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Plant Growth-Promoting Rhizobacteria

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Plant Growth-Promoting Rhizobacteria
— Present Status and Future Prospects —

Editors
A. Ogoshi, K. Kobayashi, Y. Homma,
F. Kodama, N. Kondo and S. Akino
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Microencapsulation as a potential carrier for plant growth-promoting bacteria

Angel Carrillo and Yoav Bashan

Department of Microbiology, The Center for Biological Research of the Northwest (CIB), La Paz, P.O. Box 128, B.C.S. 23000, Mexico.

Summary. A simple microencapsulation formulation and the related production equipment for plant growth-promoting bacteria (PGPB) inoculants were developed using alginate as substrate and Azospirillum brasilense *Cd as the model bacterium. The procedure involved production of small droplets of a mixture of alginate and liquid bacterial culture. The viscous mixture was forced through a small diameter nozzle (222 µm) under low pressure, producing a stream of small diameter liquid droplets. These droplets when dropped into a slowly stirred solution of $CaCl_2$ immediately created microbeads, but the process killed most all entrapped bacteria. The diameter of the microbeads was 300 to 700 µm. The surviving bacterial population in these microbeads was reincubated in bacterial culture medium for a secondary multiplication of the population. The experimental formulation can be used wet, or alternatively, the microbeads can be dried by a low-speed stream of dry air.*

Introduction

To date, most marketed bacterial inoculants are peat-based formulations for seed coating or pellets for sowing furrows (Smith, 1992). They usually produce satisfactory results primarily with rhizobia (Thomposon, 1980). The development has almost reached its technological peak, and still has several severe drawbacks because of the nature of peat. During the last decade, several experimental formulations based on polymers have been evaluated as carriers both for agriculture and for bioremedation use (Bashan and Carrillo, 1996; Cassidy *et al.*, 1996). These polymers were demonstrated to be potential bacterial carriers offering substantial practical advantages over peat (Bashan, 1986). However, they are rather expensive relative to peat-based inoculants and require more biotechnical handling by the inoculant industry (Fages, 1992). There is no commercial agricultural inoculant that uses this technology as yet.

Alginate is the most common polymer for encapsulation of microorganisms for various industrial microbiological purposes (Cassidy *et al.*, 1992). The most common formulation is in the form of macrobeads (diameter 1 to 3 mm). Their relatively large size is disadvantageous for agricultural uses and will be discussed later. To produce smaller size beads, two possibilities exist: (i) mechanical crushing of large beads and then sieving to the desired size and (ii) direct production of microsize beads by an appropriate technology.

The aims of this study were (i) to demonstrate the technical feasibility of direct microbead production, and (ii) to develop simple prototype equipment for their production for agricultural and environmental uses.

Materials and methods

Equipment for microbead production

The production of microbeads was done using a prototype device (Fig. 1). It was designed and built of common materials (metal and plastics). The basic design has the ability to be modified or modularly enlarged to produce large amounts of microbeads without compromising the basic features. This design produced microbeads of 300 to 700 µm diameter.

