P-101 **Fermentation and formulation strategies for endophytic *Beauveria bassiana*: first results**

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INTRODUCTION

There is a high demand for systematic investigations on formulations for agrobiologicals such as biopesticides, probiotics, biofertilizers and plant extracts (Burges, 1998). In a recently granted project we focus on the development and evaluation of different formulation strategies, namely encapsulation, film-coating and spraying, for a novel endophytic isolate of the entomopathogenic fungus *Beauveria bassiana*. The aim is to develop a process for fermentation and formulation of *B. bassiana* in order to mass-produce and formulate the fungus in such a fashion that it infects rape plants and protects them from insect pests just as transgenic plants do.

MATERIAL AND METHODS

***Cultivation of* Beauveria bassiana**
Beauveria bassiana isolate ATP-04 was raised at 25 °C and 150 rpm in shake flask cultures. Different liquid media consisted of 0.5 % yeast extract CMRT, KAT or CPT (Ohly, Hamburg), 0.5 % yeast extract CMRT with 2 % soy (Meier, Hille), mineral medium K according to Kononova (Kononova, 1978), mineral medium T according to Thomas (Thomas et al., 1987), LB media (Roth, Karlsruhe) resp. yeast/sucrose media with 3.5 % of both compounds. Each media was adjusted to pH 5.5. After 3 d fungal mycelium was harvested on a Buechner funnel, washed twice with sterile tap water to quantify the fungal mycelium and concentration of spores.

Preparation of calcium alginate beads
Fungal biomass, 0.1 % (w/w) end concentration, was suspended in 10 ml of 2 % sodium alginate solution (Protanal LF20/60, Pronova Biopolymer, Dammen, Norway) or 10 ml of a 2 % sodium alginate solution amended with 12 % protein. Both suspensions were dropped into 30 ml 2 % $CaCl_2$ solution and cross-linked for 20 min.
As further additive 1 % yeast extract CMRT was amended to the beads.
Radial growth of mycelium out of the beads was measured on moist filter paper for 6 days.

Influence of additives on emulsions for spray formulations
For oil in water emulsions (o/w) 35 % plant oil was mixed with 1 % of the emulsifiers polyglycerol-polyricinoleat (PGPR) or Dimodan U/J (Danisco, Frankfurt). The aqueous phase, 64 % water, was added dropwise into the oil-emulsifier solution by mixing for 1 h. The stability of resulting emulsions was observed after an incubation time of 24 h at 20 °C.

Influence of additives on residues formed in drying spray droplets
For detection of marangoni effect a model suspension was mixed with 0.5% clay additive (Rockwood, Germany), dropped on a plastic surface and dried at laboratory atmosphere for 16 h.

RESULTS

The endophytic fungus *B. bassiana* was raised in shake flask cultures. The influence of nine media with different nutrient compositions on the production of spores and mycelium was investigated (Tab. 1).

Table 1: Cultivation of spores and mycelium of *B. bassiana* in shake flask cultures

Medium	Spores*	Mycelium*
yeast extract CPT	$3{,}2 \times 10^9$	1,78 g
soy medium	$2{,}94 \times 10^9$	1,74 g
yeast extract CMRT	$2{,}1 \times 10^9$	2,21 g
yeast extract KAT	$1{,}5 \times 10^9$	1,51 g
LB medium	$6{,}5 \times 10^8$	0,51 g
yeast/sucrose medium	2×10^8	0,41 g
mineral medium T	$4{,}5 \times 10^7$	< 0,1 g
mineral medium K	$2{,}8 \times 10^6$	< 0,1 g

* per gram utilized substrate

It was determined that medium including yeast extract CPT resulted in highest spore and mycelium production per gram utilized substrate.

The effect of bead type and yeast extract on fungal growth was investigated. As shown in Figure 1 radial growth of mycelium was 41 % respectively 33 % better in beads with yeast extract.

Furthermore beads containing protein showed a 41 % larger radial mycelium growth than ordinary alginate beads (Fig. 1).

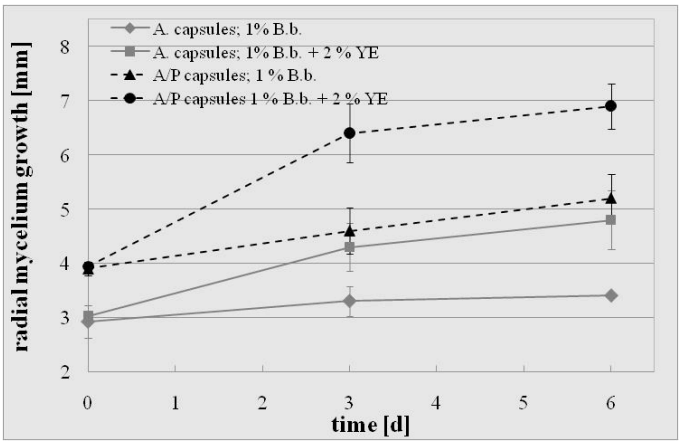

Figure 1: Influence of yeast extract on growth of fungal mycelium out of beads after 3 and 6 days at 25°C. YE: yeast extract, Bb: *B. bassiana* biomass A: alginate beads; A/P: alginate/protein beads

In comparison with sodium alginate beads, the beads containing protein showed higher mycelium density (Fig. 2).

