BIOTECHNOLOGY TECHNIQUES
Volume 8 No.8 (August 1994) pp.569-574
Received as revised 17th June

ENTRAPMENT OF BIOLOGICAL CONTROL AGENTS APPLIED TO ENTOMOPATHOGENIC NEMATODES

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Dedicated to Prof. Dr. F. Wagner on the occasion of his 65th birthday

SUMMARY

Hydrogels of alginate, phospho guar gum, carboxymethyl guar gum, *k*-carrageenan and cellulose sulphate, respectively were tested to find easily redissolvable gels. The entomopathogenic nematode, *Heterorhabditis* sp., was entrapped in calcium alginate beads, calcium alginate hollow spheres and foils made from different hydrogels. Emigration from calcium alginate beads after 7 days of storage was 100 % at room temperature and was lowered to 6 % at 6 °C, whereas no emigration from calcium alginate hollow spheres was found at either temperature. Highly concentrated polymer foils produced on gauze showed reduced emigration with a survival of 80 % after 24 h compared to foils produced on glass slides. Calcium alginate beads can be used for a controlled release of the nematode into the environment, while hollow spheres and foils are suitable for storage.

INTRODUCTION

Because of the increasing restriction of chemical insecticides and the expansion of biological control of pests, there is a demand for biological insecticides. But in order to become an economical alternative to chemical insecticides, suitable formulation processes have to be developed.
The advantages of entrapped biological control agents can be (i) a controlled release of agent (controlled by the environment and the properties of the entrapment materials), (ii) easy handling and prolonged shelf life and (iii) protection against extreme environmental conditions.
Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae can be used to fight soil-borne insect stages (e. g. larvae of the black wine weevil (*Othiorhynchus sulcatus*) in nursery and greenhouse plants), as their infective juveniles (IJ) actively search for the specific host, invade and kill the host during propagation, and then leave the dead insect searching for new hosts (for review see Gaugler &

Kaya, 1990). Currently, entomopathogenic nematodes are commercially produced *in vitro* in liquid culture (Friedman, 1990, Lunau *et al.*, 1993), and are immobilized by entrapment in calcium alginate, which proved successful with steinernematid and a few heterorhabditid nematodes for storage (Nelsen & Mannion, 1986, Pruitt & Grove, 1990) and for a controlled release of IJ (Kaya & Nelsen, 1985, Kaya *et al.*, 1987, Poinar *et al.*, 1985), but no satisfactory conservation processes for most heterorhabditids have been found, yet (Kaya & Gaugler, 1993).
In this study different biologically degradable ionotropic gels, which have been used for the encapsulation of biocatalysts (Vorlop & Klein, 1983), were screened for redissolvability. Encapsulation in calcium alginate beads was tried on *Heterorhabditis* sp. (HSH). Furthermore, the nematode was encapsulated in calcium alginate hollow spheres for the first time and entrapped in redissolvable foils made from different hydrogels.

MATERIALS AND METHODS

Strain
IJ of *Heterorhabditis* sp. (HSH) were provided by Dr. R. U. Ehlers, Institute of Phytopathology, Kiel, Germany, on polyurethane foam according to Bedding (1984) and after release were stored prior to encapsulation in 600 ml cell culture flasks ($5x10^4$ IJ/ml) at 6 °C in the dark.

Chemicals
Sodium carboxymethyl guar gum (Meyprogum R-600) and sodium phospho guar gum (Meyprofilm 50) were obtained from Meyhall Chemical AG, Kreuzlingen, Switzerland, sodium *k*-carrageenan (Genugel X-0828) from Kopenhavns Pektinfabrik, Lille Skensved, Denmark, sodium cellulose sulphate (SCS-LV) from Kelco International Ltd., San Diego, USA, sodium alginates (Protanal LF 20/60 and Lamitex LV) from Protan GmbH, Norderstedt, Germany and carboxymethyl cellulose (Relatin P 400) from Henkel KGaA, Düsseldorf, Germany.
Calcium chloride was purchased from Janssen Chimica GmbH, Geel, Belgium.
All other chemicals were products of Merck, Darmstadt, Germany.

