Cepabactin from *Pseudomonas cepacia*, a New Type of Siderophore

By JEAN-MARIE MEYER*, DANY HOHNADEL AND FÉLIX HALLÉ

Laboratoire de Microbiologie, Institut de Biologie Moléculaire et Cellulaire du Centre National de la Recherche Scientifique, 4 rue Blaise Pascal, F-67070 Strasbourg Cedex, France

In iron-deficient conditions of growth *Pseudomonas cepacia* ATCC 25416 excreted both pyochelin and a low-molecular-mass compound which strongly chelated iron(III), and facilitated iron translocation as demonstrated by growth and uptake experiments. The name cepabactin is proposed for this new siderophore. Comparisons of UV–visible spectra and chromatographic behaviour, together with ^{1}H-NMR spectra, led to the conclusion that cepabactin is 1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone, a compound which can be considered as a cyclic hydroxamate, but also as a heterocyclic analogue of catechol. This pyridinone has already been described by other workers as an antibiotic produced by *Pseudomonas alcaligenes*, and by a soil isolate closely related to *Pseudomonas cepacia*. Thus, cepabactin appears to act as a siderophore for more than one species of non-fluorescent pseudomonad.

INTRODUCTION

Pseudomonas cepacia, first described as a phytopathogenic bacterium (Burkholder, 1950), has been reported since the last decade as a potential human pathogen isolated from a wide variety of clinical sources (Holmes, 1986). It belongs to the *Pseudomonas pseudomallei* RNA homology group within the DNA homology group II (Palleroni *et al.*, 1973) and is remarkable by virtue of its ability to use over 100 different organic compounds as carbon and energy source (Stanier *et al.*, 1966).

In previous studies of the iron metabolism of pseudomonads we found that pyoverdine, the yellow-green water-soluble pigment characteristic of *Pseudomonas fluorescens*, acts as a siderophore for this bacterium (Meyer & Abdallah, 1978; Meyer & Hornsperger, 1978). The iron-related properties of pyoverdine (called pseudobactin for plant-related isolates; Teintze *et al.*, 1981) were common to almost all species belonging to the so-called fluorescent pseudomonads group (Leong, 1986; Meyer *et al.*, 1987; Cody & Gross, 1987; de Weger *et al.*, 1988; Hohnadel & Meyer, 1988). The siderophore system(s) of non-fluorescent pseudomonads, which are unable to synthesize pyoverdine, have been investigated for only a few species. *Pseudomonas stutzeri* under iron deficiency produced a colourless compound, able to strongly chelate iron(III), and chemically identified as desferri-ferrioxamine E (Meyer & Abdallah, 1980). This compound is a cyclic trimer of succinic acid coupled via an amide bond to the hydroxyamino group of 1-amino-5-hydroxyaminopentane, thus resulting in the formation of a hydroxamate. It was first described as a secondary metabolite (sideramine) produced by *Nocardia* and several other actinomycetes, and called nocardamine (Stoll *et al.*, 1951; Bickel *et al.*, 1960; Keller-Schierlein & Prelog, 1961; Müller & Zähner, 1968). Although direct evidence of the involvement of nocardamine in iron transport is scant, its role as a siderophore was strongly suggested by its ability to enhance the growth of *Arthrobacter* JG-9 (Burnham & Neilands, 1961) and other micro-organisms (Zähner *et al.*, 1962) and by the fact that it reversed inhibition by

---

Abbreviation: EDDHA, ethylenediamine-di-(*o*-hydroxyphenylacetate).

ferrimycin antibiotics (Prelog, 1963). Nocardamine stimulates the growth of *P. stutzeri* and reverses the growth inhibition caused by the synthetic iron chelator ethylenediamine-di(o-hydroxyphenylacetate) (EDDHA) (J.-M. Meyer, unpublished results). Uptake studies have demonstrated the participation of nocardamine in iron translocation by *Streptomyces pilosus* (Müller & Raymond, 1984).

P. cepacia and clinical isolates belonging to this species have recently been reported to produce pyochelin (Bukovits *et al.*, 1982; Sokol, 1986), a compound, chemically unrelated to pyoverdine, previously described in the fluorescent pseudomonad *P. aeruginosa* (Liu & Shokrani, 1978; Cox & Graham, 1979). A 14 kDa ferripyochelin-binding protein, present in increased amounts in the outer membrane of iron-starved *P. aeruginosa* and *P. cepacia* cells, has also been described (Sokol & Woods, 1983; Sokol, 1984). Pyochelin has been shown to be a potent siderophore for clinical isolates of *P. cepacia*; and a correlation was found between its production and morbidity and mortality in infected cystic fibrosis patients, suggesting a role in pathogenicity (Sokol, 1986). However, about half of the clinical isolates investigated by Sokol (1986) were pyochelin negative. How these strains satisfy their iron requirement in an environment where iron is strongly chelated by host transferrin and lactoferrin remains unexplained. Moreover, pyochelin appears to be inefficient when competing for iron with the serum proteins. A *P. aeruginosa* mutant affected in the biosynthesis of pyoverdine (Pvd$^-$), but still able to produce pyochelin (Pch$^+$), had no observable advantage over a Pvd$^-$ Pch$^-$ mutant and had severely retarded growth compared to the wild strain in media supplemented with transferrin or human serum (Ankenbauer *et al.*, 1985). Thus, even for the pyochelin-producing clinical isolates of *P. cepacia*, the existence of at least one supplementary iron-transport system would appear to be necessary for human pathogenicity.

