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Sequence heterogeneity of the ferripyoverdine uptake (*fpvA*), but not the ferric uptake regulator (*fur*), genes among strains of the fluorescent pseudomonads *Pseudomonas aeruginosa*, *Pseudomonas aureofaciens*, *Pseudomonas fluorescens* and *Pseudomonas putida*

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Abstract

Pseudomonas aeruginosa, *Pseudomonas aureofaciens*, *Pseudomonas fluorescens* and *Pseudomonas putida* are of importance to medicine, agriculture and biocycling. These microbes acquire ferric ion via the use of the siderophores pyochelin and the family known as the pyoverdines or pseudobactins. The ferric uptake regulator (*fur*) gene is responsible, at least in part, for the regulation of siderophore synthesis and uptake in *P. aeruginosa*.

To determine whether the organisms contain single or multiple homologues of the siderophore-related genes *fpvA* (ferripyoverdine uptake) and *fur*, and whether these homologues displayed sequence heterogeneity, their chromosomal DNAs were probed with *fur* and *fpvA* sequences. As a representative of a non-fluorescent pseudomonad, the bacterium *Burkholderia* (*Pseudomonas*) *cepacia* was also examined.

The pseudomonads all contained *fpvA*- and *fur*-like homologues, and heterogeneity was observed among the different species. The presence of two or more *fpvA*-like genes is indicated in all of the fluorescent pseudomonads surveyed. In contrast, *B. cepacia* DNA either did not hybridize to these probes, or did so only very weakly, suggesting that *fur*- and *fpvA*-like homologues are either absent or significantly different in *B. cepacia* compared to the fluorescent pseudomonads examined.

Introduction

Iron is an essential nutrient for virtually all microbes. In aerobic environments where the pH is near neutrality (Neilands 1982; Emery 1983; Guerinot 1994) extremely insoluble ferric hydroxide complexes form making iron particularly scarce. A wide array of microbes solve this dilemma by synthesizing and excreting siderophores, i.e., low molecular weight, high-affinity ferric ion-chelating molecules (Neilands 1982; Emery 1983; Guerinot 1994).

Two of the key aspects of siderophore use by microbes are: (1) the means by which they assimilate the ferric form (ferrisiderophores) of the siderophores they utilize; and (2) the mechanism(s) by which genetic regulation is achieved. *P. aeruginosa* synthesizes and uses three siderophores, i.e., pyochelin, salicylic acid and pyoverdine, to assimilate iron from environments where its concentration is limited (Neilands 1982; Emery 1983; Ankenbauer 1992; Meyer *et al.* 1992; Visca *et al.* 1993; Ankenbauer & Quan 1994; Guerinot 1994). Pyoverdine, the superior ferric ion chelator (Meyer *et al.* 1992, 1996), is present in the

sputa of cystic fibrosis patients (Haas *et al.* 1991) and is deemed essential to the virulence of *P. aeruginosa* (Meyer *et al.* 1996).

Pyoverdines, also known as pseudobactins, are a group of structurally related molecules (Meyer & Abdallah 1978; Teintze *et al.* 1981; Demange *et al.* 1987; Koster *et al.* 1993; Palleroni 1994; Meyer *et al.* 1996, 1997) made by the fluorescent pseudomonads, which include *P. aeruginosa*, *P. aureofaciens* (now considered *P. chlororaphis*), *P. fluorescens* and *P. putida* (Palleroni 1984). The *P. aeruginosa* outer membrane receptor protein which initiates ferripyoverdine transport into the cell is termed FpvA and the responsible gene, designated *fpvA*, has been cloned and sequenced (Poole *et al.* 1993). Using a different *P. aeruginosa* strain, Lamont and colleagues (Merriman *et al.* 1995, McMorran *et al.* 1996, Genbank accession number UO7359) also cloned and sequenced *fpvA* noting that in the region examined, it contained >99% identity to the amino acids 552–814 of FpvA. Multiple ferripyoverdine (ferripseudobactin) uptake polypeptides, and hence genes, however, have been shown in *P. putida* WCS358 (Koster *et al.* 1993; Bitter *et al.* 1994; Koster *et al.* 1995) and implied in *P. aeruginosa* (Poole *et al.* 1991). The multiple IROMPs (iron repressible outer membrane proteins) of *P. putida* WCS358 (Koster *et al.* 1993; Bitter *et al.* 1994; Koster *et al.* 1995) allow the potential use of a number of different pyoverdines, yet only one ferripyoverdine IROMP was derepressed by iron limitation alone. In order for its gene to be derepressed, a second pyoverdine IROMP required both the presence of its substrate and iron limiting conditions. Similarly, the presence of pyoverdines in the culture medium specifically induced the synthesis of an 85 kDa IROMP in *P. aeruginosa* (Gensberg *et al.* 1992).

