

Encapsulation and application of bacterial antagonists and a nematophagous fungus for biological pest control

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Introduction

Encapsulation can improve the properties of biological control agents such as (i) easy handling and application, (ii) protection of the biocontrol agent from biotic and abiotic stress factors, (iii) enhanced shelf life, (iv) controlled release (controlled by environmental conditions and formulation materials) and (v) enhanced efficacy in the soil. Recently, encapsulation techniques based on ionotropic gels well-known in classical biotechnology (Vorlop et al 1987) have successfully been used to formulate entomopathogenic nematodes (Patel & Vorlop 1994), bacterial antagonists (Patel et al 1995, Patel et al 2002a, Patel et al 2003) and the nematophagous fungus *Hirsutiella rhossiliensis* (Patel et al 2002b).

Here we present data from a joint project on the development and application of hydrogel capsules for the delivery of two biological control agents, namely bacterial antagonists to control phytopathogenic fungi and the nematophagous fungus *H. rhossiliensis* to control plant-parasitic nematodes. The focus is set on encapsulation on lab scale and on technical scale with the JetCutter, drying and storage of the formulations, application to the green-house as well as to the field and technology transfer to a biocontrol company.

Materials and methods

Encapsulation of bacterial antagonists

Pseudomonas fluorescens BA2002 was raised in ½ TSB medium at 20°C and washed twice at harvesting. 2 % Na-alginate was prepared with a total weight of 50 g. Then, 9 g autoclaved baker's yeast were added. Finally, 1 g wet cells of *P. fluorescens* were mixed into the suspension and dripped into 150 ml 2 % CaCl₂. After 20 min crosslinking time, beads having a size of 1.0-1.2 mm were treated with the drying protectant DP8 and then air-dried at 20°C. Storage experiments were conducted by exposing 10 beads in evacuated aluminium bags to 20°C for 4 weeks or 60°C for 2 hours. Before and after drying and after storage, 10 Ca-alginate beads were rehydrated and dissolved, respectively in 300 ml Na-citrate buffer, pH 7.0 for 40 min. Cell viability was assessed by a cfu determination on SNA I agar.

For field trials, three bacterial strains (BA2002, F50, F54) were raised in a bioreactor on ½ TSB medium. Then, 60 g bacterial biomass and 540 g autoclaved baker's yeast were added to 3000 g of a solution containing 2 – 6 % biopolymer. The suspension was then dropped with the Jet Cutter into a 2 % CaCl₂ solution and left to crosslink for 20 min. Afterwards, the beads were dried and incorporated into commercial sugar beet pills. For comparison, 48 h old culture broth was incorporated with the same cell amount.

Encapsulation of the nematophagous fungus Hirsutella rhossiliensis

For greenhouse experiments, fungal mycelium was raised in a bioreactor on a medium containing 1 % glucose and 0.5 % technical yeast extract. After harvesting, 50 g mycelium was added to 5000 g encapsulation liquid containing two hydrogel-forming biopolymers and 3 % autoclaved baker's yeast, dropped with the Jet Cutter into a 2 % CaCl₂ solution and left to crosslink for 20 min. Then, beads were air-dried at room temperature.

Application of encapsulated biocontrol agents in the greenhouse and field trials

Application of bacterial antagonists

Microencapsulated and free cells respectively were incorporated in the pellet surrounding sugar beet seed. Field trials (plot trial, 6 replications) took place on 6 locations in Europe (Germany, France The Netherlands). Early and final emergence of seedlings in the field, the most important parameters for effectivity, were determined. Pills without bacteria served as a check.

Application of Hirsutella rhossiliensis

The efficacy of dried beads containing 1 % and 10 % fungal mycelium were tested in greenhouse pot trials against *Heterodera schachtii* (sugar beet nematode) on sugar beet cv. Penta. The trials were conducted in steam pasteurised field soil. In order to evaluate nematode damage the number of cysts and the containing larvae, as well as the weight of plant tops were quantified.

Results and discussion

Encapsulation of bacterial antagonists

When cells encapsulated in Ca-alginate beads were dried, survival was 45 %. These beads still showed a survival of 17 % when stored at 20°C for 4 weeks (Fig. 1A).

Long-term shelf life has yet to be determined but an accelerated storage test for 2 h at 60°C still showed a survival of 26 % (Fig. 1B), corresponding to a cell loss of only 25 %. This thermostability indicates a fair long-term shelf life as was proved for other microorganisms in accelerated storage tests (Damjanovic & Badulovic 1968, Desmons et al 1998).

