Biological applications of Pyoverdines and their complexes with Iron and Gallium

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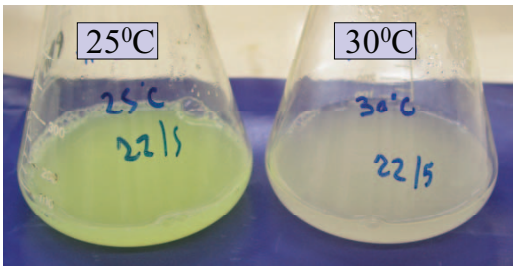
Abstract

Pyoverdines (pseudobactins) are fluorescent siderophores that have high-affinity for iron (10^{-32}M). The fluorescent *Pseudomonas* group synthesizes them under iron-deficient growth conditions. Pyoverdines are composed of three structural parts: a dihydroxyquinoline chromophore, a side chain and a variable peptidic chain. The peptide moiety is involved in receptor recognition and binding. The size and amino acid composition of pyoverdines are unique to each species, as well as the pyoverdine recognition specificity. Despite this specificity, many fluorescent *pseudomonads* are able to utilize pyoverdines produced by other strains.

We have isolated three different siderophores of the pyoverdine type from *Pseudomonas fluorescens*. These pyoverdines, differing in their side chain, were identified by HPLC and mass spectra (m/z 1161, 1190 and 1160). We show that these three forms of pyoverdines, when complexed to iron, are effective growth promoters for bacteria in a minimal medium. In contrast, a mixture of these pyoverdines complexed to Gallium acts as a useful inhibitor of growth for both *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. In addition, these pyoverdines are effective in protecting *Escherichia coli* from the deleterious effects of reactive oxygen intermediates (ROI). These results shed light on novel uses of pyoverdines and their complexes as ROI protecting and antibacterial agents.

Fermentation and Purification



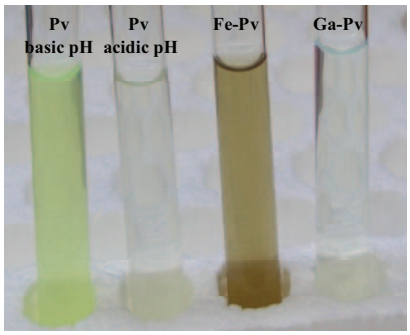
P. fluorescens was grown at 25°C (left) and 30°C (right).

Only at 25°C Pyoverdines were produced (fluorescent green).

Figure 1. Pyoverdines production is temperature dependent

P. Fluorescens 5 liter fermentation
The green color is indicative of pyoverdines production
This fermentation was also performed in large scale fermentors

Figure 2. Pyoverdines fermentation



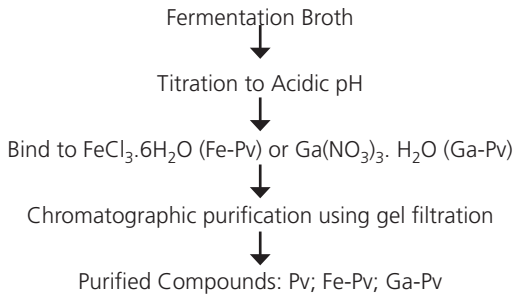
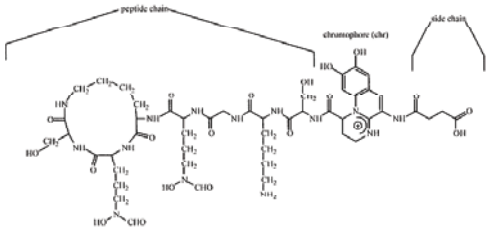


Figure 3. Purification of Pyoverdines (Pv) and their Iron and Gallium complexes

Component Identification



	Side chain	m/z
-NH-CO-CH_2-CH_2-COOH	Succinic acid	1161
-NH-CO-CH_2-CH_2-CHOH-$CONH_2$	2-hydroxy glutaramide	1190
-NH-CO-CH_2-CH_2-$CONH_2$	Succinamide	1160

Components of the pyoverdines mixture.

Each component is composed of three structural parts: Peptide chain, Chromophore and Side chain.

The three components of the mixture differ in their side chain.

Figure 4. Pyoverdines mixture components

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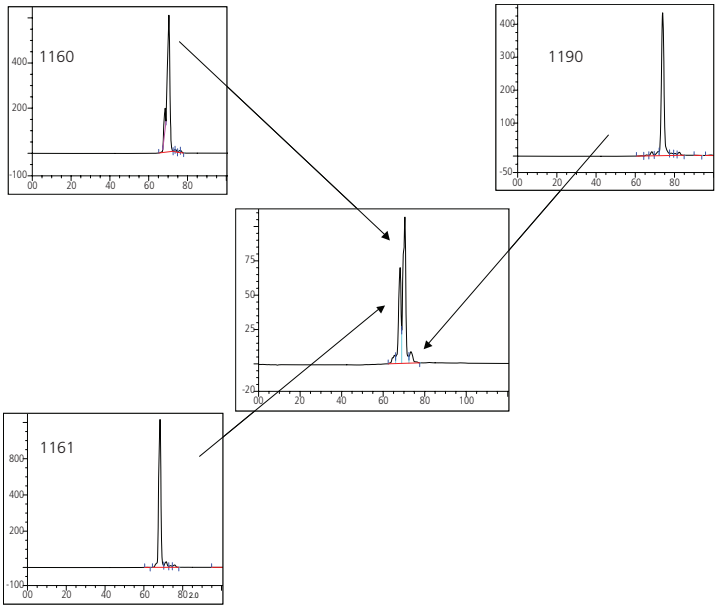
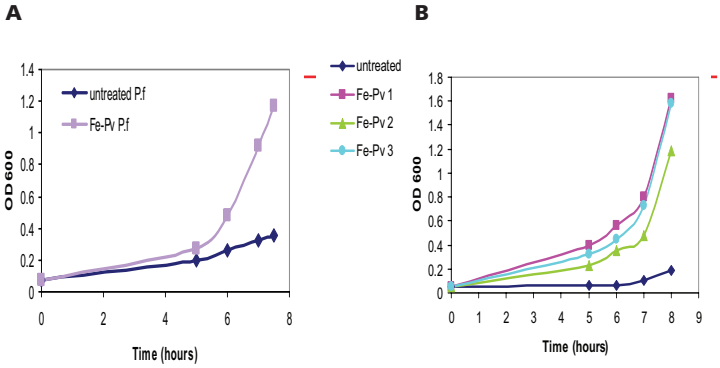


