

# Siderophores of the Human Pathogenic Fluorescent Pseudomonads<sup>1</sup>

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**Abstract:** Bacteria need a sufficient supply of iron in ionic form for their metabolism. When living in an environment where this is not possible (as in the soil due to the presence of highly insoluble ferric oxide hydrates, or in living organisms where iron is bound to peptidic chelators)  $Fe^{3+}$  complexing compounds, called siderophores, are produced. The siderophores of *Pseudomonas aeruginosa*, a dangerous opportunistic human pathogen, and of related potentially pathogenic species will be presented.

## INTRODUCTION

*Pseudomonas aeruginosa*, the most important member of the group of human pathogenic fluorescent pseudomonads, has a long medical history. In 1877, Pasteur [1] published the observation that when he inoculated animals with both *Bacillus anthracis* and other pathogenic bacilli, the animals failed to develop anthrax, and in 1889 Bouchard mentioned specifically *Bacillus pyocyaneus* (the long-used name for *P. aeruginosa*) as a potent antagonist [2]. Alkyl quinolones were identified as the active compounds [3]. These pyo compounds (or psevdans in the Russian literature) could have been the first antibiotics, but difficulties in the standardisation of the bacterial preparations resulted in their discontinuation when the sulfonamides came on the market. This was the only positive intermezzo in the medical history of *P. aeruginosa*. Even in the early days she was known to be responsible for the blue-green pus, hence the original name *pyocyaneus*.

Today *P. aeruginosa* is feared as a dangerous opportunistic bacterium responsible for frequently lethal hospital (nosocomial) infections. As a soil bacterium she is omnipresent especially due to the effect of modern air-conditioning systems. She is insensitive to many disinfecting agents and – more important – an increasing number of strains of *P. aeruginosa*, especially from hospital isolates, prove to be highly resistant against most antibiotics [4, 5] and also against therapeutic agents such as fluoroquinolones [6]. An alginate film frequently surrounding the bacteria [7], the low permeability of their outer membrane and an active export mechanism for low molecular weight substances are the main reasons for the resistance. In addition  $\beta$ -lactamase activity [8] affects  $\beta$ -lactam antibiotics. *P. aeruginosa* endangers especially severely injured patients suffering from large wounds or severe burns as well as persons whose immune system is weakened. An extremely critical situation

exists for patients suffering from mucoviscidosis (cystic fibrosis) when *P. aeruginosa* infects the bronchial tubes [9].

The pathogenicity of *P. aeruginosa* is linked to two factors. Autoinducers derived from homoserine lactone [10,11] are necessary for the proliferation in the infected organism. Of equal importance is the production of siderophores [12]. This should be obvious: bacterial cells need iron for many physiological processes. In a living organism  $Fe^{3+}$  is bound to peptidic complexing substances such as transferrins. Bacterial metabolites with a high complexing constant are therefore necessary to secure a sufficient amount of this element [13].

In addition to the well-defined *P. aeruginosa* also occasional infections of patients with an impaired immune system by *P. fluorescens* and *P. putida* have been reported [14, 15]. Both species are a rather ill-defined conglomerate of strains, and which of them are possibly opportunistic human pathogens is a moot point.

## CLASSES OF SIDEROPHORES

### Pyoverdins

The pyoverdins are responsible for the classification "fluorescent pseudomonads" as they produce that the yellowish-green fluorescence bacterial cultures develop under iron deficiency. This phenomenon has been known for more than hundred years (in 1892 Gessard [16] had summarized earlier observations) and many attempts for isolation and structure elucidation were in vain (for a survey see [17]) until Teintze in 1981 published the structure of the pyoverdin from a "plant growth promoting *Pseudomonas*" [18] which he named pseudobactin. This term is still used occasionally, but more common is the name pyoverdin coined by Turfnejer [19] (who spelled it in Dutch as pyoverdine with a final "e", and some authors prefer this spelling for priority reasons). Subsequently, "pyoverdin" has become a generic term for a series of related compounds having in common the (1S)-5-amino-2,3-dihydro-8,9-dihydroxy-1H-pyrimido-[1,2a] chinolin-1-carboxylic acid chromophore

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<sup>1</sup>Part CI of the series "Bacterial constituents". For part C see Fuchs R. and Budzikiewicz H., *J. Mass Spectrom.*, 2001, in press.