Production of beads without IJ
A polyelectrolyte solution containing 3 % w/w Lamitex LV, 5 % w/w Meyprofilm 50 and 6.5 % w/w Meyprogum R-600, respectively was dropped through a syringe in a 0.5 % w/w, 1.5 % w/w and 2 % w/w $CaCl_2$ solution, respectively, and stirred for 20 minutes. The resulting calcium hydrogel beads had a diameter of 3.0 to 4.0 mm.
Likewise, potassium hydrogel beads were produced from 3 % w/w Genugel X-0828 and 14 % w/w SCS-LV, respectively in a 2 % w/w and 5 % w/w KCl solution, respectively.

Redissolvability of the gels
Redissolvability of gels was measured as the swelling of beads consisting of calcium hydrogels (Meyprofilm 50, Meyprogum R-600, Protanal LF 20/60, Lamitex LV) and potassium hydrogels (SCS-LV, Genugel X-0828), respectively in Na^+/Ca^{2+} and Na^+/K^+ swelling solutions, respectively.
0.1 M NaCl was mixed with 0.1 M $CaCl_2$ or 0.1 M KCl, resulting in swelling solutions with varying Na^+/Ca^{2+} and Na^+/K^+ ratios. In 50 ml of these solutions only one bead, with a diameter d_0, was given (2 replicates) and incubated for 48 h at 25 °C on a shaker. Then the diameter d of the swollen beads was determined.

Entrapment and release of IJ:
In all experiments free IJ in petri dishes (approx. $5x10^3$ IJ/ml at room temperature) were used as a control.

Production of alginate beads
A solution containing 2.6 % w/w alginate (Lamitex LV) and $1.7x10^4$ IJ/g was dropped through a syringe into 100 ml 0.5 % w/w $CaCl_2$ solution and stirred for 20 minutes. The resulting beads were stored under a moist filter paper in the dark in a 24-well tissue culture plate at room temperature and 6 °C. After 7 days, the numbers of released and encapsulated IJ were determined. Therefore, beads were redissolved in 1 ml 0.1 M citrate buffer pH 7.8.

Production of alginate hollow spheres (Spiekermann *et al.*, 1987)
A solution containing 1.5 % w/w carboxymethyl cellulose Relatin 400 P, 1.5 % w/w $CaCl_2$ and $3x10^4$ IJ/g was dropped through a syringe into a 1 % Lamitex LV solution and stirred for 20 minutes. The resulting hollow spheres were stored and counted as mentioned above.

Production of foils
Polyelectrolyte paste (0.98 g) containing 10 % Meyprofilm 50, 20 % Meyprogum R-600, 10 % Protanal LF 20/60 or 20 % SCS-LV was mixed with 0.02 g IJ and was smoothed into a 1 mm layer on a glass slide. Then, it was gelled in 15 %, 20 %, 10 % $CaCl_2$ and 7 % KCl solutions, respectively.
Likewise, a 2 to 4 mm thick layer was gelled bilaterally on gauze in 15 %, 15 %, 5 % $CaCl_2$ and 7 % KCl solutions, respectively. Gelation time was 2 min in both experiments.
The resulting foils were stored at room temperature in 20 ml snap-cap bottles in the dark. After 24 hours, the gel surface was washed with a defined volume of water and weighed. Then IJ in the washing solution were counted. Thus the number of released IJ/g foil was obtained.
The number of still entrapped IJ/g foil was determined by dissolving a weighed slice of a foil in 1 ml 0.1 M citrate buffer pH 7.8 and counting the number of live (i. e. possessing the ability to move when touched with a needle) and dead IJ.

