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 Fe³⁺ oxidation. Iron toxicity is a concern as it can affect growth and product yields in animal cell culture.

Siderophores are high affinity Fe³⁺ 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 dodecanonic 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 an 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 Fe³⁺ 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 Fe³⁺ 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
The objective is to overproduce the siderophore for purification and immobilisation using the following strategies:
1. To use a mutant strain of S. melloti 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 rhtB 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 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.

5. IMAC Purification of Siderophore
Recently it has been shown that purification of hydroxamate siderophores is possible using an immobilised nickel affinity column.

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.

This research has been funded by Science Foundation Ireland under grant number 08/SRC/B1412