Siderotyping of fluorescent pseudomonads: characterization of pyoverdines of *Pseudomonas fluorescens* and *Pseudomonas putida* strains from Antarctica

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Five independent fluorescent pseudomonad isolates originating from Antarctica were analysed for their pyoverdine systems. A pyoverdine-related siderotyping, which involved pyoverdine-induced growth stimulation, pyoverdine-mediated iron uptake, pyoverdine analysis by electrophoresis and isoelectric focusing, revealed three different pyoverdine-related siderotypes among the five isolates. One siderotype, including *Pseudomonas fluorescens* 1W and 1OcW, was identical to that of *P. fluorescens* ATCC 13525. Two other strains, *P. fluorescens* 9AW and *Pseudomonas putida* 9BW, showed identical pyoverdine-related behaviour to each other, whereas the fifth strain, *P. fluorescens* 51W, had unique features compared to the other strains or to a set of 12 fluorescent *Pseudomonas* strains used as comparison material. Elucidation of the structure of the pyoverdines produced by the Antarctic strains supported the accuracy of the siderotyping methodology by confirming that pyoverdines from strains 1W and 10CW had the same structures as the *P. fluorescens* ATCC 13525 pyoverdine, whereas the 9AW and 9BW pyoverdines are probably identical with the pyoverdine of *P. fluorescens* strain 244. Pyoverdine from strain 51W appeared to be a novel pyoverdine since its structure was different from all previously established pyoverdine structures. Together with the conclusion that the Antarctic *Pseudomonas* strains have no special features at the level of their pyoverdines and pyoverdine-mediated iron metabolism compared to worldwide strains, the present work demonstrates that siderotyping provides a rapid means of screening for novel pyoverdines.

Keywords: *Pseudomonas*, iron metabolism, pyoverdine, siderophore

INTRODUCTION

Siderophore-based methods for distinguishing fluorescent *Pseudomonas* strains through their siderophore (pyoverdine)-mediated iron-uptake system were initially investigated with a small number of *Pseudomonas aeruginosa* strains (Cornelis *et al.*, 1989), and recently extended to a larger set of about 100 collection and clinical isolates of the same species (Meyer *et al.*, 1997). Data from biological and analytical tests, such as pyoverdine-mediated bacterial-growth stimulation, pyoverdine-mediated iron uptake, pyoverdine isoelectric focusing (IEF) analysis and immunodetection of the outer-membrane ferripyoverdine receptors, allowed the recognition of three different pyoverdine systems among these strains. This multiplicity correlated well with the three *P. aeruginosa* pyoverdine structures obtained by chemical analysis (Briskot *et al.*, 1989; Gipp *et al.*, 1991; Tappe *et al.*, 1993; Meyer *et al.*, 1997). Thus, by the use of a limited panel of easily and rapidly performed assays which have been defined as 'siderotyping' (Meyer *et al.*, 1997), it was possible to classify all *P. aeruginosa* strains into one of three groups or 'siderovars', each siderovar consisting of strains producing the same pyoverdine, as
evident from IEF, and having the same specific pyoverdine-mediated iron-uptake system, as judged by growth stimulation, iron uptake and ferripyoverdine receptor properties.

Diversity among pyoverdines is even greater within strains belonging to the species *Pseudomonas fluorescens* or *Pseudomonas putida*. These bacteria are very often involved, thanks to their siderophore production, in plant-related biocontrol processes (O’Sullivan & O’Gara, 1992). Therefore, the characterization and identification of their respective pyoverdine and pyoverdine-mediated iron-uptake system is of interest. So far, at least 12 different pyoverdine structures have been recognized by chemical analysis among strains identified as *P. fluorescens* and six structures among *P. putida* strains. All these structures share the chromophore (1s)-5-amino-2,3-dihydro-8,9-dihydroxy-1H-pyrimido-alquinoline-1-carboxylic acid (Michels et al., 1991) as a common feature, which results in identical UV/visible spectral properties for all the pyoverdines. However, the other part of the molecule, a small peptide chain connected to the chromophore, presents a great variability depending on the bacterial origin, both in the number of amino acid residues (so far 6–12) and in their nature. Due to the great variability of pyoverdine structures and to the fact that chemical analysis of such complicated structures is laborious and time consuming, we wished to investigate the potentiality of biological methods for distinguishing fluorescent *Pseudomonas* strains belonging to the *fluorescens–putida* groups by their pyoverdine system and for recognizing those that produce known ones. For this purpose, we chose a collection of well-characterized strains isolated from Antarctica, expecting that the exotic geographical location of these strains could favour the discovery of so far undescribed pyoverdines.