Structural determination, cross-feeding and ^{59}Fe-uptake experiments (Hohnadel *et al.* 1988; Cornelis *et al.* 1989, Meyer *et al.* 1997) indicated that the pseudomonads examined usually displayed specific pyoverdine assimilation properties. Fe^{3+}-assimilation occurred only from the use of a specific, or a structurally very similar, pyoverdine. In particular, strains of *P. aeruginosa* could be grouped into one of three ferripyoverdine uptake groups (Cornelis *et al.* 1989; Meyer *et al.* 1997). In some cases, however, overlapping of a strain's ability to assimilate Fe^{3+} from two or more distinct ferripyoverdines existed, implying that more than one ferripyoverdine assimilation protein may exist in a strain. It is possible that *P. aeruginosa*, like *P. putida* WCS 358, contains multiple IROMP genes but that specific, inducible situations are necessary if they are to be expressed simultaneously. Data supporting such a conclusion comes from the study of Smith *et al.* (1992). These investigators suggested that a second *P. aeruginosa* ferripyoverdine uptake system exists as they isolated a Tn5 mutant, which though largely deficient in the transport of its native ferripyoverdine, still maintained a basal level of ferripyoverdine transport. The authors concluded, however, that such a basal system is of lower affinity and broader specificity as it extended to heterologous pyoverdines from strains other than the original Tn5-mutant.

The gene responsible for controlling high-affinity iron uptake systems is termed *fur* (*f*erric *u*ptake *r*egulator). *fur* has been cloned and sequenced from *Escherichia coli* (Schaffer *et al.* 1985), *Legionella pneumophilia* (Hickey & Cianciotto 1994), *Neisseria gonorrhoeae* (Berish *et al.* 1993), *P. aeruginosa* (Prince *et al.* 1993), *Staphylococcus epidermidis* (Heidrich *et al.* 1996), *Vibrio cholerae* (Litwin *et al.* 1992), *V. vulnificus* (Litwin *et al.* 1993) and *Yersinia pestis* (Staggs & Perry 1992). The Fur protein forms an oligomeric complex with Fe^{2+} and acts as a repressor of many iron-regulated genes (Heidrich *et al.* 1996). While distinct features do exist, notable identity among the *fur* genes (Schaffer *et al.* 1985; Litwin *et al.* 1992; Staggs & Perry 1992; Prince *et al.* 1993), and greater than 70% similarity in the Fur proteins of E. *coli*, *P. aeruginosa*, *V. cholerae* and *Y. pestis* were observed (Prince *et al.* 1993).

In a previous study (Castignetti 1997), the presence of a ferripyochelin receptor A (fptA)-like gene was noted in five *P. aeruginosa* strains and *P. aureofaciens*, *P. fluorescens*, *P. putida* and *B. cepacia*. Considerable heterogeneity existed in the fptA-like homologues from the non-*P. aeruginosa* bacteria; marked homogeneity, however, existed in the *P. aeruginosa* strains.

The microbes previously studied (Castignetti 1997) are of considerable medical, agricultural and environmental importance (Palleroni 1984). In light of the data that places *P. aeruginosa* isolates into one of three pyoverdine homology groups (Cornelis *et al.* 1989; Meyer *et al.* 1997), it was of interest to determine whether *fpvA*-like homologues were of similar sequence organization and numbers or whether they were different in distinct strains of *P. aeruginosa*. As ferripyoverdine (ferripseudobactin) transport genes have been noted in *P. aeruginosa* (Poole *et al.* 1993), *P. fluorescens* (Morris *et al.* 1994) and *P. putida* WCS 358 (Koster *et al.* 1993, 1995; Bitter *et al.* 1994),

it was of interest to determine whether *fpvA*-like sequences existed in *P. aureofaciens* and *B. cepacia* and whether they displayed heterogeneity. Similarly, because of its importance to the control of iron-regulated genes, we investigated whether *fur*-like homologues existed in *P. aureofaciens*, *P. fluorescens*, *P. putida* and *B. cepacia* and whether such homologues displayed heterogeneity. *fur*-like heterogeneity was also investigated in the five strains of *P. aeruginosa*.

Materials and methods

The five strains of *P. aeruginosa*, four of which are of clinical origin and one which is an environmental (aquatic) isolate, were previously described (Castignetti 1997). Briefly, strains RM1407, 6680 and 1–8 are cystic fibrosis isolates, strain PU21 is a *P. aeruginosa* PAO subline originally isolated as a wound isolate (Jacoby 1974) while strain 10145 was purchased from the American Type Culture Collection (A.T.C.C.-Rockville, MD). A sixth *P. aeruginosa* strain, CD10, (kindly supplied by K. Poole) is also a PAO derivative (K. Poole, pers. comm.) and is the strain from which *fpvA* was cloned and sequenced (Poole *et al.* 1993). *P. aureofaciens* 13985, *P. fluorescens* 17400, *P. putida* 12633 and *B. cepacia* 25416 were from the American Type Culture Collection.

