Development of novel Fungal Biocontrol Agents

Ute Eiben and Peter Lüth

***Abstract* – One of the tasks within the scope of REPCO is to select fungal isolates from a group of candidates highly hyperparasitic to Venturia inaequalis on apple. The selected isolates should be suitable for large-scale biotechnological production processes based on *Solid-State* fermentation. Therefore the ability to formulate a final product suitable for application and with good shelf-life and cost-competitiveness characteristics is also to be tested.**

INTRODUCTION

Testing was carried out on 48 fungal isolate candidates selected after first year screening carried out by one of the project partners - Plant Research International. The isolates were tested under field conditions and selected with regard to their growth behaviour at different temperatures, conidia production on agar medium, as well as a determination of genus as non human pathogenic.
The four highest-ranked fungal isolates were identified for follow up tests. On the basis of these results, the second year of screening was then carried out on a smaller scale.

MATERIAL AND METHODS

The four selected isolates were investigated on different agar media for their ability to produce sufficient conidia to be used as inoculums for the fermentation experiments. Both standard agar media were used e.g. potato dextrose and, in addition, agar media based on several cereals e.g. oat flour agar. The Petri dishes were incubated at a temperature of 20-22 °C. The conidia production was evaluated after 22-30 days dependent on the appearance of the fungus cultures. The conidia density was determined using a microscope with THOMA chamber. After the first screening the isolates were tested on different cereal culture substrates using *Solid-State* technology. This technology is described in the corresponding patent (Lüth, Eiben; 1999). The laboratory *Solid-State* fermenter were filled with culture substrates and sterilized at 121 °C and 1.2 bar for 45 minutes. Each fermenter was then inoculated with 100 ml of conidia suspension containing $1x10^7$ conidia per ml of the fungus isolate to be tested.

Ute Eiben, Research & Development, PROPHYTA Biologischer Pflanzenschutz GmbH, D-23999 Malchow/ Poel, Germany (ueiben@prophyta.com)
Peter Lüth, Managing Director, PROPHYTA Biologischer Pflanzenschutz GmbH, D-23999
(peterlueth@prophyta.com)

The fermenters were connected to the fermenter control system (see Figure 1) and incubated for 28 days. The temperature in the culture substrate and the air stream volume was predefined. All process data were continuously recorded and evaluated in order both to monitor and to automatically control the fermenter run. The analysis of the measured data is important for the future scaling-up of industrial fermenters (see Figure 2). Following fermentation the product was harvested.
In order to evaluate the conidia yield of the fungus on different substrates, 3 samples of 10 grams each were taken from the substrate and suspended in 100 ml of 0.8% NaCl solution. The conidia were detached from the substrate by homogenizing the resulting suspension using an ULTRA TURRAX (IKA®) running at 11,000 rpm for 30 seconds. The conidia density was determined using a microscope with THOMA chamber.
If the result was better than $1x10^9$ conidia per gram culture substrate (input), the complete fermentation product would be harvested. The fermentation product was suspended in water, mechanically stirred and the cleaned substrate separated from the suspension using a micro sieving system. Following the sieving process the process water was separated to form a highly concentrated conidia paste.
The final constitution of the conidia; its suitability for application; as well as its shelf life, effectiveness and cost competitiveness depended on the type of formulation chosen. The first formulation tests were started on two strains. The conidia were dried using either the fluidised bed drying technology or freeze-drying technology. The final evaluation consisted of a quality check to determine the conidia concentration and germination capacity of the final fungal product.

RESULTS

The results for the four selected isolates showed considerable differences in conidia production depending on the agar media used. Two of the isolates produced more than $2x10^8$ conidia per ml per Petri dish. The yield of the other two isolates was less - meaning that more Petri dishes would be needed for production of the same amount of inoculum. The results on the culture substrates in laboratory-scaled *Solid- State* fermentation have not yet been completed. The first results (see Figure 3) will determine the suitable substance for each strain.
Two of the strains indicate a yield higher than $1x10^9$ conidia per gram substrate (input).

The commercial relevance of a certain conidia yield per fermenter units is directly affected by the amount of conidia required to treat a particular acreage. If the potential strains can be used successfully when applied at e.g. $5x10^{11}$ conidia per hectare and should it be possible to produce $1x10^{9}$ conidia per gram substrate (input), the process will be economically feasible.

REFERENCES

Lüth, P.; Eiben, U. (1999) "*Solid - State – Fermenter und Verfahren zur Solid- State – Fermentation*", Deutsches Patent und Markenamt, Offenlegungsschrift DE 199 20 020 A1

Figure 1.
Prophyta L –Laboratory *Solid-State* Fermentation Technology

Figure 2.
Industrial *Solid- State* Fermentation System

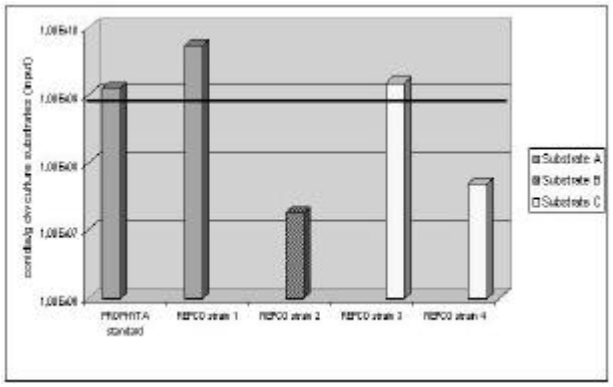

Figure 3. Conidia production of different fungus isolates on selected culture substrates after *Solid-State* fermentation