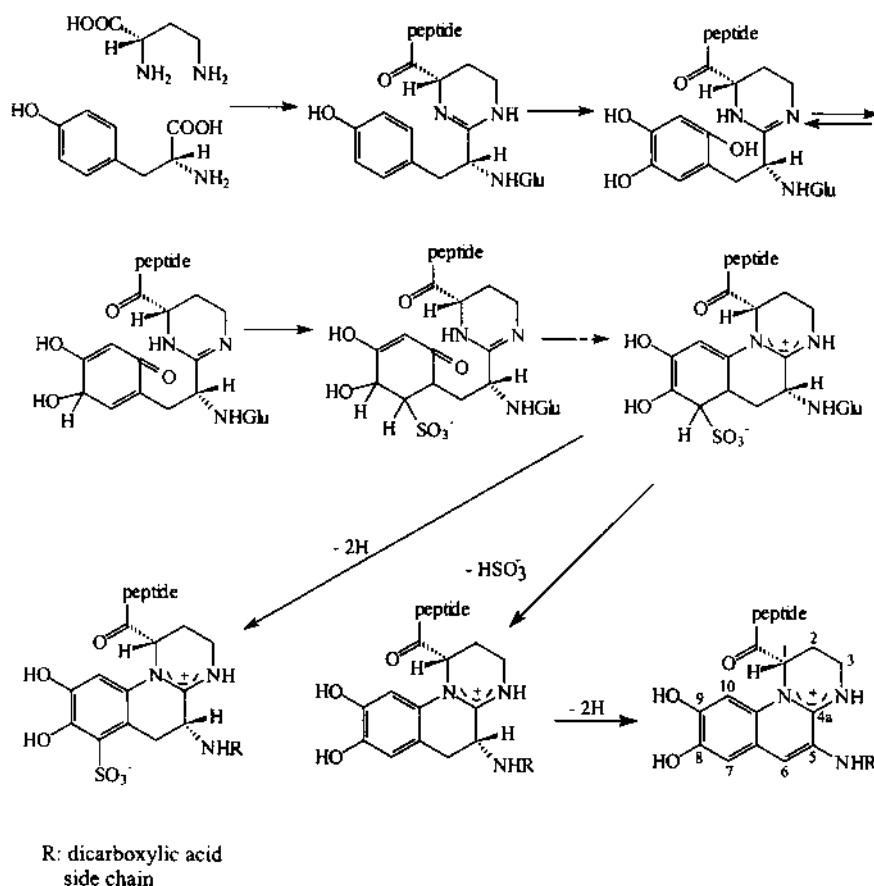


Fig. (1). Scheme of pyoverdinin biosynthesis starting from D-Tyr and L-Dab.

(see Fig. 2) responsible for the color and the fluorescence, but differing in the composition of the peptide chain attached to the carboxyl group of the chromophore by its N-terminus (for lists see [20, 21]). Some of the amino acids are non-proteinogenic (Orn; 2,3-diaminobutyric acid, Dab) and several of them are modified (-hydroxy Asp and His; -N-hydroxy- -N-acyl Orn). Occasionally condensation products between Dab and the preceding amino acid yielding a tetrahydropyridimidine ring (3 in Fig. 2) are observed. Usually several pyoverdins are found in the fermentation broth which have the same peptide chain but differ in the nature of a small dicarboxylic acid (or its amide) bound to the amino group at C-5 of the chromophore. The nature of this acid is irrelevant for the uptake by the bacterial cells and could be some biogenetic relic, or its function has not been determined yet.

Pyoverdins are potent  $\text{Fe}^{3+}$  chelators with complexing constants between  $10^{24}$  and  $10^{26}$  [ $\text{l}\cdot\text{mol}^{-1}$ ] at pH 7.0. Ligand sites are the catecholate part of the chromophore and two amino acids, hydroxamic acids derived from Orn and/or hydroxycarboxylic acids. About half of the amino acids have the R-configuration. Three-dimensional structures of a limited number of ferri-pyoverdins show that the metal ion lies at the surface of the complex. This allows for easy exchange of the ion [22].

Pyoverdins can be subdivided into four groups based on variations of their peptide chain:

- the most common variety comprises a linear sequence of amino acids with a N-hydroxy(*cyclo*)Orn (cOHOOrn) as C-terminus (2 in Fig. 2);
- next in number are those characterized by a C-terminal cyclic part consisting of 3 or 4 amino acids, formed by an amide bond between the carboxyl group of the C-terminal amino acid and the -amino group of an in-chain Lys (1 in Fig. 2);
- pyoverdins with a C-terminal (*cyclo*)depsipeptidic substructure formed by an ester bond between the carboxyl group of the C-terminal amino acid and an in-chain Ser or Thr;
- pyoverdins with a C-terminal free carboxyl group (3 in Fig. 2); some or all of them may be hydrolysis products of the rather labile cyclic esters which are readily hydrolyzed at pH values above 9.