Bacterial growth, chromosomal DNA isolation and purification were performed as described (Castignetti 1997) or as instructed by Johnson (1994) using the phenol-chloroform chromosomal DNA isolation procedure described therein. Chromosomal DNA (1–6 μg per reaction) was digested with either *Bam*HI, *Hin*dIII, *Sal*I, *Sph*I or *Sst*II (isoschizomer of *Sac*II) or *Xho*I (Gibco-BRL, Life Sciences, Bethesda, MD) as directed by the manufacturer, subjected to agarose gel electrophoresis (a 14 $\times$ 15 cm 0.9% agarose gel, 16 mA for 16–18 h) and transferred to Qiagen (Qiagen Corp., Chatworth, CA) nylon+ membranes using an alkaline transfer procedure (Reed & Mann 1985). Digestion of the chromosomal DNAs of the bacteria of this study by *Bam*HI or *Sph*I resulted in noticeably lower molecular weight bands than did digestion by *Hin*dIII. As the pseudomonads are G:C base pair rich (58–70 mol%-Palleroni 1984), the explanation for this observation may be that the former two enzymes have 4 of the 6 base pairs in their recognition sequences as G:C base pairs while *Hin*dIII has 4 of the 6 base pairs in its recognition sequence as A:T pairs.

E. coli harboring pEHFUR, which has a *P. aeruginosa fur*-containing 1.8-kb *Eco*RI-*Hin*dIII insert, was a gift from M. Vasil (Prince *et al.* 1993). pEHFUR was isolated from the *E. coli* using a Qiagen maxiprep system as directed by the manufacturer. pEHFUR was digested with *Eco*RI and *Hin*dIII (Gibco-BRL, Bethesda, MD) yielding a 1.8-kb *fur* fragment, which contained the full gene, plus approximately 0.6 kb of upstream and 0.4 kb of downstream sequences (Prince *et al.* 1993). The 1.8-kb *fur* fragment was then subjected to gel electrophoresis on a 0.85% preparative agarose gel and isolated using the Qiagen Qiaquick isolation procedure. The 1.8-kb *fur* fragment was labelled with ^{32}P-dCTP using either a nick translation procedure (Rigby *et al.* 1977) or the Rad Prime kit (Gibco-BRL-Life Technologies Bethesda, MD) as directed by the manufacturer. Variations from the manufacturer's instructions were that the isolation and collection of the radioactive probe from unincorporated nucleotides was performed using Sephadex G-50 column chromatography (Sigma, St. Louis, MO) as described (Rigby *et al.* 1977) and nucleotide incorporation occurred over a period of 60–120 min.

E. coli harboring pPVR2, which contains a *P. aeruginosa fpvA* 4.6-kb *Sph*I insert, was a gift from K. Poole (Poole *et al.* 1993) and pPVR2 was isolated from the *E. coli* as described above. Analysis of the *P. aeruginosa* CD10 *fpvA* nucleotide sequence, as well as the 4.6-kb fragment (Marck 1988) from which it was cloned (Poole *et al.* 1993), revealed that neither the gene nor the fragment contains internal sites for the restriction enzymes *Bam*HI, *Hin*dIII or *Sph*I. Further, digestion with *Bst*XI and *Bsu*36I (Gibco-BRL, Bethesda, MD) would yield a 1.7-kb *fpvA* fragment comprised of nucleotides entirely within the *fpvA* gene (Poole *et al.* 1993). The 1.7-kb *fpvA* fragment was thus generated and then subsequently electrophoresed, isolated and labelled with ^{32}P-dCTP as noted above for the 1.8-kb *fur* fragment.

E. coli harboring ptoxETA, which has a *P. aeruginosa* 741-bp PstI-NruI insert able to distinguish *P. aeruginosa* strains (Gray *et al.* 1984, Vasil *et al.* 1986, Ogle *et al.* 1987), was supplied by M. Vasil. The ptoxETA plasmid was isolated from the *E. coli* as described above and was used as such to distinguish among *P. aeruginosa* strains (M. Vasil, pers. commun.). ptoxETA was electrophoresed, isolated and labelled with ^{32}P dCTP as noted above for the *fur* and *fpvA* fragment probes.

DNA hybridizations were performed under moderately stringent conditions as described (Doering *et al.*

1982). Autoradiographs were performed using Cronex intensifying screens and Kodak XAR film using exposure times of 1–21 days. Longer autoradiography times (greater than 2–3 days) were generally necessary for the blots probed with the *fur* fragment. All DNA probing experiments were repeated at least twice and representative results are shown. Both undigested plasmids (e.g., pPVR2) containing the gene of interest and the isolated gene probe (e.g., *fpvA*) were incorporated into gels and Southern blots to serve as controls. These controls functioned as expected (data not shown) and confirmed both the selectivity and stringency of the probing experiments.