METHODS

Strains and growth conditions. Pseudomonas cepacia ATCC 25416 was used throughout this work. Growth conditions were as described for *Pseudomonas fluorescens* by Meyer & Abdallah (1978), using a synthetic medium with succinate as the carbon and energy source (succinate medium). One-litre Erlenmeyer flasks containing 500 ml medium were used for large-scale preparation; growth experiments were done in 180 × 18 mm capped glass tubes containing 7·5 ml medium. Iron supplementation, when indicated, was performed by adding sterile FeCl$_3$ solution (1 mg iron ml^{-1}) to the presterilized succinate medium. Media were inoculated with exponential-phase cells grown in the same medium, to give initial concentrations of 1 % in volume for the 500 ml cultures or 10^6 to 10^7 washed cells per 7·5 ml for tubes. Unless otherwise indicated, incubation was at 25 °C on a rotary shaker at 200 r.p.m. For comparison purposes the pyochelin-producer strain *P. aeruginosa* ATCC 15692 (strain PAO1) was grown under identical conditions.

Growth and siderophore measurements. Growth in tube cultures was measured directly by using a Lumetron colorimeter (Photovolt Co.) with a 650 nm filter. Siderophore production was measured on 5 ml culture samples. The sample was centrifuged for 10 min at 24000 g (4 °C), and 10 μl 2 M-FeCl$_3$ was added to the supernatant and mixed vigorously. The supernatant was again centrifuged (same conditions) 30 min later, and the absorbance read on a Pye–Unicam SP8-400 (Philips) spectrophotometer. Depending on the pH of the culture at the time of harvest, the λ_{max} for the iron–siderophore complex in the visible region was between 440 and 460 nm. Since only few differences in absorbance occurred within this range, the production of siderophore was quantified according to A_{450}.

^{59}*Fe incorporation.* Cells from 50 ml of an exponential culture were harvested by centrifugation (24000 g, 10 min, 4 °C), washed twice with distilled water and resuspended at OD$_{600}$ 0·3 in nitrogen-free succinate medium (incubation medium). Then 9 ml of this suspension in a 250 ml Erlenmeyer flask was incubated at 25 °C in a shaking (100 r.p.m.) water-bath for 10 min before supplementation with 1 ml of a labelled iron mixture (zero time) containing ^{59}Fe (5 μl of the commercial solution: chloride form, specific activity 20 mCi mg^{-1}, 740 MBq mg^{-1}; Commissariat à l'Energie Atomique, Gif-sur-Yvette, France); 50 μl of a 1 mM solution of the pure ligand dissolved in methanol and then diluted 1:10 with distilled water; and 945 μl incubation medium, added 10 min later. Low blank values were obtained in control experiments without bacteria.

Samples (1 ml) of the bacterial suspension supplemented with the labelled solution were withdrawn at intervals during the 30 min incubation, and rapidly filtered on to cellulose nitrate membrane filters (Whatman, 0·45 μm porosity). The filters were washed with 10 × 2 ml fresh incubation medium to remove non-incorporated labelled iron. When specified, KCN or 2,4-dinitrophenol (DNP) was added to the cell suspension 10 min before the addition of the iron source. In some experiments, cepabactin was replaced by EDDHA or various pyoverdines

(50 µM final concentration). It was verified that under the conditions used, all these compounds completely solubilized the labelled iron.