**METHODS**

**Bacterial strains and growth conditions.** The fluorescent pseudomonads investigated in the present study were isolated by one of us (S.S.) from Schirmacher Oasis, Antarctica, and were characterized on the basis of morphology, biochemical and physiological tests as belonging to the *P. fluorescens* biotype A (strain 9A W), *P. fluorescens* biotype G (strains 1W, 10CW and 51W) and *P. putida* biotype B (strain 9BW) (Shivaji et al., 1989). Collection strains, i.e. *Aeruginosa* ATCC 15692 (PAO1 strain), *Aeruginosa* ATCC 28753, *P. fluorescens* ATCC 13525, *P. fluorescens* ATCC 17400, *P. fluorescens* CCMM 2798 and *P. putida* ATCC 12633, as well as natural isolates with known pyoverdine structures, i.e. *P. aeruginosa* strain P6 (Meyer et al., 1997), *P. fluorescens* strain ii (Mohn et al., 1990), *Pseudomonas aptata* strain P44a (Budzikiewicz et al., 1992), were also used.

Strains were preserved at −80 °C in glycerol 50% / LB culture (1 : 1, v/v) and were routinely grown at 25 °C on liquid or solid (12 g agar 1−1) LB medium. For pyoverdine production and iron-uptake studies, bacteria were grown at 25 °C with shaking (200 c.p.m.) in 100 ml or 11 Erlenmeyer flasks containing, per flask, 50 (or 500) ml succinate medium (Meyer & Abdallah, 1978). IEF analysis required a low-salt medium consisting of (g 1−1) : low-iron casein hydrolysate (Difco, Bacto Casamino Acid), 5 · KH2PO4·H2O, 1·5; and MgSO4·7H2O, 0·25, designated as CAA medium.

**Purification of pyoverdines.** The extraction of the pyoverdines which accumulate during iron-starved growth of fluorescent *Pseudomonas* spp. in the culture supernatant was usually done by the XAD procedure. For this, acidified culture supernatant (pH 6) was passed through an XAD-4 Amberlite column (15 × 2·5 cm for 1–2 l supernatant), which retained the pyoverdines. After washing with 500 ml distilled water, the pyoverdines were eluted with 250 ml 50% methanol, vacuum dried and lyophilized. Such preparations of so-called XAD-purified pyoverdines were used for most of the biological studies. Further purification steps involving chromatography on CM Sephadex and Biogel P-2 (Budzikiewicz, 1993) allowed the separation of the pyoverdines differing in the dicarboxylic side chain, the main one (usually containing succinic acid) being used for chemical analysis. Alternatively, the chloroform/phenol extraction of the iron complexes of pyoverdines from the culture supernatants (Meyer & Abdallah, 1978) was used in combination with column chromatography on Biogel P-2 (3 × 20 cm) and CM-Sephadex G25 (2.6 × 33 cm) columns, elution with 0·2 M pyridine/acetate acid buffer, pH approximately 5, depending upon the type of pyoverdines (Voss, 1995). The main compound was rechromatographed twice on CM-Sephadex and the corresponding iron-free pyoverdine was finally obtained by a decomplexation procedure using potassium oxalate at pH 4 (Schröder et al., 1993).

**Pyoverdine structure determination.** The main pyoverdines produced by the five Antarctic strains were subjected to UV/visible spectroscopy by using a Perkin-Elmer Hitachi 200 spectrophotometer. For amino acid analysis, 1 mg pure pyoverdine was hydrolysed with 6 M HCl for 16 h at 110 °C as described previously by Briskot et al. (1989) and Mohn et al. (1990). The hydrolysate was evaporated to dryness and the amino acids were derivatized into trifluoroacetyl amino acid isopropylates (TAP's) for gas chromatography coupled with mass spectrometry (GC-MS), and chiral gas chromatography on a Chirasil-L-Val column, 0·25 mm i.d. (Chrompac) (Voss, 1995). GC-MS, fast atom bombardment (FAB)-MS and NMR measurements and equipment were as described previously by Meyer et al. (1997).