Results and discussion

Before examining our isolates for the presence of *fur* and *fpvA* homologues, it was necessary to establish that the microbes were individual strains. As members of distinct species, *B. cepacia*, *P. aureofaciens*, *P. fluorescens* and *P. putida* are different from one another and *P. aeruginosa*. Whether the five *P. aeruginosa* isolates (6680, 1-8, RM1407, PU21 and A.T.C.C. 10145) were individual strains, however, needed to be determined. Chromosomal DNAs from the five *P. aeruginosa* strains were thus probed with ptoxETA. All five *P. aeruginosa* strains were identified as being different since distinct ptoxETA fragment patterns were generated for each (data not shown).

Data indicating that digested (*Sst*II) chromosomal DNA from all five *P. aeruginosa* strains contain multiple copies of *fpvA*-like genes are presented in Figure 1 and Table 1. While *P. aeruginosa* 6680 and RM 1407 displayed identical binding patterns and substantial homogeneity among all five strains existed, all of these microbes had multiple (>2) probe-reactive fragments greater than 1.7 kb, the size of the *fpvA* probe. It is thus likely that two or more distinct *fpvA*-like genes exist in these bacteria, a conclusion in concert with those studies (Poole *et al.* 1991; Smith *et al.* 1992; Koster *et al.* 1995) where two or more ferripyoverdine transport proteins have been noted or presumed. A similar situation was discerned for *P. fluorescens*. With the *Sst*II digestion, however, our *P. putida*, unlike *P. putida* WCS358 (Koster *et al.* 1993, 1995; Bitter *et al.* 1994) had only two *fpvA*-like fragments and thus could not be assigned as definitely having two or more *fpvA*-like homologues. Similarly, *P. aureofaciens* had only two fragments >1.7 kb and thus may also contain a single *fpvA*-like homologue. *B. cepacia*, the one strain of this study not known to produce pyoverdine, lacked any probe-reactive fragments when the *fpvA* probe was used with *Sst*II digested chromosomal DNA. Thus while the *P. fluorescens* and the *P. aeruginosa* strains examined showed evidence of containing two or more *fpvA*-like genes, it appeared that *B. cepacia* did not contain any *fpvA*-like sequences and that *P. putida* and *P. aureofaciens* might contain only a single *fpvA*-like homologue.

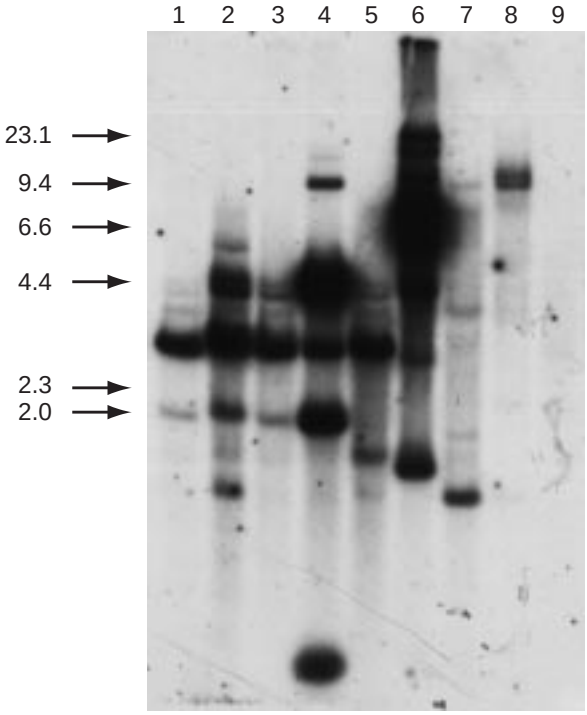

Figure 1. Southern blot of *Sst*II (*Sac*II) digests of chromosomal DNAs from *P. aeruginosa* 6680 (lane 1), *P. aeruginosa* 1–8 (lane 2), *P. aeruginosa* RM1407 (lane 3), *P. aeruginosa* PU21 (lane 4), *P. aeruginosa* 10145 (lane 5), *P. fluorescens* 17,400 (lane 6), *P. aureofaciens* 13,985 (lane 7), *P. putida* 12,633 (lane 8) and *B. cepacia* 25416 (lane 9) probed with the 1.7 kb *Bsu*36I-*Bst*XI *fpvA* fragment. Sizes of standards are in kilobases.

