Exogenous siderophore-mediated iron uptake in *Pseudomonas aeruginosa*: possible involvement of porin OprF in iron translocation

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In addition to the two siderophores pyoverdine and pyochelin synthesized by *Pseudomonas aeruginosa* ATCC 15692 (strain PAO1), several siderophores produced by other bacteria or fungi, namely cepabactin, salicylic acid, desferriferrichrysin, desferriferricrocin, desferriferroxamine B, desferriferroxamine E and coprogen, were able to promote iron uptake with variable efficiencies in this bacterium. For most of these siderophores, these results were consistent with the growth stimulation produced by the same compounds in a plate bioassay. Desferriferroxochrome A, enterobactin and desferriferrirubin, however, did not promote iron uptake, although enterobactin and desferriferrirubin stimulated bacterial growth. These paradoxical data are discussed in view of siderophore-inducible iron uptake systems, as demonstrated recently for enterobactin. Among the strains tested, including the wild-type PAO1, the pyoverdine-less mutant PAO6606 and the two porin-mutants *P. aeruginosa* H636 (oprF::Tn) and *P. aeruginosa* H673 (oprF::Tn501), only for the porin-OprF mutant were fewer siderophores able to promote iron uptake compared to the other strains. Such results suggest that beside specific routes for iron uptake *P. aeruginosa* is also able to take up siderophore-liganded iron through OprF.

**Introduction**

To fulfill their iron requirement, many micro-organisms have evolved specialized molecules named siderophores which, when excreted in the cell environment, efficiently solubilize iron due to their high binding capacities for this element (Neilands, 1981; Matzanke et al., 1989). The ferrisiderophore complex is then usually translocated through the cell membranes by a highly specific active transport system involving several specialized proteins, the best characterized being the iron-regulated outer-membrane proteins (IROMPs) which act as ferrisiderophore receptors (Neilands, 1982). Iron-starved *Escherichia coli* cells synthesize several such IROMPs, each recognizing usually one (ferri)siderophore, including the endogenous siderophore produced by this bacterium, enterobactin, as well as the iron complexes of desferriferrichrome, desferriferrichrysin, coprogen or rhodotorulic acid, siderophores synthesized by other micro-organisms (Braun et al., 1987). Other bacteria, besides *E. coli*, can acquire iron from exogenous ferrisiderophores. For example, *Mycobacterium smegmatis* incorporates iron liganded to rhodotorulic acid (Ratledge, 1987), while *Erwinia herbicola* efficiently incorporates iron complexed to enterobactin, coprogen and, to a lesser extent, desferriferrichrome in addition to its own siderophore, desferriferroxamine E (Berner et al., 1988). Also, growth of some *Haemophilus* strains is stimulated by enterobactin (Williams et al., 1990), whereas this compound induces in *Pseudomonas aeruginosa* a new iron uptake system specific for ferrienterobactin (Poole et al., 1990). *P. aeruginosa* was also seen to recognize, together with its own (ferri)siderophore pyoverdine$_{P_{AO}}$, the iron complexes of pyoverdines synthesized by other strains of fluorescent pseudomonads, such as pyoverdine$_{P_{PAO}}$ produced by *P. chlororaphis* ATCC 9446 and pyoverdine$_{P_{13525}}$ produced by *P. fluorescens* ATCC 13525, thus demonstrating an exception to the general rule of strict specificity of recognition established for many different *Pseudomonas* species with regard to their respective pyoverdine-mediated iron uptake capabilities (Hohndel & Meyer, 1988).

It is of some interest to define the iron metabolism and especially the iron incorporation potentials that an opportunistic pathogen like *P. aeruginosa* may develop

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Abbreviation: EDDHA, ethylenediamine di(hydroxyphenylacetic acid).

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(Bozenhart & Ruden, 1987). In this paper the potential of 13 exogenous siderophores and citrate to promote iron uptake in _P. aeruginosa_ is described. In addition, the question of whether these siderophore–iron complexes utilize one of the known porin pathways, OprF (Nikaido & Hancock, 1986; Woodruff & Hancock, 1988) or OprD (Trias & Nikaido, 1990), as opposed to specific siderophore receptors, is explored.