Siderophore purification. The procedure used for cepabactin purification was essentially based on the method described by Barker *et al.* (1979) for the purification of antibiotic G1549, the trivial name given by the authors to 1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone, which they isolated from *Pseudomonas alcaligenes.* Based on chloroform or ethyl acetate treatment of the acidified growth medium, this procedure is very similar to that used by Itoh *et al.* (1979) for the extraction of antibiotic BN-227 (the same pyridinone derivative as G1549) from *Pseudomonas* BN-227, or by Liu & Shokrany (1978), Cox & Graham (1979) or Sokol (1986) for the extraction of pyochelin from *P. aeruginosa* strains or clinical isolates of *P. cepacia.*

P. cepacia ATCC 25416 was grown in succinate medium (4 litres) supplemented with 2 µM-iron in order to maximize production of cepabactin (see Fig. 2). Cultures were centrifuged when cells reached the stationary phase (40 h of growth at 25 °C). The supernatant, acidified to pH 3·0 by addition of 1 M-HCl, was extracted twice with 0·2 vol. chloroform. After vigorous shaking and sedimentation, the chloroform extracts were combined, washed four times with water, filtered on anhydrous sodium sulphate, and evaporated under vacuum to dryness. The residue was dissolved in 10 ml methanol and the soluble material fractionated in methanol on a column (45 × 2·5 cm) of Sephadex LH-20. The different fractions were analysed by spotting 5 µl of each on to a cellulose plate sprayed with an iron solution (0·1 M-FeCl$_3$) or with the phenolate spray reagent (1 vol. iron solution added to 1 vol. 0·1 M-potassium ferricyanide) as described by Cox & Graham (1979). The same procedure was used to purify pyochelin from *P. aeruginosa* ATCC 15692.

Physico-chemical analysis. UV–visible spectra of the purified compounds solubilized in methanol were obtained with a Pye–Unicam SP8-400 spectrophotometer. ^{1}H-NMR analysis of cepabactin in CD$_3$OD was done with a Bruker SY200 spectrometer. Thin-layer chromatography (TLC) on Polygram SIL G/UV$_{254}$ from Macherey and Nagel (Düren, FRG) was performed using eight different solvent systems: A, chloroform/methanol (4:1, v/v); B, benzene/ethanol (4:1, v/v); C, benzene/ethyl acetate (1:9, v/v); D, benzene/acetone (3:1, v/v); E, chloroform/acetic acid (90:1, v/v); F, chloroform/ethanol (4:1, v/v); G, chloroform/acetic acid/ethanol (90:5:2·5, by vol.); and H, 2-propanol/25% ammonium hydroxide/water (100:10:10, by vol.). Systems A–D were those of Itoh *et al.* (1979), and systems E–H those of Cox & Graham (1979).

RESULTS

Growth and siderophore synthesis as a function of the iron concentration of the medium

Cultures of *P. cepacia* in succinate medium reached the stationary phase after about 40 h of growth under the conditions used. Supplementation of the medium with iron (FeCl$_3$) increased both growth rate and cell yield (Fig. 1). Siderophore production was followed colorimetrically as described in Methods. The orange colour which developed immediately upon addition of an excess of iron to the culture was quantified by measuring, after centrifugation, the absorbance of the supernatant at 450 nm. This absorbance increased progressively during exponential growth in succinate medium, and reached a maximal value when the culture entered the stationary phase. As shown in Fig. 2, which represents maximal growth yield and maximal siderophore production obtained in succinate media containing increasing concentrations of iron, the cell yield was proportional to the added iron concentration up to 4 µM added iron. Production of siderophore was maximal with 2 µM-iron supplementation, decreasing markedly at higher iron concentrations. At 10 µM added iron the siderophore was undetectable in the culture supernatant. The production of siderophore per unit of cells (expressed as the ratio A_{450}/OD_{650}) followed a regular inverse relationship with the iron concentration of the growth medium until growth was no longer iron limited. This ratio was quite stable for iron-free succinate cultures incubated at different temperatures, within the range 25–37 °C, where the growth yield stayed constant. Above 37 °C, the ratio decreased drastically, and siderophore production was completely abolished at 41 °C, the growth temperature limit found for this bacterium (data not shown).