In order to determine if the *fpvA* gene heterogeneity displayed by the *P. aeruginosa* and *P. fluorescens* strains was genuine, and not an artifact of the *Sst*II chromosomal digestions, the restriction enzymes *Bam*HI, *Hin*dIII and *Sph*I were used to digest the chromosomal DNAs from all of the bacteria and from the strain (CD10) from which *fpvA* had been isolated. These three enzymes have no restriction sites within either *fpvA* or the 4.6 kb *Sph*I fragment from which it was cloned and isolated. *fpvA*-probing of these digests should therefore recognize only one fragment if *fpvA* is indeed a single copy gene with no other homologous

Table 1. Restriction fragments patterns generated when *Sst*II (*Sac*II)-digested chromosomal DNAs from *P. aeruginosa* 6680, *P. aeruginosa* 1–8, *P. aeruginosa* RM1407, *P. aeruginosa* PU21, *P. aeruginosa* 10145, *P. fluorescens* 17,400, *P. aureofaciens* 13985, *P. putida* 12633 and *B. cepacia* 25416 were probed with the 1.7 kb *Bsu*36I-*Bst*XI *fpvA* fragment from *P. aeruginosa* CD10[a]

P. aeruginosa 6680	*P. aeruginosa* 1–8	*P. aeruginosa* RM 1407	*P. aeruginosa* PU21	*P. aeruginosa* 10145	*P. fluorescens* 17400	*P. aureofaciens* 13985	*P. putida* 12633	*B. cepacia* 25416[b]
					15.0			
					11.7			
			11.0					
					9.8		9.8	
			8.8			8.8	8.8	
					7.8			
					7.0			
					6.0			
	5.3				5.3			
4.4	4.4	4.4	4.4	4.4				
4.0	4.0	4.0	4.0	4.0	4.0			
3.7	3.7	3.7	3.7	3.7		3.7		
3.0	3.0	3.0	3.0	3.0				
					2.8			
1.8	1.8	1.8	1.8					
						1.7		
	1.5			1.5				
					1.4			
	1.1			1.1		1.1		
			<0.6					

[a]Restriction fragment sizes are in kilobases.
[b]With *Sst*II digested chromosomal DNA, no bands were observed from the *B. cepacia* sample.

genes in the genomes of the bacteria examined. While chromosomal DNA digests produced with all three restriction enzymes, followed by *fpvA* gene probing resulted in more than one band greater than 2.4 kb, the bands present in the *Hin*dIII digest were quite large and fewer in number than those present in either the *Bam*HI or *Sph*I digests (data not shown). We hypothesize that this result is due to fewer restriction sites for *Hin*dIII in these bacteria due to the relative lack of A-T rich chromosomal sequences.

The hybridization of the *fpvA* probe clearly yielded fragments other than a single band of 2.4 kb (the size of *fpvA*) or greater, indicating that more than one homologue of *fpvA* exists in all of these microbes, with the possible exception of *B. cepacia* (Tables 1 and 2, Figures 2 and 3). It is of interest to note that not only strains other than CD10, but indeed CD10 itself, contains multiple *fpvA* homologues. It is thus clear that all of the fluorescent pseudomonads examined contain greater than one *fpvA* homologue and thus may have the capacity to produce multiple FpvA-like proteins. While *B. cepacia* demonstrated no *fpvA* homology when its DNA had been digested by *Sst*II, it did hybridize weakly to the probe when its DNA had been digested by *Bam*HI. This observation is consonant with the fact that the *fpvA* probe used to generate the *Sph*I and the *Bam*HI data was, in particular, quite radioactive and that the bands noted were predominately much weaker than those seen for the other bacteria. The homology observed may thus represent weak homologies to *fpvA*-like sequences that specify membrane-spanning or energy transducing protein domains within *B. cepacia*'s IROMPs (see below).

Analysis of our data indicates that the *P. aeruginosa*, *P. fluorescens*, *P. putida* and *P. aureofaciens* strains examined in this study contain multiple *fpvA*-like sequences. We hypothesize that the domain(s) of *fpvA* responsible for the specific binding of ferripyoverdine by FpvA may, perhaps, not hybridize to nucleotides responsible for the binding of heterologous ferripyoverdines coded by other *fpvA*-like genes. The *fpvA*-like banding patterns observed in the current investigation, however, may be due to common coding sequences of ferripyoverdine (ferripseudobactin)

Table 2. Restriction fragments patterns generated when *Sph*I- and *Bam*HI- digested chromosomal DNAs from *P. aeruginosa* 6680, *P. aeruginosa* 1–8, *P. aeruginosa* RM1407, *P. aeruginosa* PU21, *P. aeruginosa* 10145, *P.aeruginosa* CD10, *P. fluorescens* 17,400, *P. aureofaciens* 13985, *P. putida* 12633 and *B. cepacia* 25416 were probed with the 1.7-kb *Bsu*36I-*Bst*XI *fpvA* fragment *from P. aeruginosa* CD10[a]