RESULTS AND DISCUSSION

Redissolvability of the gels
Fig. 1 shows the degree of bead swelling as a function of the Na^+/Ca^{2+} and Na^+/K^+ ratio, respectively.
The gels can be arranged according to their redissolvability as follows:

SCS-LV > Meyprofilm 50 > Meyprogum R-600 > Genugel X-0828 > Protanal LF 20/60 > Lamitex LV

The calcium gels of Meyprofilm 50 and Meyprogum R-600 could be redissolved quickly and gently in 0.1 M citrate buffer pH 7.8. K-gels of SCS-LV could be redissolved already in 0.1 - 1% w/w NaCl solutions, whereas the potassium gel of Genugel X-0828 did not dissolve in these solutions.

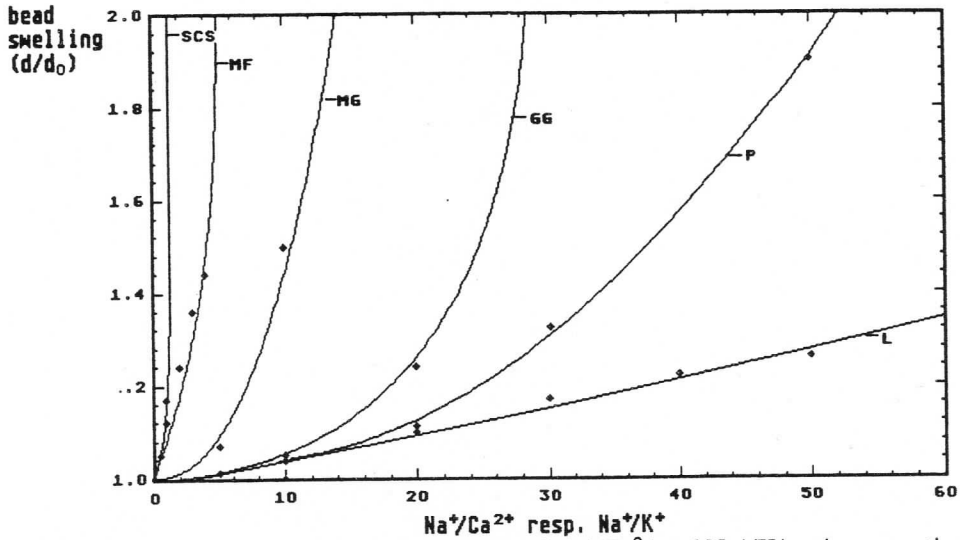


Fig. 1: Swelling of hydrogel beads as a function of Na^+/Ca^{2+} and Na^+/K^+ ratio, respectively (SCS: SCS-LV, MF: Meyprofilm 50, MG: Meyprogum R-600, GG: Genugel X-0828, P: Protanal LF 20/60, L: Lamitex LV)

Encapsulation of IJ in beads and hollow spheres

Fig. 2 shows the dependence of emigration of IJ on the type of bead and temperature. Emigration from beads was 100 % at room temperature (RT) and could be lowered to

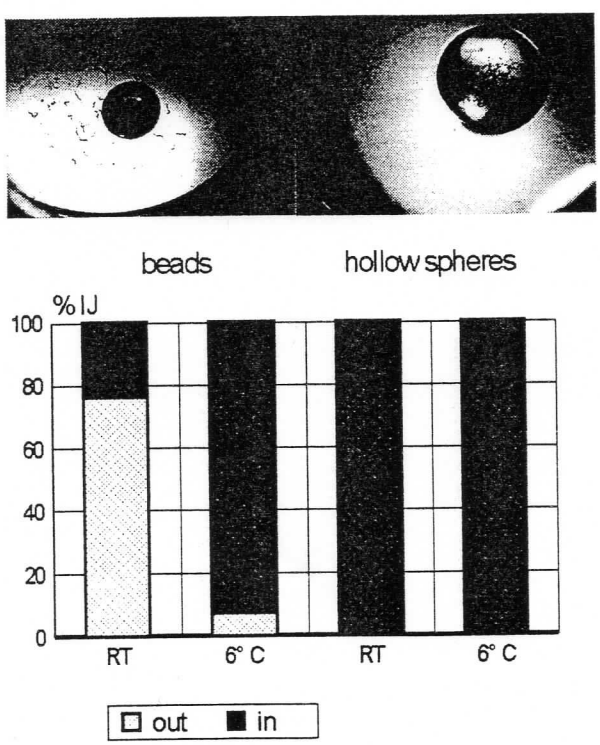


Fig. 2: Emigration of IJ from beads and hollow spheres after 7 days of storage at different temperatures

6 % at 6 °C, whereas there was no emigration at all from hollow spheres at either temperature.