**Electrophoretic analysis of pyoverdines.** CAA-culture supernatants and XAD-purified pyoverdines were analysed by IEF according to a method first described by Koedam et al. (1994). A Bio-Rad model 111 Mini IEF Cell was used with 125 × 63 × 0·4 mm polyacrylamide (5 %) gels containing ampholines (Bisolute 3/10 from Bio-Rad) which developed during the electrophoresis a pH gradient from 3·5 to 9·3. Preparation of the gels and electrophoresis conditions corresponded to the manufacturer’s recommendations. Deposits on gel were done as 1 μl aqueous solutions (6·5 mg ml−1) of the XAD-purified pyoverdines, or as 1 μl of the 20-fold-concentrated (through lyophilization) CAA-culture supernatants. Pyoverdine bands were visualized under UV light and their corresponding isoelectric pH values (pI) were determined according to a standard curve obtained by slicing the IEF gel immediately after electrophoresis into 0·5 cm bands which were incubated separately in 2 ml KCl (10 mM) solution for 30 min before measuring the pH.

Conventional electrophoresis analysis of the XAD-purified pyoverdine preparations was performed on cellulose acetate strips (17 × 4 cm; Cellogel) under slightly acidic (pH 5) conditions for 30 min at 300 V, at 4 °C.
Growth stimulation tests. Pyoverdine-induced growth stimulation tests were performed by plating the bacteria (100 µl of a 1/10 diluted culture in LB medium) on succinate agar medium supplemented with ethylenediaminedi hydroxyphenylacetic acid (EDDHA) at 500 or 1000 mg l⁻¹, depending on the sensitivity of the strain to EDDHA. UV-sterilized filter paper discs (6 mm diameter) were impregnated with 1 mM XAD-purified pyoverdine solutions (10 µl) in sterile distilled water, and the discs were placed on the surface of the seeded agar (10 discs per plate). Bacterial growth around the discs was determined after 24 h incubation at 25 °C.

Pyoverdine-mediated ⁵⁹Fe uptake. Pyoverdine-mediated iron uptake was studied as previously described by Cornelis et al. (1989) for testing the response of a single strain to a limited number of pyoverdines of various bacterial origin. A simplified, one-point kinetics method was used for testing a greater number of strains with a large number of pyoverdines (up to 14): cells from 7.5 ml culture in succinate medium were harvested by centrifugation, washed twice with distilled water, and resuspended in a defined volume of incubation medium (succinate medium with the nitrogen source omitted) to give a bacterial suspension of OD₆₆₀ 0.33. The suspension was dispensed into haemolysis tubes (one tube per pyoverdine to be tested) at a volume of 1.8 ml per tube and kept at 30 °C in a waterbath for 10 min. Then, 0.2 ml of a label mix made of commercial labelled iron chloride solution (5 µl ⁵⁹FeCl₃, specific activity 300 MBq mg⁻¹, Amersham), 1 mM solution of XAD-purified pyoverdine (50 µl) and incubation medium (945 µl) prepared 30 min before use, was added to the 1.8 ml bacterial suspension (starting point of the time scale). After rapid mixing and incubation at 30 °C for 20 min, 1 ml suspension was filtered through a cellulose membrane filter (Whatman, 0.45 µm porosity). The filter was washed twice with 2 ml fresh incubation medium followed by wrapping in aluminium foil, and was then counted for radioactivity in a Gammamatic 4000 counter (Beckman). The remaining 1 ml suspension was directly counted for radioactivity without filtration. The percentage of iron incorporated into the cells during the 20 min incubation was calculated by the ratio of c.p.m. values reached for the filtered and unfiltered assays. Assays without bacteria showed that the solubilization of the label iron through siderophore complexation was complete at 95–100%, depending on the pyoverdine preparation. Thus, percentage values of 5 % or less were considered as being due to incomplete solubilization rather than to cellular incorporation.