P. aeruginosa 6680	*P. aeruginosa* 1–8	*P. aeruginosa* RM1407	*P. aeruginosa* PU21	*P. aeruginosa* 10145	*P. aeruginosa* CD10	*P. fluorescens* 17,400	*P. aureofaciens* 13,985	*P. putida* 12,633	*B. cepacia* 25416
*Sph*I									
						16.5			
13.0	13.0	13.0	13.0	13.0	13.0				
						12.8			
							12.5		
			12.3		12.3				
						12.0			
9.6	9.6	9.6	9.6	9.6	9.6		9.6	9.6	
9.2	9.2	9.2	9.2	9.2	9.2				
						8.9			
								8.8	
		8.6							
						8.4			
								8.1	
						7.6	7.6		
7.4		7.4		7.4					
7.2	7.2	7.2	7.2	7.2	7.2				
							7.0		
								6.8	
							6.6		
								6.0	
						5.8			
	5.6		5.6		5.6				
5.2	5.2	5.2		5.2			5.2		
								5.0	
		4.8							4.8
4.6	4.6		4.6	4.6	4.6				
						4.5			
								4.4	
									4.0
						3.9	3.9		
								3.8	3.8
						3.6			
3.5	3.5	3.5		3.5					3.5
								3.2	
									3.0
					2.7				
2.5	2.5	2.5	2.5	2.5	2.5	2.5			
						2.0			
						1.8			
						1.4			
						0.9			

Table 2. Continued

P. aeruginosa 6680	*P. aeruginosa* 1–8	*P. aeruginosa* RM1407	*P. aeruginosa* PU21	*P. aeruginosa* 10145	*P. aeruginosa* CD10	*P. fluorescens* 17,400	*P. aureofaciens* 13,985	*P. putida* 12,633	*B. cepacia* 25416
*Bam*HI									
>23.1	>23.1		>23.1	>23.1	>23.1	>23.1	>23.1	>23.1	
							>23.1	>23.1	
						23.1		23.1	
						17.0	17.0		
16.0	16.0	16.0	16.0	16.0	16.0		16.0	16.0	
		11.0		11.0					
							9.3		
9.2	9.2	9.2	9.2	9.2	9.2				
		7.6							
							6.6		
6.4				6.4					
					4.2				
								3.8	
							3.6		
									3.5
								2.7	
						2.6			
									2.5
						2.3			
1.8	1.8	1.8	1.8	1.8	1.8				

[a]Restriction fragment sizes are in kilobases.

uptake genes which specify the membrane-associating amino acids or the energy transducer protein (TonB)-interacting regions of the FpvA-like proteins they specify (Poole *et al.* 1991; Koster *et al.* 1995; Larsen *et al.* 1997).

That all six *P. aeruginosa* strains contained more than two *Sst*II-digested homologues of greater than 1.7 kb in size suggests that these microbes may contain greater than one *fpvA*-like gene and protein. The same conclusion is made when one analyzes the *fpvA*-probe data from the *Bam*HI, *Hin*dIII and *Sph*I blots. Given that *P. aeruginosa* strains examined to date fall into one of three pyoverdine assimilation groups and that only a particular ferripyoverdine assimilation protein was expressed at a particular time (Hohnadel *et al.* 1988; Cornelis *et al.* 1989; Meyer *et al.* 1997), our data indicate that members of this species may indeed have the genetic capacity to use other pyoverdines via independent ferripyoverdine-assimilation proteins, a suggestion similarly made by Poole *et al.* (1991) when their ferripyoverdine IROMP mutant was nonetheless able to transport the ferrisiderophore at residual rates. *P. aeruginosa* may thus be similar to *P. putida* WCS 358 in that a specific pyoverdine may need to be present in order to cause the expression of secondary ferripyoverdine-assimilating proteins, although Ginsberg *et al.* (1992) presented data which indicated that *P. aeruginosa* employed a single ferripyoverdine IROMP for the transport of chromatographically distinct pyoverdines. In this respect, Meyer *et al.* (1999) recently demonstrated that FpvA from *P. aeruginosa* A.T.C.C. 15692 recognized and transported the bacterium's native ferripyoverdine and also that of a different species, that is, *P. fluorescens* A.T.C.C. 13525. As the nucleotide sequence of the latter bacterium's *fpvA*-like gene has not been determined, it is impossible to know the degree of similarity between the FpvA proteins and genes of the two bacteria. Whether they are strict homologues, share a great degree of similarity, or whether other *fpvA*-like homologues exist in these two strains, as the data of the current study would suggest, will be most interesting to discern.