Characterization of cepabactin and pyochelin

The extraction of the chloroform-soluble material from 4 litres of culture supernatant of *P. cepacia* (succinate medium supplemented with 2 µM-iron, acidified to pH 3) yielded 482 mg of dry residue. When the methanol-soluble portion of this residue was applied to an LH-20 column
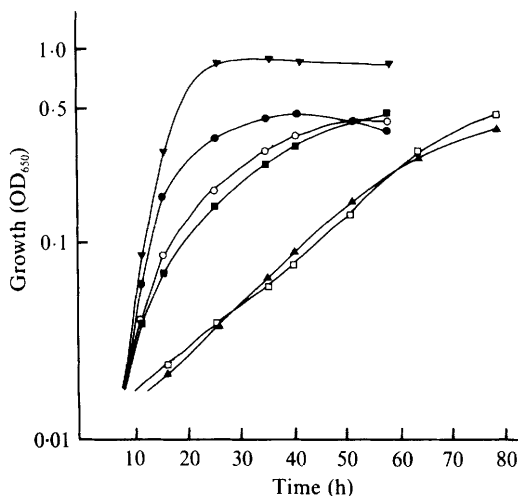
Fig. 1. Growth of *P. cepacia* ATCC 25416 in succinate medium (■) or in the same medium supplemented with 20 μM-FeCl₃ (▼) or 7 μM cepabactin (○), 70 μM-cepabactin (●), 2·8 mM-EDDHA (□), or 20 μM-pyoverdine (isolated from *P. fluorescens* ATCC 13525) (▲). The results are means of triplicate experiments.
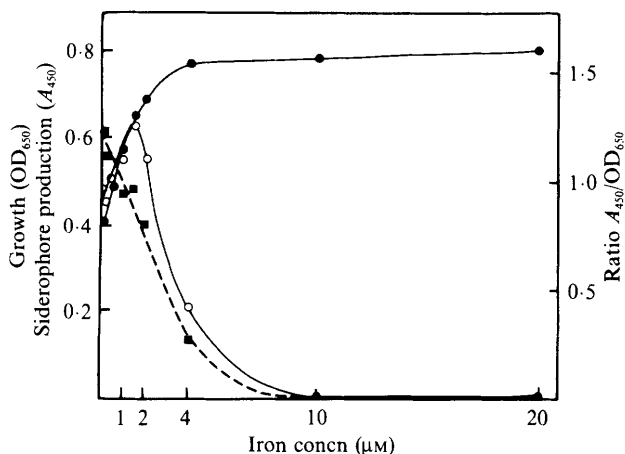

Fig. 2. Growth (OD_{650}, ●) and siderophore production (A_{450}, ○) of *P. cepacia* as a function of the iron concentration of the culture medium. ■, Production of siderophore per unit of cells, expressed as the ratio A_{450}/OD_{650}. Growth and siderophore production values are the means of triplicate experiments.

and eluted with methanol, an orange material eluted first, well separated from a yellow peak characterized by its weak fluorescence when illuminated with UV light at 365 nm. Only the fractions constituting these two major peaks reacted positively with the FeCl₃ and the phenolate spray reagents. They contained 182 mg for the orange material (compound I) and 132 mg for the yellow one (compound II). When the same procedure was applied to 5 litres of culture supernatant, acidified to pH 3, of *P. aeruginosa* ATCC 15692, three separate peaks were obtained. The first peak was slightly purple, and contained 4 mg of dry material; the two others, both yellow, contained 55 and 14 mg respectively. Only the material collected as the major yellow peak gave a positive reaction with the two spray reagents and thus was further characterized; the two other peak fractions were discarded.

UV–visible spectra were obtained for methanol-solubilized samples of the three iron-reacting compounds isolated. Compound I from *P. cepacia* was characterized by a maximum absorption

Table 1. *TLC R_F values of cepabactin and pyochelin*

| | R_F value | | | | | | | |
| | | | | Solvent system*: | | | | |
Compound	A	B	C	D	E	F	G	H
P. cepacia peak I (cepabactin)	0·84 (0·69)†	0·56 (0·54)†	0·05 (0·07)†	0·16 (0·22)†	0·19	0·95	0·51	0·17
P. cepacia peak II‡ (pyochelin)	0·73	0·47	0·19	0·22	0·13	0·72	0·47	0·46
P. aeruginosa pyochelin	0·73	0·47	0·18	0·21	0·13 (0·19)§	0·71 (0·75)§	0·47 (0·69)§	0·46 (0·60)§

* See Methods for the composition of the solvent systems and the TLC plates used.
† Value from Itoh *et al.* (1979) for 1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone.
‡ The R_F values indicated refer to the major iron-reacting spot (see text).
§ Value from Cox & Graham (1979) for pyochelin of *P. aeruginosa* PAO1.
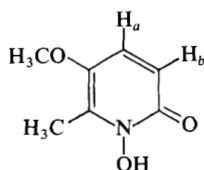
at 330 nm and a weak absorption at 440 nm responsible for the typical orange colour of the concentrated solution. Compound II from *P. cepacia*, and the product isolated from *P. aeruginosa*, had UV–visible absorption spectra identical to those of pyochelin isolated from *P. aeruginosa* (Cox & Graham, 1979) or from clinical isolates of *P. cepacia* (Sokol, 1986), with peaks at 210, 250 and 310 nm. Furthermore, both compounds had identical R_F values when analysed on TLC plates, whereas *P. cepacia* compound I demonstrated specific R_F values for each of the eight TLC-solvent systems used (Table 1). The chromatograms also revealed the homogeneity of compound I, which migrated as a single iron-reacting spot in each solvent system. In contrast, *P. cepacia* compound II and pyochelin both usually gave two or three iron-reacting spots, with one being predominant. This behaviour fitted well with the lability on TLC described by Cox & Graham (1979) for pyochelin. Solvents A to D were those used by Itoh *et al.* (1979) to characterize the iron-chelating compound 1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone produced by the soil isolate *Pseudomonas* BN-227, whereas solvents E to H were those used by Cox & Graham (1979) for the characterization of pyochelin from *P. aeruginosa* strains. The respective values found by these authors (indicated in parentheses in Table 1) roughly corresponded to compound I for the *Pseudomonas* BN-227 product, and to the yellow iron-reacting compound of *P. cepacia* or *P. aeruginosa* for pyochelin, the few discrepancies probably being due to the differences in TLC supports, which can influence the mobility and the degree of lability of the tested compounds, especially pyochelin. For example, the chromatograms developed with solvents G and H revealed, on the Polygram SIL G/UV$_{254}$ pre-coated plastic sheets used in this study, very minor spots for the *P. cepacia* compound II and the *P. aeruginosa* product, having R_F values of respectively 0·73 and 0·57, which corresponded more closely to the values (0·69 and 0·60) obtained by Cox & Graham (1979).