### Methods

**Bacterial strains.** The bacterial strains used in this study were the wild-type strain _P. aeruginosa_ ATCC 15692 (strain PA01) and three mutants derived originally from this strain. The pyoverdine-less mutant PA06606, obtained by UV-mutagenesis, has been characterized previously (Hohnadel et al., 1986). The two porin-mutants, strains H636 and H673, were kindly provided by R. E. W. Hancock (University of British Columbia, Canada). Strain H673 is an oprD:-Tn501 mutant which lacks the outer-membrane protein D2 (H. Meadows & R. E. W. Hancock, unpublished data), whereas strain H636 is an oprF::Omega mutant lacking the outer-membrane protein F (Woodruff & Hancock, 1988).

**Plate bioassays.** The influence of siderophores on bacterial growth was studied by a disk diffusion method on solid media. Plate bioassays were performed on nutrient agar and on succinate-agar media supplemented or not with 100 μg ml⁻¹ of purified ethylenediamine di(hydroxyphenylacetic acid) (EDDHA), as previously described (Hohnadel & Meyer, 1988). Succinate-agar media were supplemented with 1 mM-methionine for growth of the methionine auxotroph strain PA06606. Cells harvested from an overnight culture in the corresponding liquid medium were washed twice with sterile saline and plated evenly with the help of sterile glass beads at a concentration of 10⁴ cells per plate. Sterile paper disks were impregnated with 20 μl of filter-sterilized 1 mM-siderophore solutions (3 mM for siderophores forming a 3:1 complex with iron, i.e. cepabactin) and deposited at the surface of the inoculated agar, four to five different compounds being tested per plate. Triplicate independent assays were run for each strain and for the different siderophore tested. Bacterial growth was observed at various times during incubation of the plates at 37 °C, and stimulation effects were visualized by development of the bacterial lawn first around the impregnated paper disks. This occurred, approximately 6 h after plating on nutrient agar without EDDHA, but required an 18 to 24 h incubation for growth to be visible on succinate-agar medium supplemented with EDDHA. No attempts were made to quantify the growth stimulation effects of the siderophores tested since, due to differences in the solubility of these compounds in aqueous media (e.g. pyoverdines are highly soluble in water, whereas for preparation of 1 or 3 mM solutions of cepabactin, enterobactin or pyochelin, methanol was required), the diameter of the stimulation zone measured around the disks may not correlate with the potency of the siderophores. To prevent any growth inhibitory effect due to methanol for compounds dissolved in this solvent, the impregnated disks were first air-dried before being deposited on the surface of the plates.

**Results**

**Cross-feeding assays**

As visualized by plate bioassays, many siderophores of various bacterial origins were able to promote growth of the wild-type _P. aeruginosa_ strain PA01 (Table 1). Some of them, i.e. the endogenous pyoverdine of _P. aeruginosa_ PA01 as well as the pyoverdines of _P. fluorescens_ ATCC 13525 and _P. chlororaphis_ ATCC 9446, resulted in a strong stimulatory effect as judged by the bacterial lawn which developed first around the respective siderophore-impregnated paper disks. Incubation at 37 °C for 2 d was necessary for bacterial growth on the entire surface of the EDDHA/succinate agar plates to be visible, whereas some growth was already visible around the disks after a 12 h incubation period and had reached its maximal intensity 12 h later. In contrast, bacterial growth in the immediate vicinity of disks impregnated with the pyoverdines of _P. fluorescens_ ATCC 17400, _P. fluorescens_ CCM 2798 or _P. aeruginosa_ ATCC 27853, required, like growth on the entire surface of the plates, 48 h to develop. The other siderophores tested, chemically unrelated to pyoverdines, resulted in various responses:
The siderophores were used in this study as the deferrated forms. Growth effects were observed after 24 h at 37 °C on EDDHA-supplemented succinate-agar medium and are indicated as follows: +, growth stimulation; 0, no stimulation; +/−, variable responses (slight stimulation or no stimulation) observed among the triplicate independent experiments performed.