Entrapment of IJ in foils

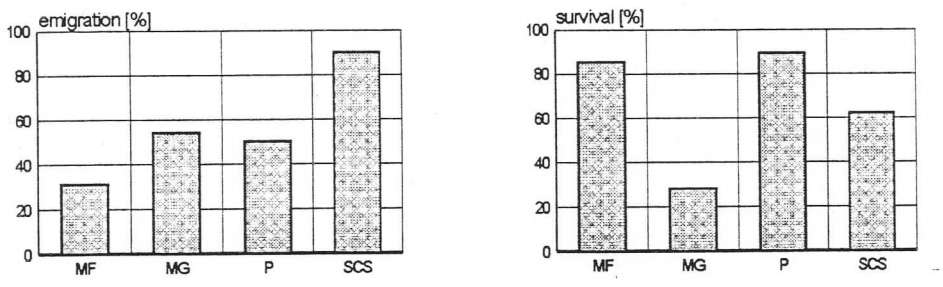
Emigration and survival of IJ in 1 mm thin foils which were gelled unilaterally and of 2-4 mm thick foils gelled bilaterally are shown in Figs. 3 and 4.

The highest survival (80%) with low emigration of IJ was found in foils which were gelled to thick layers.

CONCLUSION

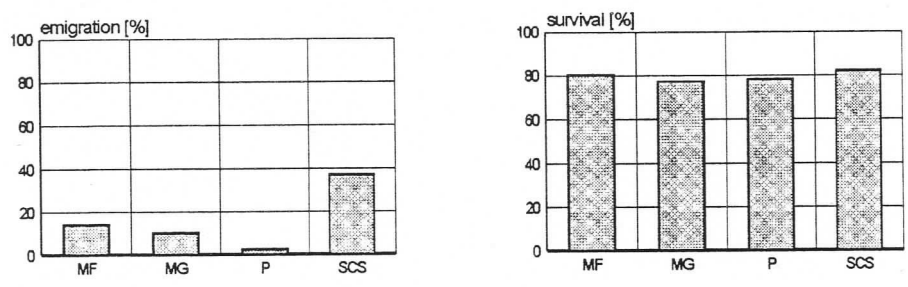
IJ encapsulated in biologically degradable calcium alginate beads can be used for a controlled release of IJ in the environment as proposed by several authors (Kaya & Nelsen, 1985, Kaya *et al.*, 1987, Poinar *et al.*, 1985), while hollow spheres can prove useful in the storage of IJ which can be released for application by crushing the hollow spheres.

IJ entrapped in foils of highly concentrated Protanal LF 20/60, Meyprogum R-600 or



A B

Fig. 3: Emigration (A) and survival (B) of IJ in thin, unilaterally gelled foils after 24 hours of storage at room temperature (abbreviations see Fig. 1)



A B

Fig. 4: Emigration (A) and survival (B) of IJ in thick, bilaterally gelled foils after 24 hours of storage at room temperature (abbreviations see Fig. 1)

Meyprofilm 50 are suitable for storage which can then be released by redissolving them in citrate solutions. If polymer properties of SCS-LV can be optimized, it will be possible to release IJ from a potassium SCS-LV hydrogel by redissolving it in a physiological NaCl solution.
First experiments on the preservation of *Heterorhabditis* sp. (HSH) by partial desiccation and cryopreservation indicate that a combination of these methods with certain entrapment methods is useful in order to prolong shelf life.

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