother* **31:** 582–586.
Hirashima, A. *et al.* (1973) *J Biol Chem* **248:** 5654–5659.
Hirst, T.R., and Welch, R.A. (1988) *Trends Biochem Sci* **13:** 165–269.
Hohnadel, D., and Meyer, J.M. (1988) *J Bacteriol* **170:** 4865–4873.
Kropinski, A.M.B. *et al.* (1987) *J Bacteriol* **169:** 1960–1966.
Lee, H.S. *et al.* (1989) *J Bacteriol* **171:** 2096–2100.
Magazin, M.D. *et al.* (1986) *J Biol Chem* **261:** 795–799.
Marugg, J.D. *et al.* (1989) *J Bacteriol* **171:** 2819–2826.
Meyer, J.M. *et al.* (1979) *FEMS Microbiol Lett* **5:** 395–398.
Mizuno, T. (1979) *Biochem* **86:** 991–1000.
Mizuno, T. (1981) *J Biochem* **89:** 1039–1049.
Mizuno, T., and Kageyama, M. (1979) *J Biochem* **85:** 115–122.
Mutharia, L.M., and Hancock, R.E.W. (1985a) *Can J Microbiol* **31:** 381–390.
Mutharia, L.M., and Hancock, R.E.W. (1985b) *Int J Syst Bacteriol* **35:** 530–532.
Nakajima, K. *et al.* (1983) *Int J Syst Bacteriol* **33:** 1–8.
Nicas, T.I., and Hancock, R.E.W. (1980) *J Bacteriol* **143:** 872–878.
Nicas, T.I., and Hancock, R.E.W. (1983a) *J Gen Microbiol* **129:** 509–517.
Nicas, T.I., and Hancock, R.E.W. (1983b) *J Bacteriol* **153:** 281–285.
Nikaido, H. (1989) In *Microbial Resistance to Drugs*. Bryan, L.E. (ed.). New York: Springer-Verlag, pp. 1–34.
Nikaido, H., and Hancock, R.E.W. (1986) In *The Bacteria: A Treatise on Structure and Function. X. The Biology of* Pseudomonas. Sokatch, J.R. (ed.). New York: Academic Press, pp. 145–193.
Nikaido, H., and Vaara, M. (1985) *Microbiol Rev* **49:** 1–32.
Ohkawa, I. *et al.* (1979) *J Biochem* **86:** 643–656.
Ohkawa, I. *et al.* (1980) *J Biochem* **87:** 323–331.
Page, W.J., and Huyer, M. (1984) *J Bacteriol* **158:** 494–502.
Palleroni, N.J. *et al.* (1973) *Int J Syst Bacteriol* **23:** 333–339.
Piddock, L.J.V. *et al.* (1987) *Lancet* **ii:** 907.
Poole, K., and Hancock, R.E.W. (1986a) *Mol Gen Genet* **202:** 403–409.
Poole, K., and Hancock, R.E.W. (1986b) *J Bacteriol* **165:** 987–993.
Poole, K. *et al.* (1987) *Can J Microbiol* **33:** 63–69.
Quinn, J.P. *et al.* (1986) *J Inf Dis* **154:** 289–294.
Siehnel, R. *et al.* (1988a) *Mol Microbiol* **2:** 347–352.
Siehnel, R. *et al.* (1988b) *J Bacteriol* **170:** 2312–2318.
Siehnel, R. *et al.* (1990a) In Pseudomonas*: Biotransformations, Pathogenesis, and Evolving Biotechnology*. Silver, S. (ed.). Washington, D.C.: American Society for Microbiology, pp. 328–342.
Siehnel, R. *et al.* (1990b) *Mol Microbiol* **4:** in press.
Sokol, P.A., and Woods, D.E. (1983) *Infect Immun* **40:** 665–669.
Sokol, P.A., and Woods, D.E. (1986) *Infect Immun* **53:** 621–627.
Strom, M.S., and Lory, S. (1986) *J Bacteriol* **165:** 367–372.
Trias, J. *et al.* (1988) *Biochim Biophys Acta* **938:** 493–496.
Trias, J. *et al.* (1989) *Antimicrob Agents Chemother* **33:** 1201–1206.
Watts, T.H. *et al.* (1982) *J Bacteriol* **152:** 687–691.
Woodruff, W.A., and Hancock, R.E.W. (1988) *J Bacteriol* **170:** 2592–2598.
Woodruff, W.A., and Hancock, R.E.W. (1989) *J Bacteriol* **171:** 3304–3309.
Woodruff, W.A. *et al.* (1986) *J Bacteriol* **167:** 473–479.
Worobec, B. *et al.* (1988) *Biochim Biophys Acta* **939:** 366–374.
Wretlind, B., and Pavlovskis, O.R. (1984) *J Bacteriol* **158:** 801–808.
Yates, J.M. *et al.* (1989) *FEMS Microbiol Lett* **58:** 259–262.
Yoneyama, H. *et al.* (1986) *Biochem Biophys Res Commun* **134:** 106–112.
Yoshihara, E., and Nakae, T. (1989) *J Biol Chem* **264:** 6297–6301.
Yoshihara, E. *et al.* (1988) *Biochem Biophys Res Commun* **156:** 470–476.
Yoshimura, F., and Nikaido, H. (1982) *J Bacteriol* **152:** 636–642.
Yoshimura, F. *et al.* (1983) *J Biol Chem* **258:** 2308–2314.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.