Microbead formation

Azospirillum brasilense Cd served as a model bacterium and was grown by using the standard techniques of this genus (Bashan *et al.*, 1993). Fifty mL of bacterial suspension were mixed under aseptic conditions with sterile 0.75% sodium alginate (14,000 cps, Sigma, USA) under slow stirring at ambient temperature for 1 to 2 hrs until all the ingredients were thoroughly mixed. The bacterial concentration at this stage was $3x10^7$ cfu/mL solution. Alternatively, skim milk (0.75%) was added to the alginate-bacterial suspension to produce more degradable beads (Bashan, 1986).The suspension was then placed in an Erlenmeyer flask, attached to the bead producing equipment (described in Fig. 1), and a pressure of 13 PSI was created in the equipment using a commercial air compressor. The bacterial suspension sucked from the Erlenmeyer was forced through a capillary exit (222 m). This created a fine stream of miniature droplets of the suspension. The droplets were allowed to free-fall into a slowly rotating (40 rpm) large-mouth container containing 0.1 M $CaCl_2$. Microbeads were formed instantly upon contact of the droplets with the solution. The microbeads were allowed to cure in the $CaCl_2$ for 1 hr. After the wet beads were removed from the $CaCl_2$ solution, they were rinsed in a vacuum funnel on filter paper (Whatman #1) for 10 times under aseptic conditions and transferred into a fresh Nutrient Broth. Bacteria were multiplied in this medium for 12 h at 30±2°C at 100 rpm. The microbeads were separated from the suspension by filtration, and rinsed with a saline solution (0.85% NaCl) for 10 times covering the beads completely with rinse solution each time. The population level after this secondary multiplication was always $>1x10^9$ cfu/g beads (fresh wt).

Drying procedures

The prototype equipment to airdry the beads is described in Fig 2. Samples (0.5 to 3g) of wet microbeads were placed inside a fine sieve bag. A stream of dry air (9% humidity, passed through a trap of Silica Gel) at a flow rate of 770 mL/min and at ambient temperature (27 to 29°C) was passed through the microbeads