RESULTS

IEF profiles of the Antarctic Pseudomonas pyoverdines

Growth of the Antarctic strains in iron-poor media such as succinate medium or CAA medium resulted in the production of pyoverdines which were extracted from the growth supernatants by the conventional XAD-chromatography method. This one-step procedure allows the purification en masse of the different forms (succinate, succinamide, 2-oxoglutarate, etc.) of the pyoverdine produced during the culture (Budzikiewicz, 1993, 1997a). The resulting material was analysed by electrophoresis on polyacrylamide gel containing ampholines covering a pH range of 3.5–9.3. As shown in Fig. 1, pyoverdines from strains 9AW and 9BW (lanes 5 and 6, respectively) consistently presented identical profiles with three well-separated bands characterized by pl values of 9.2, 8.7 and 7.7, respectively. Pyoverdine IEF profiles from strains 1W and 10CW (lanes 3 and 4) were also very similar to each other, with three different bands with identical electrophoretic mobilities (pl 8.6, 8.5 and 7.4) but with variations in their intensities. Pyoverdines from strain 51W showed a distinct pattern, with two bands corresponding to pl values of 7.6 and 5.4, respectively (lane 7). Comparisons with the IEF profiles of pyoverdines from collection strains showed a close similarity between the pyoverdines of strains 1W and 10CW and that of P. fluorescens ATCC 13525 pyoverdines (Fig. 1, lane 2), with three bands for each migrating at the same level. The other collection strains demonstrated IEF profiles which were different from those of the Antarctic strains, as shown in Fig. 1 for P. aeruginosa ATCC 15692 (lane 1).

Identical conclusions were reached from the analysis of the pyoverdines by conventional electrophoresis on Cellogel. Three different mobility patterns were observed: one for the pyoverdines of strains 9AW and 9BW, the second for the pyoverdines of strains 1W, 10CW and P. fluorescens ATCC 13525, and the third for the pyoverdines of strain 51W (data not shown).

Growth stimulation properties of the pyoverdines of the Antarctic strains

In cross-feeding bioassays using EDDHA-supplemented agar plates, the pyoverdines of the Antarctic strains showed different patterns of growth stimulation activities. Pyoverdine from strain 51W was able to
Table 1. Pyoverdine (PVD)-mediated growth stimulation of the Antarctic Pseudomonas strains and some of the control strains

1W, 51W, 9AW, 9BW and 10CW are the test strains (Antarctic strains). P. fluorescens ATCC 13525, P. aeruginosa ATCC 15692 and P. fluorescens ATCC 17400 are control strains. Other control strains, i.e. P. aeruginosa ATCC 27853, P. fluorescens CCM 2798, P. putida ATCC 12633, P. aeruginosa Pa6, P. fluorescens ii and ‘P. aptata’ Ps4a, and their respective pyoverdines, reacted as did P. aeruginosa ATCC 17400. +, Bacterial growth occurring around the pyoverdine-impregnated paper disc (see Methods); 0, no bacterial growth.

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<td>1W</td>
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<td>51W</td>
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stimulate only the growth of its producer strain (Table 1) whereas pyoverdines from strains 1W and 10CW also showed crosswise growth stimulation. Analogous behaviour was also observed for the two strains 9AW and 9BW and their corresponding pyoverdines. Among the collection strains which were used for cross-feeding assays, two, P. aeruginosa ATCC 15692 and P. fluorescens ATCC 13525, showed cross-reactivity with strains 1W and 10CW. Their growth was stimulated by both 1W and 10CW pyoverdines, and their corresponding pyoverdines stimulated the growth of both Antarctic strains. All other collection strains and their respective pyoverdines reacted in a manner similar to P. fluorescens ATCC 17400 whose behaviour is illustrated in Table 1, i.e. they showed no growth stimulation of the tested strains.