Since compound I of *P. cepacia* ATCC 25416 has never been previously described for well-defined *P. cepacia* strains, its possible identity as 1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone was further analysed by ^{1}H-NMR spectroscopy. The characteristics of its spectrum when dissolved in CD$_3$OD are given in Table 2, together with the attributed formula of the compound. The perfect correlation between these results and those of Itoh *et al.* (1980) and Barker *et al.* (1979), together with the similarity shown by UV-visible spectrometry and TLC, led to the conclusion that *P. cepacia* compound I, which we term cepabactin, was 1-hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone, whereas *P. cepacia* compound II corresponded to pyochelin, already described as a potent siderophore for some clinical isolates of *P. cepacia* (Sokol, 1986).

Effects of cepabactin and other ligands on growth of P. cepacia

Supplementation of the succinate medium with cepabactin resulted in a growth stimulation which was proportional to the amount of ligand added. Stimulation was already effective with 7 μM-cepabactin (Fig. 1), and it increased linearly with increasing concentration of added

Table 2. ^1H-NMR spectrum of cepabactin in CD_3OD

	CH$_3$	OCH$_3$	H$_a$ $\xleftarrow{x}$	H$_b$
δ (p.p.m.)	2·44	3·81	6·59	7·45
Multiplicity	s	s	d (7·5 Hz)	d (7·9 Hz)

x, Peaks not assigned.

cepabactin, the maximal effect being observed at 70 µM-cepabactin, a concentration corresponding to the estimated limit of solubility of this compound in aqueous solution at 25 °C. Since the cepabactin was added as a methanolic solution it was checked that, at the concentrations used, the solvent had no effect on the bacterial growth (data not shown). Fig. 1 shows that the growth rate during the first hours of exponential growth was similar in succinate medium supplemented with 20 µM-FeCl$_3$ or with 70 µM-cepabactin. Maximal cell yield, however, was doubled in the iron-supplemented medium compared to the unsupplemented and cepabactin-supplemented media, which gave about the same yield of biomass. Effects of several other iron chelators were also studied. Growth inhibition, resulting from a drastic lowering of growth rate together with an increased lag phase, was observed when succinate medium was supplemented with the synthetic iron chelator EDDHA (final concentration 2·8 mM) or with pyoverdine (final concentration 20 µM). Fig. 1 shows the results obtained with pyoverdine isolated from *P. fluorescens* ATCC 13525. Using pyoverdines from different bacterial strains, viz. *P. fluorescens* W (laboratory strain), *P. fluorescens* ATCC 17400 and *P. aeruginosa* ATCC 15692 (PAO1 strain), which have slightly different chemical structures (Hohnadel & Meyer, 1988), resulted in a similar inhibition effect. Identical results were obtained when *P. cepacia* was inoculated on to agar-solidified succinate medium supplemented with the various iron chelators. No growth appeared on plates supplemented with EDDHA (2·8 mM) even after one week of incubation, whereas 48 h at 25 °C was sufficient to observe well-formed colonies on the unsupplemented medium. Pyoverdines tested by an impregnated (7 µl of a 5 mg ml^{-1} aqueous solution) paper disc method (Hohnadel & Meyer, 1988), gave a growth-inhibition zone around the disc that was still visible 2 d after the bacterial lawn had developed.

