EXPLOITATION OF SIDEROPHORES FOR THE SPECIATION OF IRON

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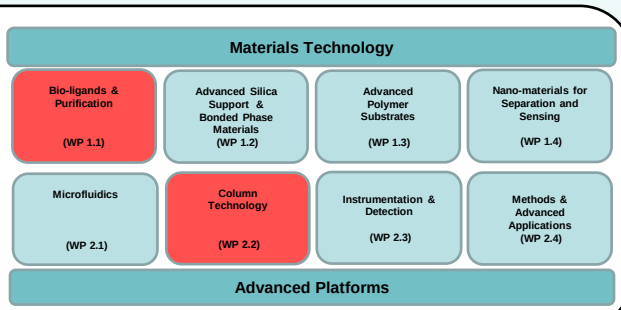
Aim:

To produce and purify the siderophore rhizobactin 1021 to assess its application in iron detection and speciation.



1. Introduction

Iron is essential for life. It acts as an electron donor/acceptor in metabolic processes facilitated by its variable valency. Although vital, it is toxic at high levels due to Fe^{2+} oxidation. Iron toxicity is a concern as it can affect growth and product yields in animal cell culture.

Siderophores are high affinity Fe^{3+} chelators produced by microorganisms. This affinity gives them the potential to be used as a basis in platforms to detect and speciate iron in industrial cell culture. Rhizobactin 1021 is of interest due to its decanoic acid "tail" that is not involved in chelation which makes it an ideal target for immobilisation.

2. Background

Current trends in animal cell culture have shifted from the use of animal derived media supplements such as serum to defined protein free media formulations. This is attempt to increase product purity and remove batch variability but has led to the need to supplement media with nutrients normally found in serum. Iron is usually delivered as a salt or complexed to the eukaryotic iron binding protein transferrin. Iron levels must be tightly controlled as both undersupply and oversupply lead to suboptimal growth and poor product yields. The Fe^{2+} state is rapidly oxidised resulting in free radical formation which, in the absence of serum protection, is cytotoxic. As a result of this it is important to monitor Fe^{2+} levels in real time to allow for counter measures to be taken against its presence. This presents a requirement for a sensor to monitor iron levels in cell culture e.g. in biopharmaceutical production.

3. Scaled production of siderophore

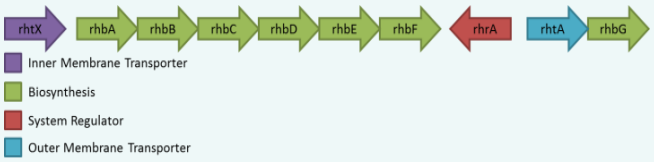


Figure 1: *Genetic organisation of the biosynthesis and transport genes of rhizobactin 1021 in S. meliloti comprising seven biosynthesis genes, two transport genes and a AraC type regulator.*

The objective is to overproduce the siderophore for purification and immobilisation using the following strategies:
1. To use a mutant strain of *S. meliloti* that is unable to utilise rhizobactin 1021 but retains the ability to produce the siderophore. *rhtA* and *rhtX* would be targets under this strategy.
2. Clone the rhizobactin 1021 synthesis genes into an *E. coli* expression system to allow their constitutive overexpression to increase product yield.
3. The *rhbG* gene is responsible for the lipid addition and its overexpression will ensure the complete acylation of rhizobactin 1021.

4. Siderophore Detection by CAS Assay

CAS media allows of the detection of siderophore by showing iron chelation through formation of an orange halo. This assay can be modified to give an approximate measure of siderophore concentration in liquid media.

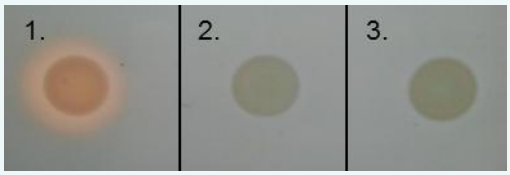


Figure 2: *Siderophore production detected by Chrome Azure S assay*
1. Wild type S. meliloti displaying siderophore production
2. S. meliloti rhbA knockout mutant with no siderophore product
3. S. meliloti rhbE knockout mutant with no siderophore product

5. IMAC Purification of Siderophore

Recently it has being shown that purification of hydroxamate siderophores is possible using an immobilised nickel affinity column.

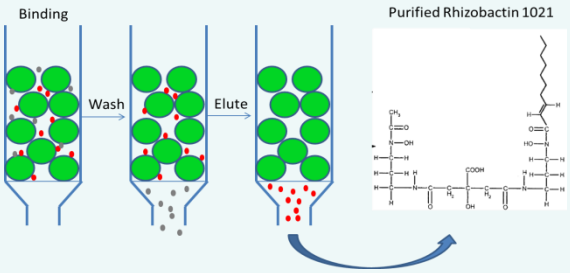


Figure 3: *Schematic for IMAC purification. Supernatant containing rhizobactin 1021 is passed through the column and allowed to bind. Weakly bound contaminants are removed by a weak elution buffer wash. Final elution is achieved by a high imidazole wash to competitively remove the siderophore.*

6. Conclusion

Current work is focused on mutagenesis of the *rhtX* gene to create a rhizobactin 1021 utilisation mutant. The success of this mutation will be shown as a wider orange halo than the wild type by CAS assay analysis. The quality of rhizobactin 1021 purification will be first assayed by CAS assay to give approximate yields followed by HPLC analysis to assess the quality and homogeneity of final elution.

7. Project Outputs

ISSC Internal Meetings:

- Researcher Forum, UCC, October 2011
- Review Meeting, UCC, December 2011

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