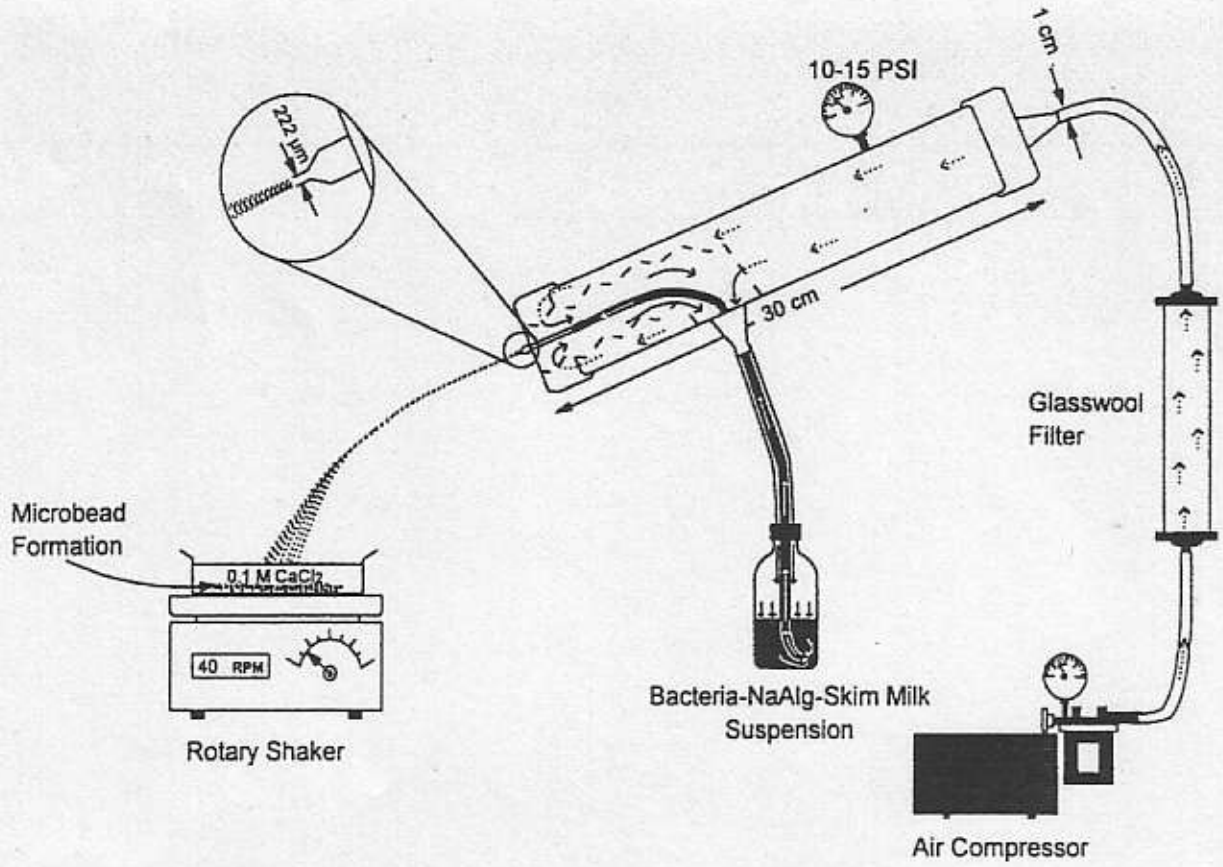

Figure 1. Prototype equipment for production of alginate microbeads

until they were dry.

Bacterial counts from beads

Bacteria entrapped (or covering) were counted after dissolving the beads in 0.4M KH_2PO_4 (pH 6.9) for 30 to 60 minutes and counting the bacteria in the formed solution by conventional Plate Count Method on Nutrient Agar.

Results and Discussion

The main advantages of alginate preparations are their non-toxic nature, biodegradability in the soil, and their slow release of microorganisms into the soil (Bashan, 1986). The preparation of macro alginate beads (1 to 3 mm in diameter) containing bacteria is fairly easy and involves a multistep procedure (Bashan, 1986, Digat, 1991). Several alginate-based preparations were evaluated for agricultural purposes, including the encapsulation of the VAM fungi (Ganry *et al.*, 1982), ectomycorrhizal fungi (Le Tacon *et al.*, 1985; Marx and Kenney, 1982), and biocontrol fungi against soil-borne pathogens (Fravel *et al.*, 1985; Lewis and Papavizas, 1985). This technology was also employed to encapsulate the plant-beneficial bacteria *A. brasilense* and *Pseudomonas fluorescens* (Bashan, 1986), which were later successfully used to inoculate wheat plants under field conditions (Bashan *et al.*, 1987) or to encapsulate genetically engineered *P. fluorescens* released into soil microcosms (van Elsas *et al.*, 1992).

The use of macro alginate beads has three major disadvantages. Aerobic diffusion into the bead is limited, making about 80% of the bead volume uninhabitable for aerobic PGPR (Chen and Huang, 1988); the need for the bacteria to move from the inoculation site through the soil towards the plants (Bashan and Holguin, 1994; Bashan and Levanony, 1987); and the need for an additional, specialized treatment during sowing (Bashan and Carrillo, 1996).

To overcome these problems, the microbead concept was conceived. If the beads are small enough but still capable of encapsulating a sufficient number of bacteria, it would be possible to produce an almost powder-like formulation to be used in seed coating.

Beads can be produced at various micro diameters. Very small beads in a wet formulation (2 to 50 μm diameter) were previously proposed for degradation of pentachlorophenol by *Flavobacterium* (Stormo and Crawford, 1992). A device to produce microdroplets that can be used in microbead production is commercially available (Misonix, 1996). From an agricultural standpoint, these ultra small beads have two main disadvantages; insufficient bacterial capacity and their wet preparation.

A main industrial concern about the activity of the entrapped bacteria in macrobeads is the restriction of oxygen diffusion into the bead. In the microbead formulation proposed in this study, this is not a problem because the diameter of the microbead is small, allowing air diffusion. Furthermore, in dry agricultural preparations, the bacteria are produced to be inactive (and alive) since their activities are needed only after seed germination and the degradation of the beads in the rhizosphere.