Iron uptake mediated by cepabactin and other ligands

When *P. cepacia* cells, harvested at the end of the exponential phase from succinate medium (iron-depleted cells), were incubated in non-proliferating conditions (succinate medium with the nitrogen source omitted) in the presence of ^{59}Fe chelated by cepabactin, uptake of labelled iron occurred rapidly, attaining 170 pmol ^{59}Fe per mg dry weight of cells after 30 min incubation (Fig. 3). The same experiment conducted at 0 °C instead of 25 °C resulted in no iron uptake. Iron uptake was also completely inhibited when cells were washed and incubated at 25 °C in a medium without succinate. Pre-incubation of the cells for 10 min in incubation medium plus 0·2 mM-KCN before the addition of the ^{59}Fe source resulted in a 73% inhibition of uptake, whereas 0·2 mM-DNP had only a slight effect (14% inhibition). A 1 mM concentration of DNP had to be used in the incubation medium in order to observe a similar level of inhibition as that obtained with 0·2 mM-KCN (Fig. 3). Cepabactin-mediated iron uptake by cells harvested from succinate medium supplemented with 20 µM-iron was inefficient. Incubation of iron-deficient cells with pyoverdines from different bacteria (*P. fluorescens* W, *P. fluorescens* ATCC 13525, *P. fluorescens* ATCC 17400, *P. aeruginosa* PAO1) or EDDHA instead of cepabactin as labelled iron chelator resulted in no uptake.

DISCUSSION

This study shows that cepabactin isolated from *P. cepacia* ATCC 25416 has all the characteristics of a siderophore: it is synthesized specifically by iron-deficient cells; it stimulates
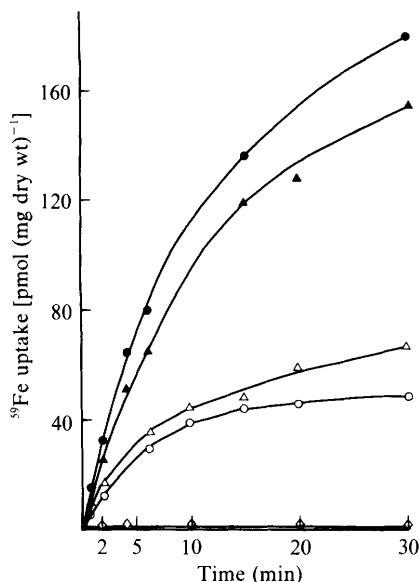
Fig. 3. Iron uptake in *P. cepacia*. Incorporation of ^{59}Fe chelated by cepabactin was measured with iron-starved cells resuspended in incubation medium at 25 °C (●), at 0 °C (◇), or in incubation medium (25 °C) supplemented with 0·2 mM-DNP (▲), 1 mM-DNP (△), 0·2 mM-KCN (○), or in incubation medium with the energy source (succinate) omitted (◇). The symbol (◇) also represents incorporation experiments performed in standard conditions (normal incubation medium, 25 °C, ^{59}Fe-cepabactin) but with cells previously grown in iron-rich medium, or with iron-starved cells but with cepabactin replaced by pyoverdine or EDDHA as iron chelators. Uptake assays were done at least in duplicate.

bacterial growth in an iron-poor medium and it specifically promotes iron uptake in iron-deficient cells. In the presence of ferric ions, cepabactin formed an orange complex resulting from the presence in its structure of a hydroxamate group formed by the *N*-hydroxyl of a pyridine ring and the *N*-adjacent carbonyl group. Its full structure has been established by ^{1}H-NMR to be identical to that of antibiotics G1549 and BN227, respectively synthesized by *P. alcaligenes* (Barker *et al.*, 1979) and *Pseudomonas* BN227 (Itoh *et al.*, 1979, 1980), and determined to be 1-hydroxy-6-methoxy-5-methyl-2(1H)-pyridinone. Moreover, the complexation properties of cepabactin, and its UV–visible spectrum and behaviour on TLC, were identical to those previously described for the two antibiotics. Thus, the two compounds G1549 and BN227 may be considered not only as antibiotics but also as siderophores of *P. alcaligenes* and *Pseudomonas* BN227. These two functions are not contradictory since it is well known that siderophores may have an antibiotic-like activity through their iron-chelating properties. Such behaviour has been shown in this study for the pyoverdines of various fluorescent pseudomonads, which considerably inhibited the growth of *P. cepacia* in liquid cultures and on agar plates. Since pyoverdines did not permit iron uptake by *P. cepacia*, their antibiotic-like effects are presumably the consequence of a higher affinity constant for iron compared to cepabactin. Another indication that compounds G1549 and BN227 possess their antibiotic activity thanks to their iron-complexation properties comes from a comparison of their MIC values and the MIC values of their iron chelates, determined by Barker *et al.* (1979) and Itoh *et al.* (1979) against a wide variety of micro-organisms. Usually the iron chelate had a much lower activity (higher MIC) and in some cases, no activity at all. Thus, cepabactin appears to be a siderophore not only for *P. cepacia* ATCC 25416, but also for other non-fluorescent pseudomonads.

