Novel hydrocolloid carriers for efficient biocontrol of soil-borne plant-pathogenic fungi

Nussinovitch A*. and Zohar-Perez C.
Hebrew Univ. Jerusalem, Rehovot - Israel (amos.nussi@mail.huji.ac.il)

INTRODUCTION AND OBJECTIVE

Yields of vegetable, fruit, flower and cotton crops are often destroyed by fungal disease. Modern chemical disinfectants used to control soil borne pathogenic fungi have created serious ecological problems; turning public attention towards biocontrol agents. However, there is a gap between their potential and successful field application, largely due to soil heterogeneity, fluctuations in moisture, temperature, nutrient levels, soil texture and pH, the presence of other microbial species, competition with indigenous microorganisms, and other factors that influence microbial growth, survival and effectiveness (Nussinovitch 1997). Our objective was to study the properties of dried hydrocolloid carriers entrapping potential biocontrol agents. It was hypothesized that only a comprehensive understanding of the interactions between the soil, the biocontrol agents and their carriers can pave the way for an efficient use of such materials and exploitation of their abilities.

MATERIALS AND METHODS

Alginate (Sigma, LV, St. Louis, MO) was dissolved in distilled water (2% w/w). Glycerol (1-70% w/w) was added to the alginate solution. A colloidal chitin suspension was added to the alginate or alginate-glycerol solution to a final concentration of 1% (w/w). All mixtures were autoclaved before adding the *Pantoae agglomerans* IC1270 or *Serratia marcescens* bacterium at a 1:9 volumetric ratio to the alginate, alginate-glycerol, alginate-chitin, or alginate-glycerol-chitin solution. Final mixtures were dripped into a sterile 1% (w/w) calcium chloride solution, and stirred for 30 min. The spherical beads produced were then freeze dried (Nussinovitch 2003).

Bacterial enumeration in the carriers and the soil was performed by plating on nutrient agar (NA) amended with 40 µg/mL rifampicin and incubating at 28˚C for 24 hours. The amount of bacterial cells was then enumerated. Freeze-dried carriers were mixed with sandy loam soil in polypropylene boxes that were kept in a greenhouse at 28˚C. In some boxes the soil was irrigated with tap water every 8 hours to maintain ~25% (w/w) soil water content for 1 month. Other containers were irrigated only at the beginning of the experiment and then again 2 weeks later. *Rhizoctonia solani* was used as the pathogenic fungus. A scanning electron microscope was used to study the carrier structure (Zohar-Perez 2005). Confocal microscopy was used to study the way in which the cells are

distributed in the beads. The external and internal shape and texture

of the carriers was studied in detail. To analyse the relationships between the bacteria's biological efficiency and the carriers' physical properties, a special *in-vitro* experimental apparatus was constructed. The apparatus was designed to examine the slow-release properties of the alginate carriers as the bacteria and their antagonistic enzymes were released into the soil (Nussinovitch 2003).

RESULTS AND DISCUSSION

Entrapping 10^9 cells per bead of the gram-negative bacterium produced from an alginate solution containing 30% glycerol and 1% chitin improved survival prospects (95%) during freeze-drying (fig. 1 & fig. 2). This improvement was probably due to the higher initial bacterial load entrapped in each bead and the elevated glycerol concentration. Confocal microscopy showed a greater concentration of cells on the surface of the alginate beads than in their cores. As a result of the spontaneous cross-linking reaction on the beads' surface, and the following contraction, a more constricted network is created on the gel bead's surface than at its core (Nussinovitch 2010).

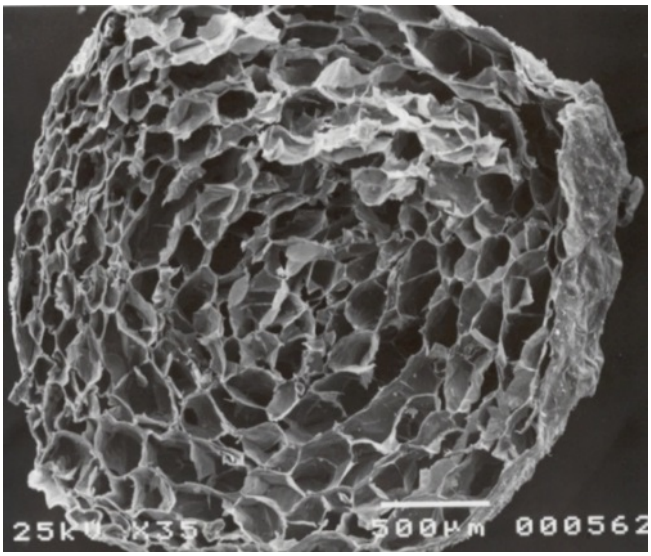

Figure 1 : Electron micrograph of alginate dried carrier entrapping IC1270.