The lack, or weak, binding of the 1.7 kb *fpvA* probe to DNA of *B. cepacia* suggests that while it makes two siderophores (pyochelin and salicylic acid) in common with *P. aeruginosa*, it does not share ap-

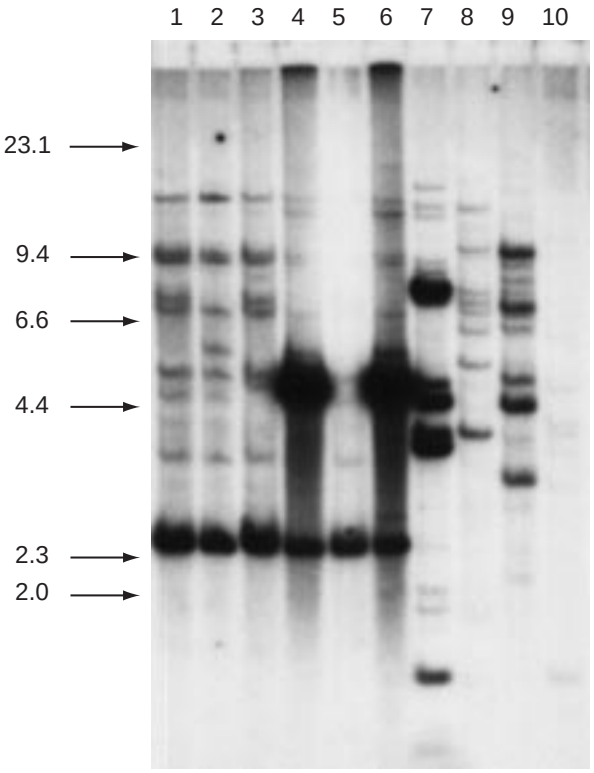

Figure 2. Southern blot of *Sph*I digests of chromosomal DNAs from *P. aeruginosa* 6680 (lane 1), *P. aeruginosa* 1–8 (lane 2), *P. aeruginosa* RM1407 (lane 3), *P. aeruginosa* PU21 (lane 4), *P. aeruginosa* 10145 (lane 5), *P. aeruginosa* CD10 (lane 6), *P. fluorescens* 17 400 (lane 7), *P. aureofaciens* 13 985 (lane 8), *P. putida* 12 633 (lane 9) and *B. cepacia* 25416 (lane 10) probed with the 1.7 kb *Bsu*36I-*Bst*XI-*fpvA* fragment. Sizes of standards are in kilobases.

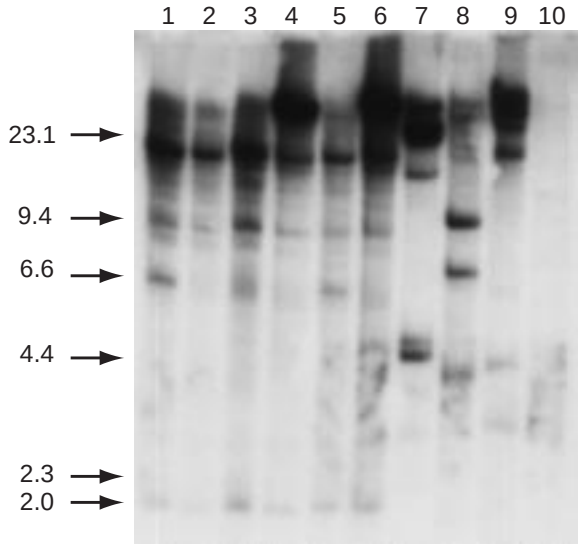

Figure 3. Southern blot of *Bam*HI digests of chromosomal DNAs from *P. aeruginosa* 6680 (lane 1), *P. aeruginosa* 1–8 (lane 2), *P. aeruginosa* RM1407 (lane 3), *P. aeruginosa* PU21 (lane 4), *P. aeruginosa* 10145 (lane 5), *P. aeruginosa* CD10 (lane 6), *P. fluorescens* 17 400 (lane 7), *P. aureofaciens* 13 985 (lane 8), *P. putida* 12 633 (lane 9) and *B. cepacia* 25416 (lane 10) probed with the 1.7 kb *Bsu*36I-*Bst*XI-*fpvA* fragment. Sizes of standards are in kilobases.

preciable homologies in its presumed ferrisiderophore uptake genes or proteins. As *B. cepacia* lacks the ability to use ferripyoverdine (Meyer *et al.* 1989), strong binding to the *fpvA* probe was not expected. The need for membrane localizing- and TonB-interacting domains in ferrisiderophore uptake proteins, however, would suggest that all such genes contain some degree of homology. Whether such is the situation between *B. cepacia*, *P. aeruginosa* and the other pseudomonads of this study, will only be clarified once all the responsible genes have been sequenced and the homologies among the group have been determined. The absence or noticeably weak binding of the *fpvA* probe to chromosomal DNA of *B. cepacia*, however, indicates that the ferrisiderophore-transporting proteins of this organism lack appreciable homology to those of the ferripyoverdine-IROMPs of *P. aeruginosa* and the other fluorescent pseudomonads examined.