By its structure, cepabactin can be considered as a cyclic hydroxamic acid, but also as a heterocyclic analogue of catechol. This compound thus occupies an interesting median position

between the two siderophore families (hydroxamates and catecholates) usually recognized in the microbial world (Neilands, 1981). Synthetic hydroxy-pyridinones that have both cyclic hydroxamate and catechol-like structures have already been studied for their potent affinity and specificity for iron(III) (Scarrow, 1985): the overall formation constants for the iron(III)-complexes of 1-hydroxy-2(1H)-pyridinone, 3-hydroxy-2(1H)-pyridinone and 3-hydroxy-4(1H)-pyridinone were determined to be respectively 26·9, 29·6 and 35·1. The competition for iron between such synthetic compounds and transferrin has also been studied, and some derivatives [e.g. 1,2-dimethyl-3-hydroxy-4(1H)-pyridinone and 2-methyl-3-hydroxy-4(1H)-pyridinone] have been tested with success in *in vivo* experiments for iron removal (Kontoghiorghes, 1985). Whether cepabactin, which is a 1-hydroxy-2(1H)-pyridinone derivative, the compound among the series with the lowest affinity for iron(III) (26·9), competes successfully for iron chelated by transferrin remains to be determined. In conclusion, since pyochelin has already been shown by Sokol (1986) to facilitate iron translocation in *P. cepacia* strains, it appears that this bacterium possess at least two different siderophore-mediated iron-uptake systems.

REFERENCES

ANKENBAUER, R., SRIYOSACHATI, S. & COX, C. D. (1985). Effects of siderophores on the growth of *Pseudomonas aeruginosa* in human serum and transferrin. *Infection and Immunity* **49**, 132–140.

BARKER, W. R., CALLAGHAN, C., HILL, L., NOBEL, D., ACRED, P., HARPER, P. B., SOWA, M. A. & FLETTON, R. A. (1979). G1549, a new cyclic hydroxamic acid antibiotic, isolated from culture broth of *Pseudomonas alcaligenes*. *Journal of Antibiotics* **32**, 1096–1103.

BICKEL, H., BOSSHARDT, R., GAUMANN, E., REUSSER, P., VISCHER, E., VOSER, W., WETTSTEIN, A. & ZÄHNER, H. (1960). Stoffwechsel Produkte von Actinomyceten. Über die Isolierung und Characterisierung der Ferrioxamine A-F, neuer Wuchstoffe der Sideramin Gruppe. *Helvetica chimica acta* **43**, 2118–2128.

BUKOVITS, G. J., MOHR, N., BUDZIKIEWICZ, H., KORTH, H. & PULVERER, G. (1982). 2-phenyl thiazole derivatives from *Pseudomonas cepacia*. *Zeitschrift für Naturforschung* **37b**, 877–880.

BURKHOLDER, W. H. (1950). Sour skin, a bacterial rot of onion bulbs. *Phytopathology* **40**, 115–117.

BURNHAM, B. F. & NEILANDS, J. B. (1961). Studies on the metabolic function of the ferrichrome compounds. *Journal of Biological Chemistry* **236**, 554–559.

CODY, Y. S. & GROSS, D. C. (1987). Characterization of pyoverdin_pss, the fluorescent siderophore produced by *Pseudomonas syringae* pv. *syringae*. *Applied and Environmental Microbiology* **53**, 928–934.

COX, C. D. & GRAHAM, R. (1979). Isolation of an iron-binding compound from *Pseudomonas aeruginosa*. *Journal of Bacteriology* **137**, 357–364.

HOHNADEL, D. & MEYER, J. M. (1988). Specificity of pyoverdine-mediated iron uptake among fluorescent *Pseudomonas* strains. *Journal of Bacteriology* **170**, 4865–4873.

HOLMES, B. (1986). The identification of *Pseudomonas cepacia* and its occurrence in clinical material. *Journal of Applied Bacteriology* **61**, 299–314.

ITOH, J., MIYADOH, S., TAKAHASI, S., AMANO, S., EZAKI, N. & YAMADA, Y. (1979). Studies on antibiotics BN-227 and BN-227-F, new antibiotics. I. Taxonomy, isolation and characterization. *Journal of Antibiotics* **32**, 1089–1095.

ITOH, J., AMANO, S., OGAWA, Y., KODAMA, Y., EZAKI, N. & YAMADA, Y. (1980). Studies on antibiotics BN-227 and BN-227-F, new antibiotics. II. Chemical structure of antibiotics BN-227 and BN-227-F. *Journal of Antibiotics* **33**, 377–382.

KELLER-SCHIERLEIN, W. & PRELOG, V. (1961). Stoffwechsel Produkte von Actinomyceten. Über das Ferrioxamin E; ein Beitrag zur Konstitution de Nocardamins. *Helvetica chimica acta* **44**, 1981–1985.