Pyoverdins are produced only under severe iron limitation in the growth medium, and together with them appear iron regulated outer membrane proteins (IROMPs) which serve as receptors for the ferri-pyoverdins. They are also responsible for the transport of iron through the membrane. Iron is then set free by reduction in the periplasmic space. The variability of the peptide chain safeguards that a given pyoverdinin can only be recognized and thus utilized by the producing strain. Exceptions to this

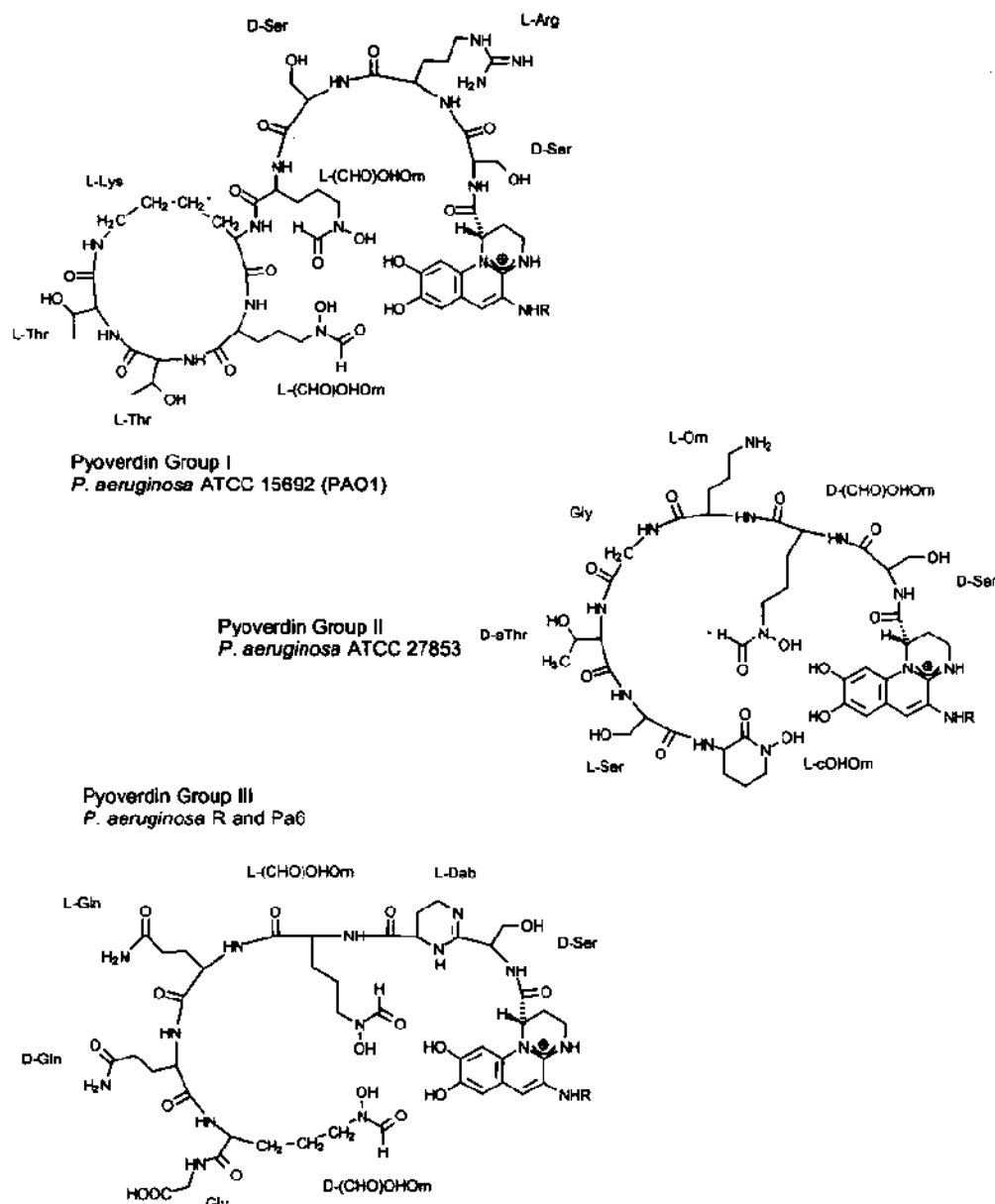


Fig. (2). The three *P. aeruginosa* pyoverdins 1 - 3 (R, dicarboxylic acid side chain).

rule are known, but the respective pyoverdins have partially identical or similar regions of the peptide chain which are apparently required for recognition [22, 23].

