Objectives & Rationale

The overall aim of this labs projects in this arena is to develop a consistent, and effective control agent against phytopathogenic fruit decay. In order to achieve this, purification methods for the bio-fungicide lipopeptides (LPs) are needed. This project therefore focussed on understanding and comparing separation and purification methodologies, for firstly the recovery of LPs produced in Bacillus fermentations, and secondly their purification, preferably to individual homologues (since different homologues have very different anti-fungal activity). The work investigated a number of standard purification technologies, and applied them to lipopeptide purification, including: acid precipitation, solvent extraction, and resin adsorption. Further, a novel purification methodology – aqueous two phase extraction - was tested for product recovery and purification. On the basis of the experiments, a proposed flowsheet can be built.

Methods

A variety of methods used, specific to each purification type. Detailed descriptions in the methods section below.

Key Results

Acid precipitation can be used for LP product recovery, however, poor purity is seen due to co-precipitation of other biomolecules (mostly proteins). For process intensification, acid precipitation may still be appropriate, since co-precipitated compounds do not appear to confound anti-fungal activity (from the parallel project in Plant Pathology).

Solvent extraction is a potential route for increased purification of LPs from co-precipitated compounds, however, poor selectivity between the different LP types (fengycin, iturin and surfactin) was seen. Further, solvent extraction requires significant volumes of potentially hazardous chemicals. The route is not recommended for industrial application, particularly not for food applications.

Resin adsorption was found to be not very effective in collecting LPs, although elution did allow several-fold concentration of the product. Nonetheless, comparatively low recoveries indicate that unless higher affinity resins are used, this purification methodology is not suitable for industrial application.

The novel purification route, ATPS, has been demonstrated to have excellent potential for LP purification, and even separation of homologues. Iturin and fengycin reported to the polymer phase in a PEG6000-phosphate ATPS system, separating out the surfactin. Recoveries of up to 90% of LPs have been seen.

Bacillus was grown under high salt conditions (in order to verify whether ATPS extractive fermentation can be used). Sulphate and tartrate showed good growth and LP production results, equal or better than standard fermentations. This technology has the potential for industrial application, since it improves productivity and allows for LP recovery in a single step.

Conclusion and Discussion / Recommendation

ATPS is a good, and novel, route for LP separation and purification. Acid precipitation remains a standard method for crude recovery, but solvent extraction and adsorption and not recommended for industrial application. The work has tied well with parallel projects in plant sciences, showing good anti-fungal efficacy from the extracts, and no toxic effects under usable concentrations.