Isolation and purification of the siderophore produced by *Pseudomonas* sp. T17

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Introduction

Iron is an essential micronutrient in the metabolism of most microorganisms with low bioavailability. One of the strategies for its uptake is the synthesis of siderophores, which production is tightly regulated by the concentration of iron in the medium. Siderophores are low molecular weight bidentate ligands with chelating activity and high affinity for the metal; they allow entry into the cell through specific membrane receptors and they are synthesized mostly by NRPS (nonribosomal peptide synthetase)[1]. They have numerous applications, highlighting the "Trojan horse" strategy, of conjugating the siderophore with antibiotics, facilitating their entry into the bacterial cell[2], treatments against cancer[3] or in bioremediation[4].

Methodology

Growth under iron limiting conditions

Pseudomonas sp. T17 was grown in 6 L of poor medium with succinate (4 g/L) and low in iron content to stimulate siderophore production.

Solid phase chromatography

The supernant was subjected to solid phase chromatography.

An extraction column composed of layers of sand, CHROMABOND® C18 resin activated by methanol, sand and glasswool was used **(Fig. 1).** The siderophore was eluted with 60% acetonitrile and subsequently lyophilized.

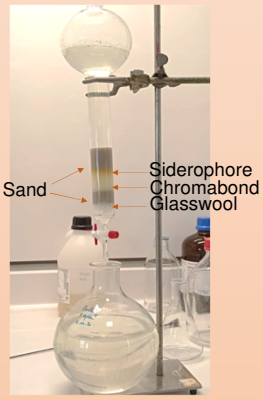

Figure 1: *Solid phase extraction column*

Compound purification: preparative HPLC

Preparative HPLC was performed on the sample using a acetonitrile gradient **(Table 1).** The components were separated into 40 different fractions and these were run on the analytical HPLC.

Table 1: *Preparative HPLC gradient conditions. A: Water. B: Acetonitrile*

	% A	%B
Initial conditions	90	10
10 min	90	10
20 min	40	60
25 min	0	100
30 min	100	0

Results and discussion

Preparative/analytical HPLC results and colorimetric test on CAS plates

40 fractions, named from A1-D10, were obtained. Fractions containing compounds with iron chelating properties change the colour of the CAS from green to yellow **(Fig. 2B).** Positive fractions were from B7-C7**.**

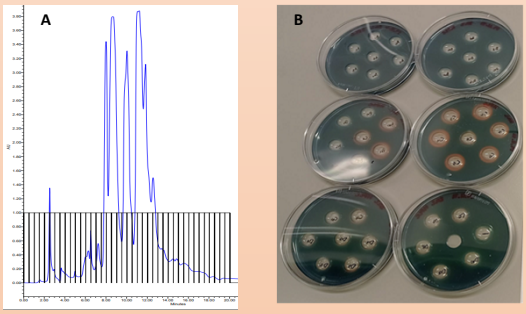

Figure 2: *Preparative HPLC results and colorimetric CAS test.* ***A****: Chromatogram at λ=220 nm with the collected fractions indicated.* ***B****: Positive fractions in CAS plates are those with a yellow colour (B7-C7)*

An *in silico* analysis carried out previouslyusing NRPSpredictor2[5] indicated that part of the detected siderophore is a six-animo acids long peptide. Preparative HPLC resulted in several CAS-positive fractions with different but quite close retention times. On analytical HPLC, peak overlap is observed **(Fig. 3).** This might be explained by the presence of a few active isoforms of the siderophore, which could be separated optimising the HPLC gradient.

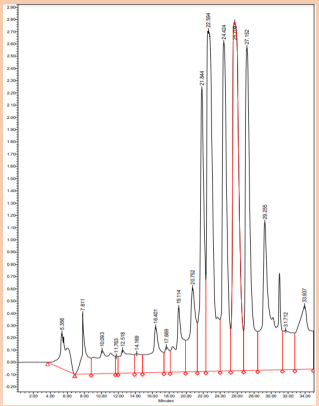

Figure 3: *Analytical HPLC result. Chromatogram at λ=220 nm of the semi-purified sample.*

Conclusions

Pseudomonas sp. T17 produces a new siderophore. Determination of its structure is complicated because of its complex nature, probably several closely related compounds (isoforms) are present. Therefore, the purification methodology needs to be optimised to be able to purify pure compounds. In addition, the eluent gradient should be optimised to avoid the appearance of overlapping peaks. Subsequently, mass spectrometry and NMR analysis will be used to obtain the chemical structure.

Bibliography

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