Purified Pyoverdines mixture (center) and the three purified pyoverdines components of the mixture

Figure 5. HPLC chromatograms

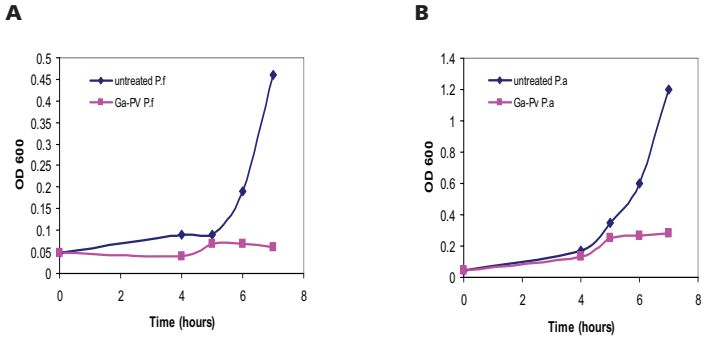
Biological Activity



P. fluorescens was grown in iron depleted minimal medium without or with the addition of (A) a mixture of 0.14 mM Fe-Pv from *P. fluorescens* or with (B) the addition of Fe-Pv 0.8 mM components:

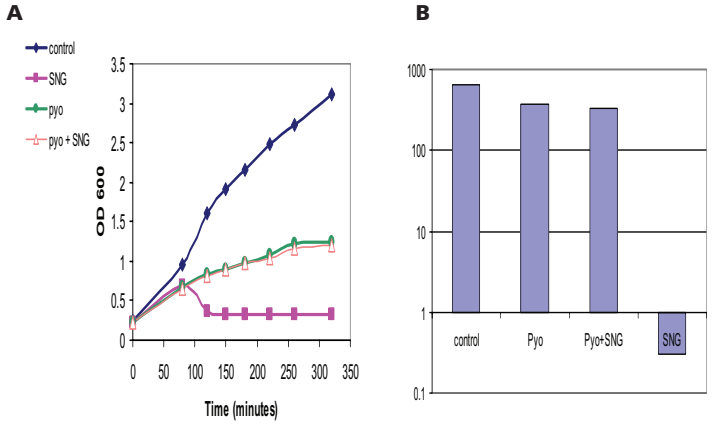
Fe-Pv 1: 2-hydroxy glutaramide (1190); Fe-Pv 2: Succnic acid (1161); Fe-Pv 3: Succinamide (1160)

Figure 6. A mixture of Ferri-pyoverdines (Fe-Pv) and its three components promote *P. fluorescens* growth



P. fluorescens (A) and *P. aeruginosa* (B) were grown in iron depleted minimal medium without or with the addition of 0.7 mM Ga-Pv from *P. fluorescens*

Figure 7. Gallium-pyoverdines (Ga-Pv) inhibit *P. fluorescens* and *P. aeruginosa* growth



Streptonigrin (SNG) antibiotic activity is iron-activated. The DNA cleavage reaction and chromosome damage by SNG are influenced by the nature of the metal ion present and dependent on the production of free radicals.

E. coli was grown in LB to early log phase. Free Pyoverdines mixture (pyo) was added at 19 mM (A) or 38 mM (B). 20 minutes after the addition of pyo, the indicated cultures were treated with Streptonigrin (Sigma code S1014)(SNG) 1.5 µg/ml (A), or 1.25 µg/ml (B). Samples were taken at different time points for absorbance measurement (A) or at 150 minutes for viable counts (B).

Figure 8. Pyoverdines protect against Streptonigrin-induced DNA damage

Summary

- Three pyoverdine compounds were purified from *P. fluorescens* fermentations, differing in their side chain: the succinic acid form (1161), the succinamide (1160) and the 2-hydroxy glutaramide form (1190).
- All three forms were active as growth promoters of *P. fluorescens* when complexed to iron.
- The complex Gallium-Pyoverdines acts as a useful growth inhibitor for both *P. fluorescens* and *P. aeruginosa*, suggesting that Ga-Pyoverdines can be used as specific antimicrobial agents.
- Pyoverdines protect *E. coli* from the deleterious effects of Streptonigrin, possibly by its iron chelating activity. This chelating activity also leads to *E. coli* inhibition of growth.

Possible Applications of Pyoverdines

- Prevention of iron overload toxicity
- Inhibition of pathogenic bacterial growth (as iron chelator).
- Specific inhibitor of fluorescent *pseudomonads* strains (when complexed to Gallium)

The Following Products are Available from Sigma:

- P8374 Pyoverdine Iron complex (Fe-Pv)
- P8249 Pyoverdines Gallium (Ga-Pv)
- P8124 Pyoverdines (Pv)

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