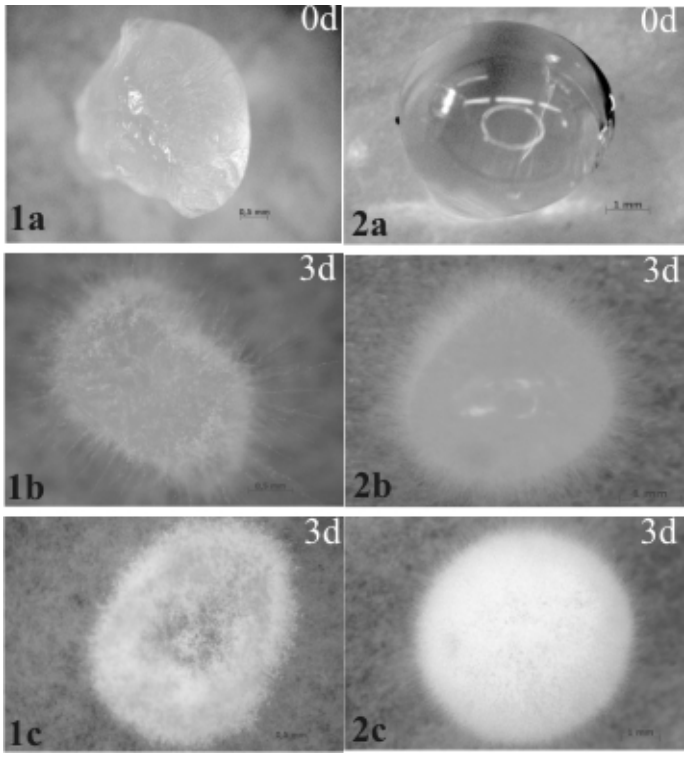

Figure 2: Fungal growth out of 1) sodium alginate beads 2) sodium alginate/protein beads a) 0 d b) 3 d without yeast extract c) 3d yeast extract

Besides, the influence of additives on emulsions for spray formulations was investigated. The addition of the emulsifier PGPR to the oil in water mixture resulted in an emulsion which was stable for 24 h (Fig. 3).

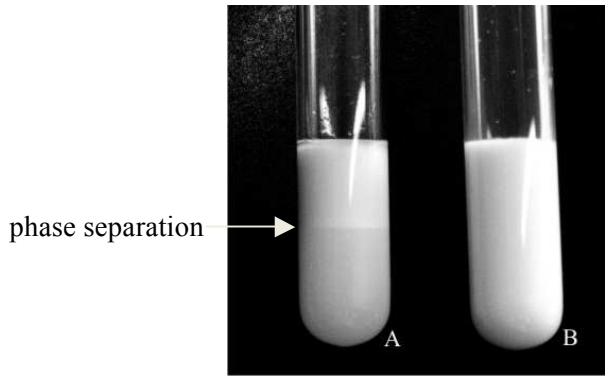

Figure 3: Stability of emulsions after 24 h. Mixtures with 1 % A) Dimodan; B) PGPR

In contrast to the residues formed through evaporative deposition of droplets of a model suspension without additives, the addition of 0.5 % clay additive resulted in a uniform deposition on the surface (Fig. 4).

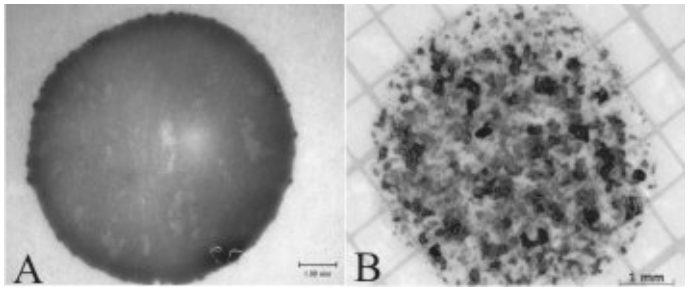

Figure 4: Residues formed through evaporative deposition on a plastic surface exposed to laboratory atmosphere A) model suspension B) model suspension with 0.5 % clay additive

CONCLUSIONS

First results on cultivation indicate that the endophyte can grow in liquid culture as good as conventional biocontrol strains of *B. bassiana*. First experiments on encapsulation and spraying allow the conclusion that these may be technologies for a mass-production process. Further experiments will focus on development of fermentation media and variation of formulation materials and methods.

REFERENCES

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