<table>
<thead>
<tr>
<th>Siderophore</th>
<th>PAO1</th>
<th>PAO6606</th>
<th>H636</th>
<th>H673</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferricrocin</td>
<td>+</td>
<td>+/-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ferrichrysine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Ferrirubin</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Ferroxamine E</td>
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<td>+</td>
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<tr>
<td>Ferroxamine B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ferrichrome A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enterobactin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pyochelin</td>
<td>+/-</td>
<td>0</td>
<td>+/-</td>
<td>0</td>
</tr>
<tr>
<td>Cepabactin</td>
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<td>0</td>
<td>+/-</td>
<td>0</td>
</tr>
<tr>
<td>Pyoverdine$_{PAO}$</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>Pyoverdine$_{PAO783}$</td>
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<td>Pyoverdine$_{PAO2795}$</td>
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</tbody>
</table>

The 13 iron complexes tested in iron uptake experiments performed on the wild type strain _P. aeruginosa_ PAO1 could be classified in four groups, depending on the iron uptake kinetics experimentally observed (Fig. 1). The first group contained siderophores which very efficiently promoted iron uptake, namely the endogenous _P. aeruginosa_ PAO1 siderophore, pyoverdine$_{PAO}$, and cepabactin, the major siderophore of _P. cepacia_ ATCC 25416. The second group included citrate, salicylic acid, desferriferriechrysin and pyochelin, only one of which, pyochelin, is synthesized by _P. aeruginosa_ PAO1. These ligands promoted iron incorporation at, on average, 30% of the efficiency obtained with the first group of siderophores. The third group, represented by desferriferrioxamine B, desferriferrioxamine E, desferriferriechrysin and coprogen, demonstrated only a weak ability to promote iron uptake, at 10 to 15% of the efficiency of pyoverdine$_{PAO}$. Finally, desferriferriechrysin A, enterobactin and desferriferrirubin, constituting the fourth group, were unable to promote iron uptake in _P. aeruginosa_ PAO1.

The same experiments, done with the pyoverdine-less mutant PAO6606, resulted in roughly similar grouping of the different siderophores tested with certain changes. As shown in Fig. 2, iron uptake efficiencies were significantly increased for cepabactin, pyochelin, salicylic acid and citrate. In contrast, the efficiency of pyoverdine-iron uptake was about 30% less. Desferriferriechrysin, together with the siderophores of the third group (desferriferrioxamines B and E, desferriferriechrysin and coprogen), demonstrated a similar capacity to promote iron uptake in PAO6606 compared to PAO1, and desferriferriechrysin A, enterobactin and desferriferrirubin were again ineffective.

Iron uptake with the porin OprD (= protein D2) mutant (strain H673, Fig. 3) followed a similar pattern to that observed with PAO1: enhanced uptake with desferriferrioxamine B, decreased uptake with desferriferriechrysin, and no uptake with desferriferrioxamine E were the only major differences observed for this strain compared to its parent strain.

For the porin-OprF-deficient mutant, strain H636 (Fig. 4), fewer siderophores were able to promote iron incorporation as compared to the other strains. Cepabactin and pyoverdine$_{PAO}$ were the most efficient siderophores as for strain PAO1, and citrate, salicylic acid and pyochelin still promoted uptake at about 30% of the efficiency of cepabactin and pyoverdine$_{PAO}$. However, with the exception of coprogen, which still demonstrated a weak capacity to promote iron incorporation, none of the other siderophores promoted iron uptake in strain H636. This last group included desferriferrirubin, entero-
Fig. 1. $^{59}$Fe uptake mediated by various iron ligands in *P. aeruginosa* PA01 (ATCC 15692). The ligands are abbreviated as follows: cep, cepabactin; pvd, pyoverdine; cit, citrate; sal, salicylic acid; fch, desferriferriochrysin; pch, pyochelin; dfB, desferriferrioxamine B; fcr, desferriferricrocin; cop, coprogen; dfE, desferriferrioxamine E. The line designated 'X' represents incorporation of $^{59}$Fe liganded by desferriferrichrome A, enterobactin or desferriferrirubin.

Fig. 2. $^{59}$Fe uptake mediated by various iron ligands in the pyoverdine-less mutant *P. aeruginosa* PAO6606. The ligands are abbreviated as in Fig. 1. The line designated 'X' represents incorporation of $^{59}$Fe liganded by desferriferrichrome A, enterobactin or desferriferrirubin.