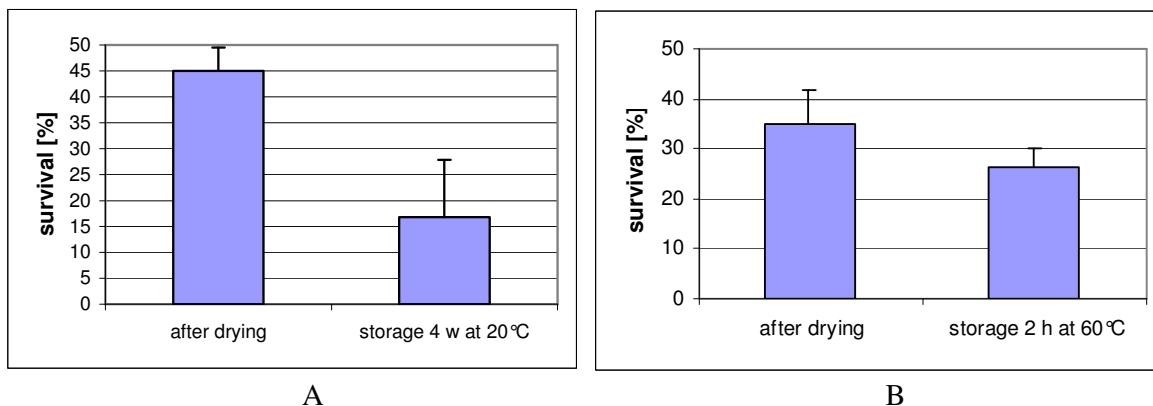


Figure 1: Shelf life of encapsulated and dried *P. fluorescens* BA2002. A: after 4 weeks of storage at 20°C, B: accelerated storage test (2 h at 20°C)

Field trials with encapsulated bacterial antagonists

Table 2 shows the results of field trials 2004 conducted at Seligenstadt, Germany. The field trials 2004 indicated that encapsulated bacterial antagonists were superior with regard to field emergence than culture broth.

Table 2: Field trial 2004 with encapsulated bacterial antagonists at Seligenstadt, Germany

formulation	antagonist	early field emergence [%]	% of standard
bead	F30	71,1	112,1
bead	F54	70,0	110,3
bead	BA2002	68,0	107,3
culture broth	BA2002	67,7	106,8
culture broth	F54	66,1	104,2
culture broth	F30	64,9	102,3
standard (no pesticides)	-	63,4	100,0

Greenhouse trials with encapsulated H. rhossiliensis

When fungal mycelium was applied to the soil directly, i.e. as wet biomass, there was no reduction of the nematode population. When the same amount of biomass was encapsulated to give beads with 1 % biomass, air-dried and then applied to the soil, there was a significant reduction of the nematode population down to 14 %. A tenfold increase of biomass concentration led to a nematode reduction down to 8 %. Weight of plant tops correlated with these data (data not shown). Thus, it could be shown that encapsulation enables the weak saprophyte to become established in the soil and protect sugar beet plants from infection with nematodes.

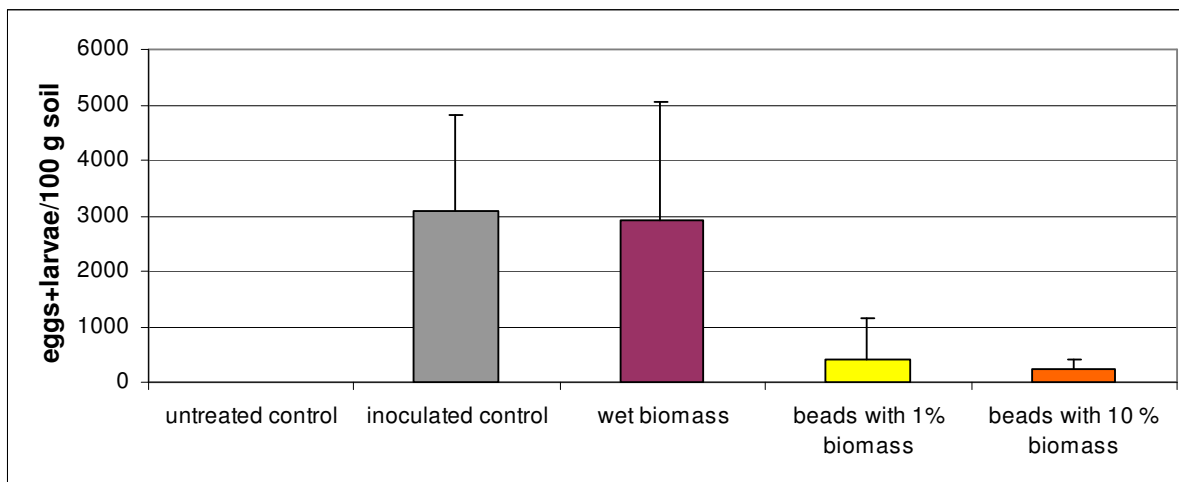


Figure 4: Greenhouse trials with encapsulated *H. rhossiliensis*: influence of encapsulation on nematode reduction

Conclusions

In view of the results, it can be concluded that beads based on hydrogels may enhance shelf life of drying-sensitive bacterial antagonists which may then be applied to the field as “pellets” (i.e. dried beads with a size >1 mm) or incorporated into commercial sugar beet pills as a powder formulation (bead size < 200 µm). In the case of the nematophagous fungus *H. rhossiliensis*, the key problem is the establishment of the weak saprophyte in the soil. Here, it can be concluded that mycelium may be delivered by encapsulation.

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