The *fpvA*-like homology data of the current investigation has both similarities and contrasts with the data recently presented (Castignetti 1997). Homologues of fptA-like sequences in the *P. aeruginosa* strains used in that study, which are identical to the ones used in the current study (with the exception of *P. aeruginosa* CD10) contained sequence homogeneity among four of the five strains and only a single fptA gene likely exists in all five of the *P. aeruginosa* strains examined. *P. fluorescens*, *P. aureofaciens* and *P. putida*, however, contained multiple fptA-like sequences, indicating that for these bacteria multiple FptA-like proteins may be present.

Probing the five *P. aeruginosa* strains (6680, 1–8, RM1407, PU21 and A.T.C.C. 10145) with the 1.8-kb *fur* fragment resulted in the two distinct banding patterns seen in Figure 4. The patterns noted are consistent with *fur* being well-conserved within *P. aeruginosa* strains. Even though the *fur* probe used contains sequences other than those of the *fur* gene, our results are consistent with the possibility that a single *fur* homologue exists in these strains since these *fur*-like homologues contained no more than two bands larger than the probe (1.8 kb). Such a situation is consonant with those of previous studies (Schaffer *et al.*

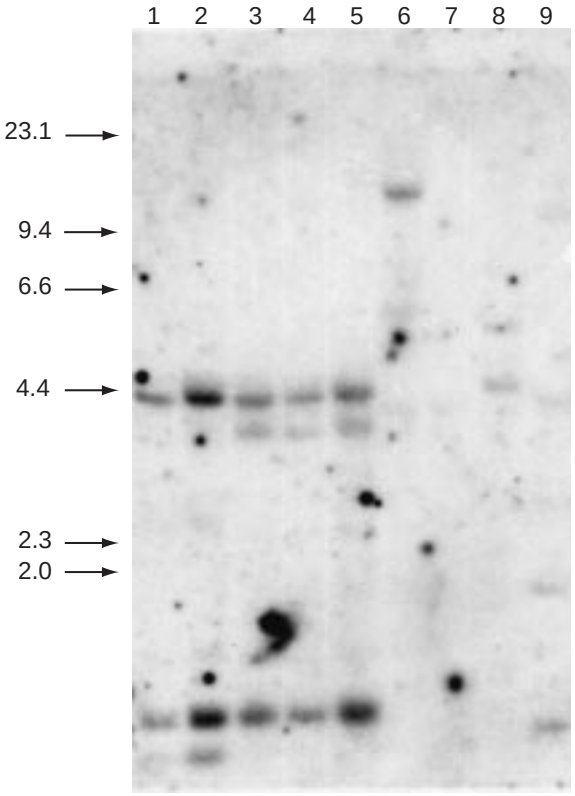

Figure 4. Southern blot of a *Sst*II (*Sac*II) digests of chromosomal DNA from *P. aeruginosa* 6680 (lane 1), *P. aeruginosa* 1–8 (lane 2), *P. aeruginosa* RM1407 (lane 3), *P. aeruginosa* PU21 (lane 4), *P. aeruginosa* 10145 (lane 5), *P. fluorescens* 17,400 (lane 6), *B. cepacia* 25416 (lane 7), *P. putida* 12 633 (lane 8) and *P. aureofaciens* 13 985 (lane 9) probed with the 1.8 kb *Eco*RI-*Hin*dIII *fur* fragment. Sizes of standards are in kilobases. Two patterns (first pattern:bands of 4.0, 1.1 and 0.9 kb for strains 6680 and 1–8, second pattern:bands of 4.0, 3.5 and 1.1 kb for strains RM1407, PU21 and 10145) were noted for the strains of *P. aeruginosa*. *P. fluorescens* had a single band of 14.5 kb, *B. cepacia* DNA did not bind the probe, *P. putida* had bands of 5.5 and 4.2 kb while *P. aureofaciens* bound the probe with bands of 2.0 and 0.9 kb.

1985; Litwin *et al.* 1992, 1993; Staggs & Perry 1992; Berish *et al.* 1993; Prince *et al.* 1993; Hickey & Cianciotto 1994; Heidrich *et al.* 1996), which indicated that a considerable degree of homology exists in the *fur* homologues of different bacterial species. The banding patterns noted in *P. aureofaciens*, *P. fluorescens* and *P. putida* are of interest as they were not those of *P. aeruginosa*, suggesting that while a *fur*-like homologue exists in these microbes, such genes are heterologous to those of the probe, i.e., *P. aeruginosa*. The lack of binding of the 1.8-kb *fur* fragment to chromosomal DNA of *B. cepacia* indicates, however, that if this organism does contain a *fur*-like gene, its sequence is significantly different from that of either of the *P. aeruginosa* strains or the other fluorescent pseudomonads examined.

Note added in proof

A Blast search of the unfinished genomes of *Pseudomonas aeruginosa* and *Pseudomonas putida* resulted in only one significant homology being found, that is, the *fpvA* sequence of *P. aeruginosa*.

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