The biosynthesis of pyoverdins proceeds by a multi-enzyme thiotemplate mechanism which involves also the first steps of the formation of the chromophore. Isotope labelling studies have shown that the chromophore is derived from a condensation product of D-Tyr and L-Dab with subsequent ring closure by a Bucherer reaction [24] (Fig. 1). The dicarboxylic acids bound to the 5-NH<sub>2</sub> group of the chromophore belong to the citric acid cycle.

From *P. aeruginosa* three siderovars (sv, subgroups which differ in the nature of the produced pyoverdin, Fig. 2) were found by testing a large number of strains mainly from clinical isolates [25]. Their ferri-pyoverdins are not accepted

mutually. As these three pyoverdins are not produced by any other species of *Pseudomonas* they can be used for a quick identification of *P. aeruginosa* [21]. Most of the about fifty pyoverdins whose structures have been elucidated so far stem from the *P. fluorescens/putida* conglomerate of strains. One example (4) [26] from a clinical isolate (from sputum) is given in (Fig. 3) containing the rather rare iso-variety of the chromophore (peptide chain bound to C-3 rather than C-1 of the chromophore, "isopyoverdin").

### Pyochelin and Salicylic Acid

*P. aeruginosa* and several other *P. spp.* have a second iron uptake system using pyochelin [27] (5, Fig. 4) derived from salicylic acid and two molecules of Cys. Its precursor is probably aeruginic acid (the condensation product of

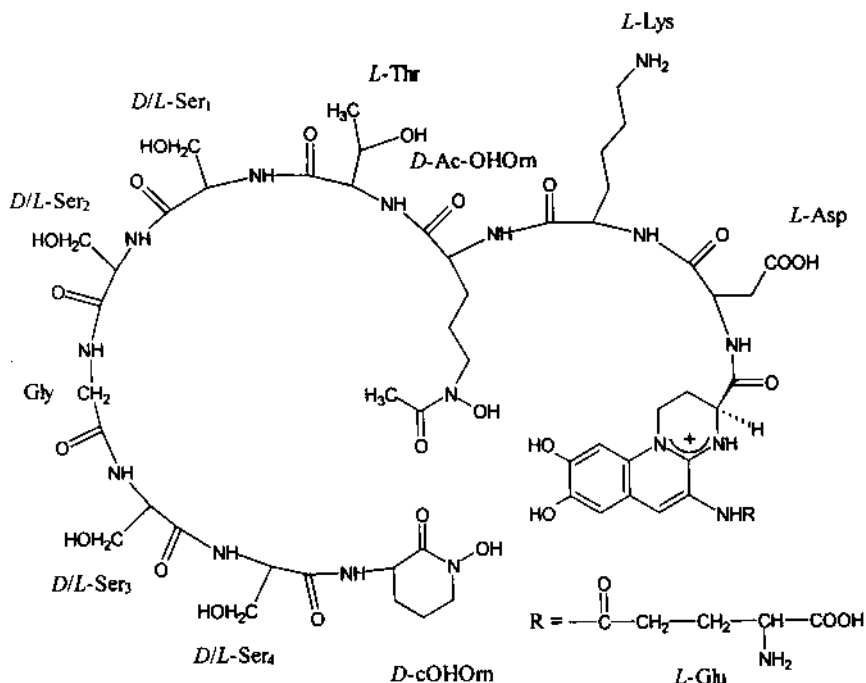


Fig. (3). Isopyoverdin 4 from *P. putida* CFML 90-44.

salicylic acid and Cys, 2-(*o*-hydroxyphenyl)thiazol-4-carboxylic acid [28]). The absolute configuration of the three chiral centers in the native pyochelin is *RRR*, but the 4''-center adjacent to the free carboxyl group in the third ring isomerizes readily to *S* giving an equilibrium mixture of the two epimers. Only the *RRR*-stereoisomer can form a complex with  $\text{Fe}^{3+}$ . X-ray analysis demonstrated a 1:1 stoichiometry with the phenolate and carboxylate ions and the two nitrogen atoms as binding sites. The remaining two octahedral positions are occupied by solvent ions [29]. Pyochelin is only produced under iron deficiency. It has a lower complexing constant than the pyoverdins (ca.  $10^5 \text{ l}\cdot\text{mol}^{-1}$ ), but it can also bind several bivalent metals, and may thus be a transport vehicle for trace elements [30]. Ferripyochelin (but not ferri-pyoverdins) can be involved in the generation of hydroxyl radicals and be responsible for cell damage [31, 32].