Fig. 3. $^{59}$Fe uptake mediated by various iron ligands in the porin OprD-deficient mutant *P. aeruginosa* H673. Abbreviations of the ligands are the same as in legend of Fig. 1. The line designated 'X' represents incorporation of $^{59}$Fe liganded by desferriferrichrome A, enterobactin, desferriferrirubin or desferriferrioxamine E.

Fig. 4. $^{59}$Fe uptake mediated by various iron ligands in the porin-OprF-deficient mutant *P. aeruginosa* H636. The ligands are abbreviated as in Fig. 1. The line designated 'X' represents incorporation of $^{59}$Fe liganded by desferriferrichrome A, enterobactin, desferriferrirubin, desferriferrioxamine E, desferriferrioxamine B, desferriferricrocin or desferriferrichrysin.
bactin and desferriferrichrome A, as for the other strains, but also desferriferroxamine B, desferriferroxamine E, desferriferrococin and desferriferrichrysin.

Discussion

From $^{59}\text{Fe}$ uptake studies involving 13 iron ligands mainly of microbial origin (siderophores) it can be concluded that 

P. aeruginosa ATCC 15692 (strain PAO1) is able to use the majority of the siderophores as an iron source. The only ones that were apparently not used by this bacterium were ferrichrome A and ferrirubin, two hydroxamate siderophores of fungal origin (van der Helm et al., 1987; Konetschny-Rapp et al., 1988), and enterobactin, the tricatecholate siderophore produced by several enterobacterial species, such as E. coli (O’Brien & Gibson, 1970), Salmonella (Pollack & Neilands, 1970) and Shigella (Payne, 1980).

Comparison of the uptake results with the growth stimulation properties of the siderophores towards the P. aeruginosa strains as visualized in plate bioassays (Table 1), indicated some discrepancies. For most of the siderophores, i.e. desferriferrococin, desferriferrichrysin, desferriferroxamine B, desferriferroxamine E and pyoverdine$_{PAO1}$, both methods resulted in the same conclusion — that the compounds had a stimulatory effect. Moreover, desferriferrichrome A and the pyoverdines from P. aeruginosa ATCC 27853, P. fluorescens ATCC 17400 and P. fluorescens CCM 2798, which had already been seen to be inefficient in promoting iron uptake in P. aeruginosa PAO1 (Hohnadel & Meyer, 1988; Cornelis et al., 1989), gave consistent results with both methods. Discrepancies, however, were found for desferrirrubin and enterobactin, which promoted growth of all the strains tested, but were unable to promote iron uptake in these strains. In contrast, pyochelin and cepabactin, both efficient iron transporters as revealed by uptake assays, failed to promote bacterial growth in EDDHA-containing plate bioassays. Such results are not surprising considering the strong inhibitory effect of EDDHA on growth of exclusively cepabactin- and pyochelin-producing strains like PAO6606 (Hohnadel et al., 1986) or P. cepacia ATCC 25416 (Meyer et al., 1989), since these siderophores have a weaker affinity for iron than EDDHA.

As already discussed elsewhere (Hohnadel & Meyer, 1988), results of plate cross-feeding experiments always need to be interpreted with caution since uncontrolled phenomena like competition for iron between the added siderophore and EDDHA, or with the endogenous siderophore excreted by the bacteria during growth, or toxicity of a given siderophore, may result in artifactual conclusions. Such undesired competition appears unlikely during uptake assays, done over a short period of time (15 min). Moreover, internal controls like the uptake results with strain PAO6606, a pyoverdine-deficient mutant, as well as the specific lack of iron incorporation with some siderophores for strain H636, attest to negligible iron exchange between siderophores during these assays.

Plate bioassays are still interesting, however, since they can reveal unsuspected features of siderophores, such as induction of specific iron transport pathways. K. Poole and colleagues have recently described the induction of an enterobactin-mediated iron uptake system by enterobactin itself in P. aeruginosa PAO1 (Poole et al., 1990). This inducibility of enterobactin may explain the discrepancy between the cross-feeding and uptake assays observed with this compound. In plate bioassays the growing bacteria were in contact with the inducer for a long period of time (18 to 24 h), resulting in a growth stimulation effect likely to be due to induction of the enterobactin-mediated iron incorporation system, whereas this system was not induced during the uptake assays, since enterobactin and the non-proliferating cells were in contact for only a short period of time (15 min). It remains to be determined whether the desferriferrirubin-related discrepancy results from such an induction, or is due to an iron exchange phenomenon.