Pyoverdine-mediated iron-uptake capacities of the Antarctic strains

The five Antarctic strains were analysed for iron-uptake capacity by using $^{59}$Fe$^{3+}$ chelated by pyoverdines (PVD) of different bacterial origin. For each strain, uptake mediated by 14 purified pyoverdines was tested separately. These pyoverdines included the structurally undetermined pyoverdines produced by the Antarctic strains and nine pyoverdines with different structures, originating from P. fluorescens ATCC 13525 (PVD13525), P. fluorescens ATCC 17400 (PVD17400), P. fluorescens CCM 2798 (PVD2798), P. fluorescens strain ii (PVDii), P. putida ATCC 12633 (PVD12633), ‘P. aptata’ strain Ps4a (PVDps4a), P. aeruginosa ATCC 15692 (PVD15692), P. aeruginosa ATCC 27853 (PVD27853) and P. aeruginosa strain Pa6 (PVDpa6) [for the structures of these pyoverdines, see Budzikiewicz (1997a, b)]. Comparison of the iron incorporation capacities of the five Antarctic strains in cross-uptake experiments revealed that each strain showed the best iron incorporation in the presence of its own pyoverdine. Strain 51W was strictly specific to its pyoverdine, whereas strains 1W and 10CW, as well as strains 9AW and 9BW, formed pairs of strains each presenting a different cross-specificity pattern. Strains 9AW and 9BW cross-acted with each other and recognized only their own pyoverdines, whereas strains 1W and 10CW cross-recognized their respective pyoverdines as well as the two pyoverdines of foreign origin, PVD13525 and PVD15692 (Table 2). PVD13525 was as efficient as PVD1W or PVD10CW in stimulating iron uptake in these two strains, whereas PVD15692 presented in both cases a much lower, but still significant, efficiency (Table 2). All other pyoverdines from collection strains were inefficient regarding iron uptake for all Antarctic strains, as illustrated in Table 2 for PVD27853. The special affinity of strains 1W and 10CW for the pyoverdine of P. fluorescens ATCC 13525 prompted us to investigate the reciprocity of cross-reactions among these three strains. As shown in Table 2, P. fluorescens ATCC 13525 incorporated the iron liganded to the pyoverdines of the two Antarctic strains with an efficiency similar to its own pyoverdine, whereas the efficiency of PVD15692 was similarly lower as in the case of the 1W and 10CW strains. In a similar manner, uptakes mediated by these four pyoverdines on P. aeruginosa ATCC 15692 revealed that PVD1W and PVD10CW showed behaviour identical to PVD13525, which is known to have a lower efficiency compared to the homologous pyoverdine, PVD15692 (Hohnadel & Meyer, 1988) (Table 2). P. aeruginosa ATCC 27853, the pyoverdine of which was not used by any of the Antarctic strains, reciprocally showed no
Table 2. Pyoverdine (PVD)-mediated iron-uptake capacities of the Antarctic
Pseudomonas strains and of some of the control strains

Uptake is expressed as the percentage of labelled iron incorporated into the cells under the
conditions as described in Methods (mean values of at least duplicate experiments). Bold values
indicate cross-uptake. 1W, 51W, 9AW, 9BW and 10CW are the test strains (Antarctic strains).
P. fluorescens ATCC 13525, P. aeruginosa ATCC 15692 and P. aeruginosa ATCC 27853 are control
strains. Other control strains, i.e. P. fluorescens CCM 2798, P. fluorescens ATCC 17400,
P. fluorescens ii, P. aeruginosa Pa6, P. putida ATCC 12633, 'P. aptata' Ps4a, and their respective
pyoverdines, gave similar results as P. aeruginosa ATCC 27853.

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<td>1W</td>
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<td>51W</td>
<td>3.2</td>
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<td>9AW</td>
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<td>10CW</td>
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<td>15.2</td>
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Table 3. Structures of the Antarctic Pseudomonas pyoverdines as represented by the
simplified on-line notation of the peptide chain

Affinity for any of the Antarctic pyoverdines. Identical
conclusions were reached when P. fluorescens CCM
2798, P. fluorescens ATCC 17400, P. fluorescens ii, P.
aeruginosa Pa6, P. putida ATCC 12633 and 'P. aptata'
Ps4a were tested (data not shown).

Pyoverdine structure determination

The main pyoverdine forms produced by the five
Antarctic strains were subjected to UV/visible spec-
troscopy, positive-ion FAB-MS and 1H-NMR. More-
over, a GC-MS analysis was performed following total
HCl hydrolysis and derivatization of the amino acids to
TAP derivatives. All these compounds showed the same
UV/visible spectrum, strictly identical to the one already
described for other pyoverdines (Briskot et al., 1989;
Mohn et al., 1990; Hohlneicher et al., 1995) and
characteristic of the quinoline chromophore of py-
overdines (Michels et al., 1991). Amino acid analysis as
well as MS and NMR data demonstrated that strains
1W and 10CW produced the same pyoverdines, identical
to that obtained previously from P. fluorescens ATCC
13525 and from Pseudomonas chlororaphis ATCC 9446
(Voss, 1995; see structure 1 in Table 3). Strains 9AW and
DISCUSSION