KONTOGHIORGHES, G. J. (1985). New orally active iron chelators. *Lancet* **1**, 817.

LEONG, J. (1986). Siderophores: their biochemistry and possible role in the biocontrol of plant pathogens. *Annual Review of Phytopathology* **24**, 187–209.

LIU, P. V. & SHOKRANI, F. (1978). Biological activities of pyochelins: iron-chelating agents of *Pseudomonas aeruginosa*. *Infection and Immunity* **22**, 878–890.

MEYER, J. M. & ABDALLAH, M. A. (1978). The fluorescent pigment of *Pseudomonas fluorescens*: biosynthesis, purification and physico-chemical properties. *Journal of General Microbiology* **107**, 319–328.

MEYER, J. M. & ABDALLAH, M. A. (1980). The siderochromes of non-fluorescent pseudomonads: production of nocardamine by *Pseudomonas stutzeri*. *Journal of General Microbiology* **118**, 125–129.

MEYER, J. M. & HORNSPERGER, J. M. (1978). Role of pyoverdine_pf, the iron binding fluorescent pigment of *Pseudomonas fluorescens*, in iron transport. *Journal of General Microbiology* **107**, 329–331.

MEYER, J. M., HALLE, F., HOHNADEL, D., LEMANCEAU, P. & RATEFIARIVELO, H. (1987). Siderophores of *Pseudomonas* – biological properties. In *Iron Transport in Microbes, Plants and Animals*, pp. 188–205. Edited by G. Winkelmann, D. van der Helm & J. B. Neilands. Weinheim: VCH Verlagsgesellschaft.

MÜLLER, G. & RAYMOND, K. N. (1984). Specificity and mechanism of ferrioxamine-mediated iron transport in *Streptomyces pilosus*. *Journal of Bacteriology* **160**, 304–312.

MÜLLER, A. & ZÄHNER, H. (1968). Stoffwechselprodukte von Mikroorganismen. Ferrioxamine aus Eubacteriales. *Archiv für Mikrobiologie* **62**, 257–263.

NEILANDS, J. B. (1981). Microbial iron compounds. *Annual Review of Biochemistry* **50**, 715–731.

PALLERONI, N. J., KUNISAWA, R., CONTOPOULOU, R. & DOUDOROFF, M. (1973). Nucleic acid homologies in the genus *Pseudomonas*. *International Journal of Systematic Bacteriology* **23**, 333–339.

PRELOG, V. (1963). Iron containing antibiotics and microbic growth factors. *Pure and Applied Chemistry* **6**, 327–338.

SCARROW, R. C. (1985). *Hydroxypyridinones: ligands with high affinity and specificity for iron(III)*. PhD dissertation, University of California, Berkeley.

SOKOL, P. A. (1984). Production of the ferripyochelin outer membrane receptor by *Pseudomonas* species. *FEMS Microbiology Letters* **23**, 313–317.

SOKOL, P. A. (1986). Production and utilization of pyochelin by clinical isolates of *Pseudomonas cepacia*. *Journal of Clinical Microbiology* **23**, 560–562.

SOKOL, P. A. & WOODS, D. E. (1983). Demonstration of an iron-siderophore-binding protein in the outer membrane of *Pseudomonas aeruginosa*. *Infection and Immunity* **40**, 665–669.

STANIER, R. Y., PALLERONI, N. J. & DOUDOROFF, M. (1966). The aerobic pseudomonads: a taxonomic study. *Journal of General Microbiology* **43**, 159–271.

STOLL, A., BRACK, A. & RENZ, J. (1951). Nocardamin, ein neues Antibioticum aus einer Nocardia-Art. *Schweizerische Zeitschrift für allgemeine Pathologie und Bakteriologie* **14**, 225–233.

TEINTZE, M., HOSSAIN, M. B., BARNES, C. L., LEONG, J. & VAN DER HELM, D. (1981). Structure of ferric pseudobactin, a siderophore from a plant growth promoting *Pseudomonas*. *Biochemistry* **20**, 6446–6457.

DE WEGER, L. A., VAN ARENDONK, J. J. C. M., RECOURT, K., VAN DER HOFSTAD, G. A. J. M., WEISBEEK, P. J. & LUGTENBERG, B. (1988). Siderophore-mediated uptake of Fe^{3+} by the plant growth-stimulating *Pseudomonas putida* strain WCS358 and by other rhizosphere microorganisms. *Journal of Bacteriology* **170**, 4693–4698.

ZÄHNER, H., BACHMAN, E., HÜTTER, R. & NUESCH, J. (1962). Sideramine, eisenhaltige Wachstumfaktoren aus Mikroorganismen. *Pathologia et microbiologia* **25**, 708–736.