The siderophores that promoted iron uptake by P. aeruginosa are of both bacterial and fungal origins, and belong to several different structural families. The fungal siderophores desferriferrococin and desferriferrichrysin are structurally closely related to the inefficient siderophores desferriferrichrome A and desferriferrirubin (van der Helm et al., 1987; Konetschny-Rapp et al., 1988). Coprogen is a fungal trihydroxamate siderophore made from three $\delta$N-hydroxy-$\delta$N-acylornithine units (van der Helm et al., 1987). In addition, the present study included two desferriferoxamine compounds of bacterial origin, desferriferroxamine B produced by actinomycetes (Bickel et al., 1960), and desferriferroxamine E, produced by actinomycetes and also by some other bacterial species, such as Erwinia herbicola (Berner et al., 1988) and Pseudomonas stutzeri (Meyer & Abdallah, 1980). The other effective siderophores are structurally unrelated to the siderophores cited above and included cepabactin, a pyridinone compound produced by the non-fluorescent Pseudomonas cepacia ATCC 25416 (Meyer et al., 1989), and salicylate, which is a precursor of pyochelin (Ankenbauer & Cox, 1988), but also acts as a siderophore for a strain of Pseudomonas fluorescens (unpublished results). Together with these exogenous siderophores and the siderophores pyochelin and pyoverdine, produced by P. aeruginosa PAO1 itself (Cox & Graham, 1979; Cox & Adams, 1985), citrate was also able to mediate iron
incorporation in this strain, a result in agreement with previous findings (Cox, 1980; Harding & Royt, 1990).

Among the 10 iron-chelating compounds able to promote iron uptake in P. aeruginosa PAO1, two of them, pyoverdine \textsubscript{PAO} and cepabactin, were particularly efficient compared with the others. For the wild-type strain PAO1, as well as for the two insertion mutants, H636 and H673, with altered porins, these two compounds led to an incorporation of iron that was two to three times greater than that observed with the next most efficient iron chelator, citrate.

It is not surprising that pyoverdine \textsubscript{PAO} was one of the most effective siderophores in promoting iron uptake since it is the endogenous siderophore of P. aeruginosa PAO1. The iron uptake efficiency for the second endogenous siderophore, pyochelin, was considerably lower, representing for strains PAO1, H636 and H673 less than 30\% of the iron incorporation observed with pyoverdine. Such results support the conclusion that pyoverdine is the major siderophore of P. aeruginosa PAO1, in agreement with results on the comparative pathogenicity of pyoverdine-less or pyochelin-less mutants of P. aeruginosa PAO1 (Ankenbauer \textit{et al.}, 1985). However, the present study demonstrates that, under appropriate circumstances, pyochelin can be more efficient than pyoverdine, as shown for the pyoverdine-less mutant PAO6606. After 15 min incubation this strain had incorporated twice as much radioactivity as the other strains when iron was chelated by cepabactin, salicylate or citrate, whereas pyochelin-mediated iron incorporation was 4-5 times greater in this strain compared to pyochelin-mediated incorporation in the other strains. In contrast, using pyoverdine, iron was taken up with similar efficiency by all strains, including PAO6606. Therefore, in PAO6606, pyochelin supported nearly 2-fold higher iron uptake than pyoverdine. From these results it seems that inability to synthesize pyoverdine results, in response to iron starvation, in increased efficiency of only some of the other possible iron acquisition routes, eg. cepabactin- or pyochelin-mediated iron uptake. Previous studies concerning the phenotypic characterization of the mutant PAO6606 (Hohnadel \textit{et al.}, 1986) demonstrated that this mutation, affecting the biosynthesis of pyoverdine, had no apparent effect on the outer membrane proteins of this bacterium. Thus, the mechanism by which P. aeruginosa PAO6606 is able to increase the efficiency of its secondary iron uptake systems is apparently not related to an overexpression of IROMPs.