Pyoverdines, which are known as the characteristic yellow–green fluorescent pigments (Elliott, 1958) and siderophores of the fluorescent pseudomonads (Meyer & Hornsperger, 1978; Meyer et al., 1987), constitute a large family of compounds which could be considered as the most complex structures among microbial siderophores. Although no phenotypic differences can be observed between pigmented cultures of fluorescent pseudomonads, due to the identical spectral properties of the pyoverdines, it is well established that these compounds present a great diversity in structure at the level of their respective peptide chain, whereas the other part of the molecule [the chromophore, i.e. (1S)-5-amino-2,3-dihydro-8,9-dihydroxy-1H-pyrimido[1,2-a]quinoline-1-carboxylic acid (Michels et al., 1991)] is highly conserved. Diversity is even greater since, for a given strain, several pyoverdine forms co-occur in the culture supernatant, having the same peptide chain and the same chromophore, but differing from one another by the acyl chain (succinic acid, succinamide, 2-oxoglutaric acid, malic acid, malamide) attached to the chromophore. Close to 30 different pyoverdine structures have been established thanks to studies which required the most up-to-date technology, e.g. HPLC, gas chromatography, FAB-MS, analysis of the sequence characteristic ions in the ESI-MS spectrum after collision-induced dissociation, one- and two-dimensional NMR. Indeed and unfortunately, such laborious and time-consuming studies have resulted in the elucidation of already known pyoverdine structures, as in the case of P. aeruginosa pyoverdines (Briskot et al., 1989; Demange et al., 1990; Eng-Wilmot et al., 1990; Gipp et al., 1991; Tappe et al., 1993), or for some other pyoverdines and related compounds synthesized by various strains of P. chlororaphis, P. fluorescens and P. putida species or by undetermined fluorescent pseudomonad isolates (Demange et al., 1986; Persmark et al., 1990; Andriollo et al., 1992; Linget et al., 1992a, b; Hohnheicher et al., 1992, 1995; Glennon et al., 1994). To avoid such a waste of time and energy, any method which could allow a rapid recognition and comparison of a given pyoverdine with already known compounds would be of great interest for deciding whether or not a chemical investigation is required.

The different analytical and biological methods used in the present study appear to reach this goal. They allowed the recognition of three different pyoverdines among five well-characterized fluorescent Pseudomonas strains, one being produced by a single strain (51W), whereas the two others were each respectively produced by two strains, 9AW and 9BW for one, 1W and 10CW for the other. The biological tests, i.e. cross-feeding and iron-uptake assays, already suggested a strong similarity between the 1W/10CW pyoverdine-mediated iron-uptake systems and the one developed by P. fluorescens ATCC 13525. The analytical tests reinforced this view by demonstrating identical electrophoretic behaviour and IEF pattern for these pyoverdines, the identity of which was finally attested by a chemical investigation involving amino acid identification, FAB-MS and NMR analysis. Structure determination also confirmed that strains 9AW and 9BW were producing the same pyoverdine as suggested by the siderotyping and, moreover, revealed its probable identity with the pyoverdine from Pseudomonas A244 (Budzikiewicz et al., 1997). Strain 51W showed unique siderotyping features and its pyoverdine structure was effectively recognized as a novel type of pyoverdine. Thus, the siderotyping methods used in the present study are accurate and efficient in the characterization of pyoverdine-mediated iron-uptake systems, allowing a rapid screening of numerous fluorescent Pseudomonas and a rapid detection of novel pyoverdines. Although the two selected biological methods, i.e. cross-feeding and cross-uptake, resulted in identical conclusions, the use of both is of interest. Both give information on the iron-uptake systems constitutively expressed by the bacteria, whereas the cross-feeding assays alone could only directly detect for the presence of supplementary siderophore-inducible iron-uptake systems, as already demonstrated for some fluorescent Pseudomonas strains (Poole et al., 1990; Koster et al., 1993). The perfect correlation between data reached by the two methods did not suggest the presence of such supplementary inducible uptake systems within the Antarctic strains and, therefore, reinforced the presumption of identical structures for the cross-reacting pyoverdines.

As shown in the present study, the Antarctic strains present a great diversity in the pyoverdines they produce (three different pyoverdines for five strains analysed) and, moreover, most of them (four of five) can be classified in previously recognized siderovars, i.e. strains 9AW and 9BW within the siderovar of P. fluorescens 244, strains 1W and 10CW within the siderovar of P. fluorescens ATCC 13525. The fifth strain, 51W, thanks to its novel pyoverdine structure, opens a new siderovar which, however, could not be considered as specific to Antarctic strains. A recent siderotyping of more than 500 worldwide isolates allowed us to recognize two strains, one originating from a dairy farm in Spain, the other one from a mushroom farm in Finland, having an identical pyoverdine as strain 51W, as judged by IEF.
patterns and cross-uptakes. Thus, it can be concluded that Antarctic strains have no peculiar features as regards their pyoverdines compared to other, cosmopolitan, strains.

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