In sum, we are proposing a simple prototype equipment and method to produce microbeads for agricultural application of

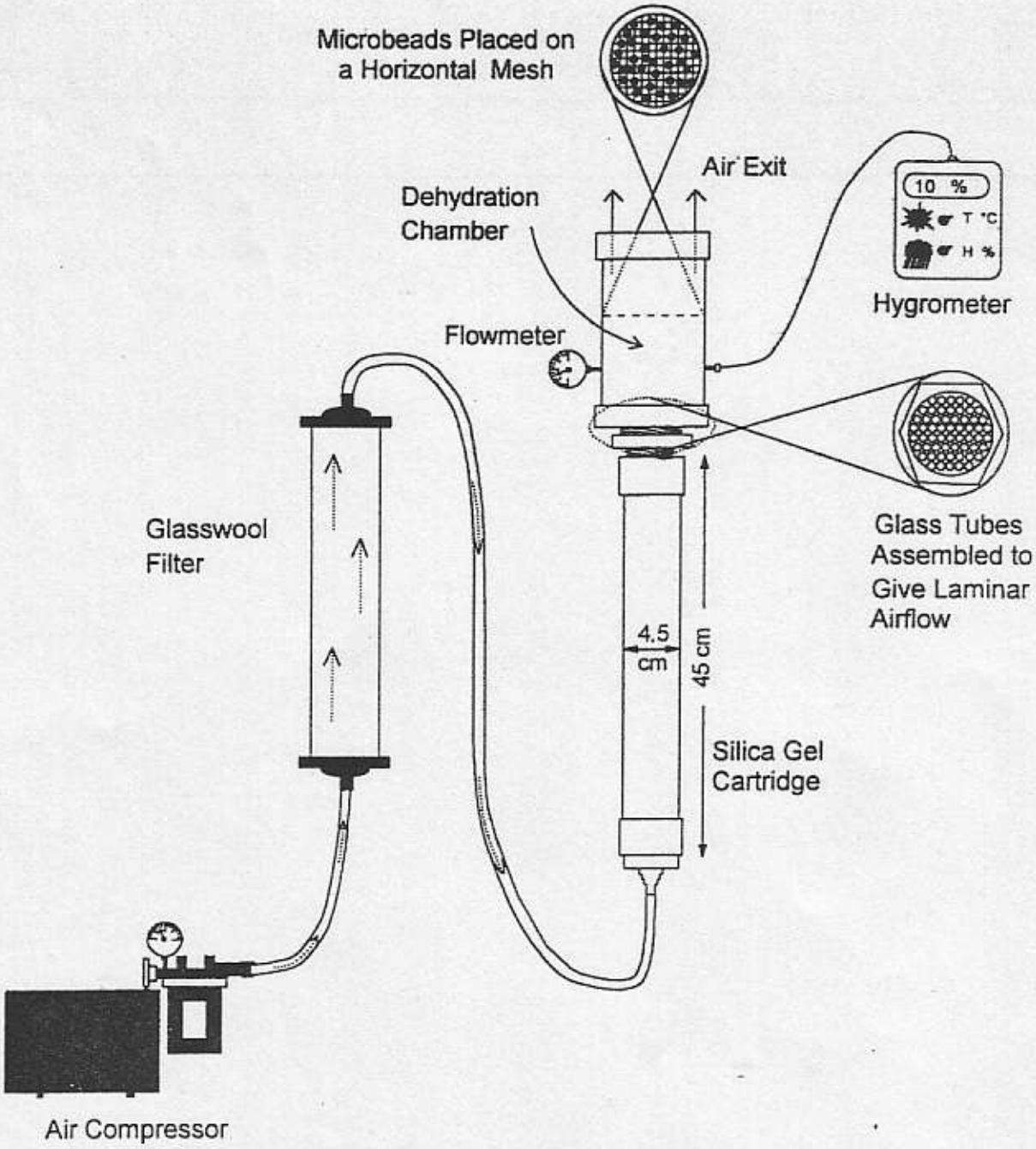

Figure 2. Prototype equipment used to air-dry microbeads

bacteria. Field application and scaling up of this novel formulation is still pending.

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