Among the siderophores which are not produced by P. aeruginosa but were effective in iron incorporation, cepabactin, as mentioned above, demonstrated an effectiveness equivalent to that of the major endogenous siderophore of P. aeruginosa. Salicylate and citrate, which can be considered as siderophore-like compounds, were two other compounds which, regardless of the strain tested, resulted in a lesser but nevertheless consistent iron uptake efficiency. Cepabactin, 1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridone (Meyer et al., 1989), has no chemical relationship with pyoverdine (Briskot \textit{et al.}, 1989) or pyochelin (Cox \textit{et al.}, 1981). Therefore, it appears likely that a special receptor may be involved at the level of the P. aeruginosa outer membrane for recognition of the 3:1 cepabactin–iron complex.

Under iron-starvation, P. aeruginosa strains usually express several IROMPs (Cornelis \textit{et al.}, 1987, 1989), the major one, a protein with an apparent molecular mass of 80 kDa being the receptor for the pyoverdine–iron complex in strain PAO1 (Meyer \textit{et al.}, 1990). A search for mutants affected in the other IROMPs should clarify which protein could serve as the ferricepabactin receptor in the outer membrane of P. aeruginosa PAO1. A ferric–citrate receptor, corresponding to a 43 kDa outer-membrane protein, has recently been postulated in P. aeruginosa PAO1 (Harding & Royt, 1990), suggesting the existence of a specific route for iron incorporation through this compound, similar to the FecA function in \textit{E. coli} (Wagegg & Braun, 1981). The receptor, if any, for iron–salicylate remains to be determined. Since this compound is a precursor and part of the structure of pyochelin, one could postulate that the 14 kDa and/or the 75 kDa IROMPs of P. aeruginosa may act as receptor(s) for both pyochelin– (Sokol & Woods, 1983; Heinrichs \textit{et al.}, 1991) and salicylate–iron complexes.

The remaining exogenous siderophores able to promote iron uptake in P. aeruginosa, desferriferroxamine B, desferriferroxamine E, desferriferrichrysin and desferriferricrocin, generally had a substantially lower efficiency compared to the other siderophores and, interestingly, were totally inefficient when tested with the strain H636. This strain has an insertion in the gene for the outer-membrane protein OprF (Woodruff & Hancock, 1988), which has been shown to function as a porin, being a major uptake route across the outer membrane for hydrophilic compounds having a molecular mass less than 3000 Da (Nikaido & Hancock, 1986). This would suggest that ferrisiderophores may penetrate the bacterial cell through this pathway, and that the porin OprF is the only uptake route for iron complexes of desferriferroxamine B, desferriferroxamine E, desferriferrichrysin and desferriferricrocin. However, why ferrichrome A, ferrirubin and ferrienterobactin, which were shown to be inefficient for all the OprF\textsuperscript{+} strains, were unable to use this route, remains to be explained.

The siderophores taken up by specific pathways involving IROMPs like pyoverdine or pyochelin in P. aeruginosa may also penetrate through porin channels.
K. Poole and colleagues have recently isolated a *P. aeruginosa* mutant lacking the ferricyanide receptor (Poole et al., 1991). Interestingly, this mutant still presented a very low level of pyoverdine-mediated iron uptake, in a range of efficiency similar to that observed in the present study for desferriferrioxamine- and desferrichrysin- and desferriferricrocin-mediated iron uptake. The authors suggested the presence of a second uptake system for ferricyanide in *P. aeruginosa* which, in the light of results presented here, may be the porin pathway. More direct experimental evidence, such as permeability studies involving ferrisiderophores and porin-OprF proteoliposomes, are needed to assess this route of penetration for iron in *P. aeruginosa*.

In conclusion, this study underlines the particularly important role of iron in cellular metabolism by demonstrating that, beside the two endogenous systems involving pyoverdine and pyochelin and their respective receptors, *P. aeruginosa* is also able to fulfill its iron requirement by using exogenous siderophores and exogenous-siderophore-mediated iron uptake pathways, among them the porin-OprF channels. Another important feature revealed in this study is the increased capacity of iron uptake through secondary systems when the major route of iron incorporation becomes deficient. This unexpected mechanism of regulation remains to be explored.

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