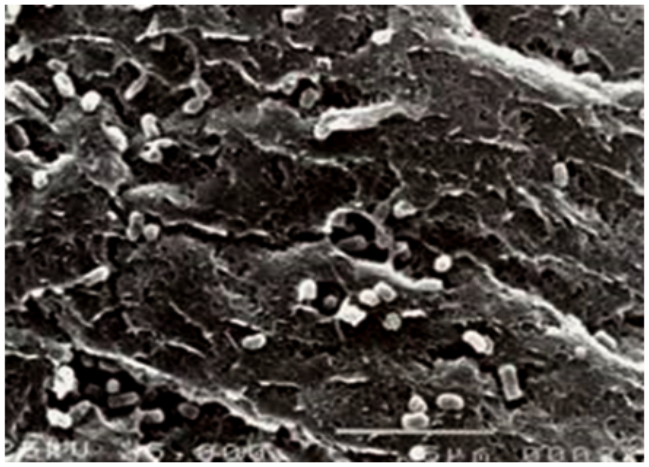

Figure 2 : Presence of *P. agglomerans* in the inner surface of alginate gel carrier.

The addition of glycerol and filler reduced the carriers' freezing point and freezing rate. As a result, the beads had larger pores. Moreover, beads containing glycerol and a filler had thicker pore walls. Alginate-glycerol-filler, both as gel and dried, were rounder than their counterparts containing no glycerol and filler. When the carriers were immersed in the soil, and upon rehydration, their weight increased six fold (Nussinovitch 2010). Decreases in their stiffness and porosity values were also observed (fig. 3).
In-vitro experiments showed that the dispersal of *Serratia marcescens* entrapped in alginate carriers into the soil resulted in high soil cell densities throughout the 30 days of the experiment (Zohar-Perez 2005). This is due to the continuous release of the bacteria from the beads and reproduction of the released bacteria in the soil. Chitin incorporation into the beads induced a higher chitinolytic activity of *S. macescens* relative to beads not containing chitin. It is possible that the cellular porous structure of the dried beads enables higher diffusion rates of these enzymes (Zohar-Perez 2005). *In-vivo* experiments demonstrated improved bacterial survival during application in the soil under different irrigation conditions. It seems that the dried alginate carriers' ability to absorb high amounts of water in a short time and retain it when the soil is dehydrated enables a maintained viability of the entrapped cells and their revival once the water supply was restored. The cell immobilization also proved its biological efficiency under greenhouse conditions. The *S. marcescens* immobilized in the dried carriers with chitin reduced bean disease by 60% (Zohar-Perez 2005).

CONCLUSIONS

This study offers a new approach to solving a major problem when growing crops. Only an integration of the physical and biological properties of the immobilization system can lead to practical application. Controlling the physical properties of the carriers enables tailoring the cellular carriers for slow release, controlling specific fungal diseases, and achieving stability under stressful environmental conditions such as application in non-sterile soil.

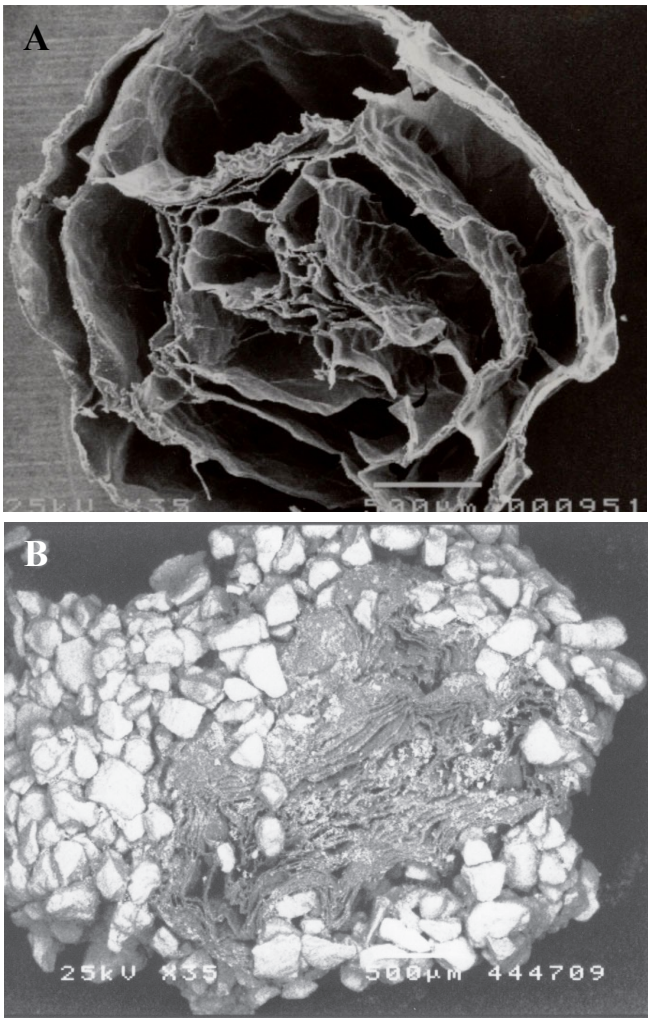

Figure 3 : Electron micrographs of halved alginate freeze-dried beads entrapping *Serratia marcescens* before (A) and after (B) incubation in soil.

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