Salicylic acid is produced by *P. aeruginosa* and other species. Due to its low complexing ability it could actually serve as a rescue siderophore when more potent iron scavenger systems fail to operate [33].

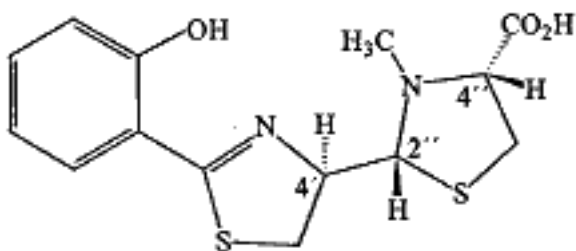
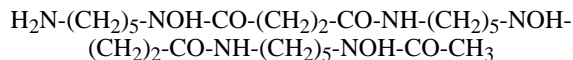


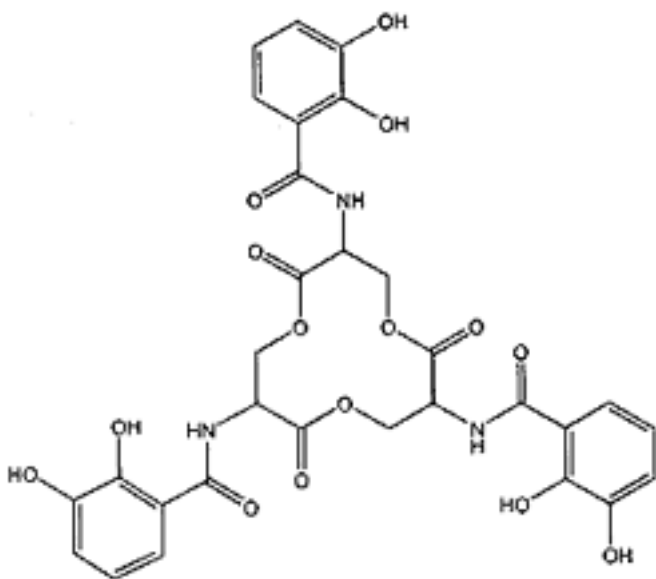
Fig. (4). Structure of pyochelin (5).

### "Foreign" Siderophores

*P. aeruginosa* as well as other *P. spp.* can be opportunists and even predators. They are able to use iron-complexing compounds which are produced by other organisms and which are commonplace and can be found in their surrounding, such as citrate [34, 35] and *myo*-inositol hexakisphosphate [36]. Even the artificial siderophore nitrilotriacetic acid,  $\text{N}(\text{CH}_2\text{COOH})_3$  can be used [37]. While ferrioxamine B [38]



from *Streptomyces spp.* could be taken up by the porin system as possible for the smaller compounds, for larger molecules specific receptors must be created. As has been mentioned above, the acceptance of a number of pyoverdins from foreign strains can be explained by structural similarities in the respective peptide chains, in the case of *P. putida* WCS 358 the gene inducing the formation of the receptor for the pyoverdin from *P. fluorescens* could be identified [39]. Even more interesting is the observation that *P. aeruginosa* can use enterobactin (6 Fig. 5) produced by *Escherichia coli* [40] as well as its biogenetic precursors 2,3-dihydroxybenzoic acid and *N*-(2,3-dihydroxybenzoyl)-L-Ser [41], although *P. aeruginosa* by itself does not produce catechol siderophores. The complexing constant of enterobactin exceeds those of the pyoverdins by orders of magnitude. The greater iron chelating ability relative to the ferri-siderophore of *E. coli* is a major chance for *P. aeruginosa* to survive in the intestinal flora.



## CONCLUSION

*P. aeruginosa* as well as the other possibly human pathogenic species from the *P. fluorescens/putida* cluster are soil bacteria. They had to adapt themselves to survival in hostile surroundings. A number of strategies have been acquired such as the production of compounds toxic to other microorganisms [3], defense against reciprocal (including human pharmaceutical) attacks [5] and survival strategies as the development of a signal system [11] and the production of potent specific siderophores. These allow for sequestering iron even under the most restrictive conditions and deprive competitors from this essential metal. Being resistant against most disinfecting agents (occasionally even using them as nutrients) and being well distributed everywhere by modern air conditioning systems, their potential danger for hospital patients is obvious as can be seen by the number of deaths after nosocomial infection.

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