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Encapsulated plant growth-promoting bacteria for agriculture and the environment

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INTRODUCTION

In agriculture, plants are inoculated with plant growth-promoting bacteria (PGPB) and fungi to enhance productivity. This application is currently extended to environmental uses. Today, most inoculants (a carrier containing microorganisms) are made of peat or liquids. During the last two decade, several experimental formulations based on polymers have been evaluated. These polymers have demonstrated potential as carriers of bacteria that offered substantial advantages over peat and liquids. These formulations encapsulate the living cells, protect the microorganisms against many environmental stresses, and gradually release them to the soil but in large quantities as the polymers are degraded by soil microorganisms, usually at the time germination and emergence.

The inoculants can be stored in a dry container at ambient temperatures for prolonged periods, offer a consistent batch quality and a better environment for the bacteria, and can be easily manipulated to the needs of specific bacteria. These inoculants can be amended with nutrients to improve short-term survival of the bacteria at inoculation, which is essential to the success of the process of inoculation, especially when associated with PGPB. However, *one major constraint* is that polymers are expensive compared to peat-based inoculants and require more handling by processors. Therefore, encapsulation of microorganisms into a polymer matrix is still experimental in the field of agricultural technology of bacterial inoculation. At present, there is no commercial bacterial product using this technology.

Encapsulated bacterial formulations for agriculture and the environment have at least two distinctly different goals from those of the fermentation industry: (1) temporarily protect the encapsulated microorganisms from the soil environment and microbial competition, and (2) gradually release bacteria for colonizing roots.

This presentation focuses on our current studies in inoculation technologies.

Study 1 — Recycling leftovers of immobilized microalgae and plant growth-promoting bacteria from wastewater treatment as potential resource to enhance quality of severely eroded arid soil and plant growth

Leftover alginate beads, used initially for tertiary wastewater treatment and containing the microalgae *Chlorella sorokiniana* and the PGPB *Azospirillum brasilense* as the wastewater treating agents, were used to improve quality of eroded arid soil and enhance plant growth as an additional application. *A. brasilense* survived well in already-used dried beads for at least one year. Three consecutive applications of these dry leftovers increased the organic matter, organic carbon, and microbial carbon in the soil (Fig. 1). Growth of sorghum plants in this amended soil was enhanced compared to growth in eroded soil or soil amended with beads containing any other combination of alginate, microalgae, or bacteria (Fig. 2). The surface of plant roots growing in the amended soil was heavily colonized by the PGPB, with no endophytic colonization, where the preferred colonization sites were the root tips. This study demonstrates that biological residues from a novel biological wastewater treatment can serve, in a secondary role as a resource for improving arid soil quality and enhancing plant growth.

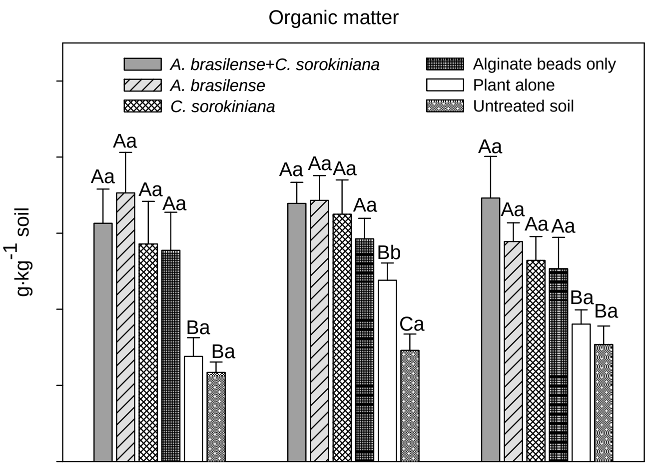

Figure 1: Changes in organic matter following addition of alginate beads with and without microorganisms to eroded soil. For each treatment, columns denoted with different lower case letter differ significantly at $P \leq 0.05$ by one-way ANOVA and Tukey's analyses. Columns denoted with different capital letter for each cycle of growth (groups of 6 columns) differ significantly at $P \leq 0.05$ by one-way ANOVA and Tukey's analyses. Bars represent standard error (SE).

Study 2 — Long term desert reforestation

To measure the feasibility of long term desert reforestation technology of mixed vegetation, cardon cactus seedlings from nurseries were planted in the field adjacent to one seedling of a potential legume nurse tree. The combination of tree and cactus were inoculated with a consortium of desert arbuscular mycorrhizal fungi, a PGPB, a phosphate-solubilizing bacteria, or a mixture of all. Plants in seven field experiments were observed and measured periodically during 30 months for survival and growth. Association with a nurse tree increased survival and enhanced growth of untreated cardon cactus and also significantly improved the long-term growth of two species of leguminous trees. We propose that young legume trees have the capacity to enhance survival and growth of cardon cactus, depending on the legume-cactus combination. Additional amendments, such as PGPB, can either amplify the effect or attenuate it.

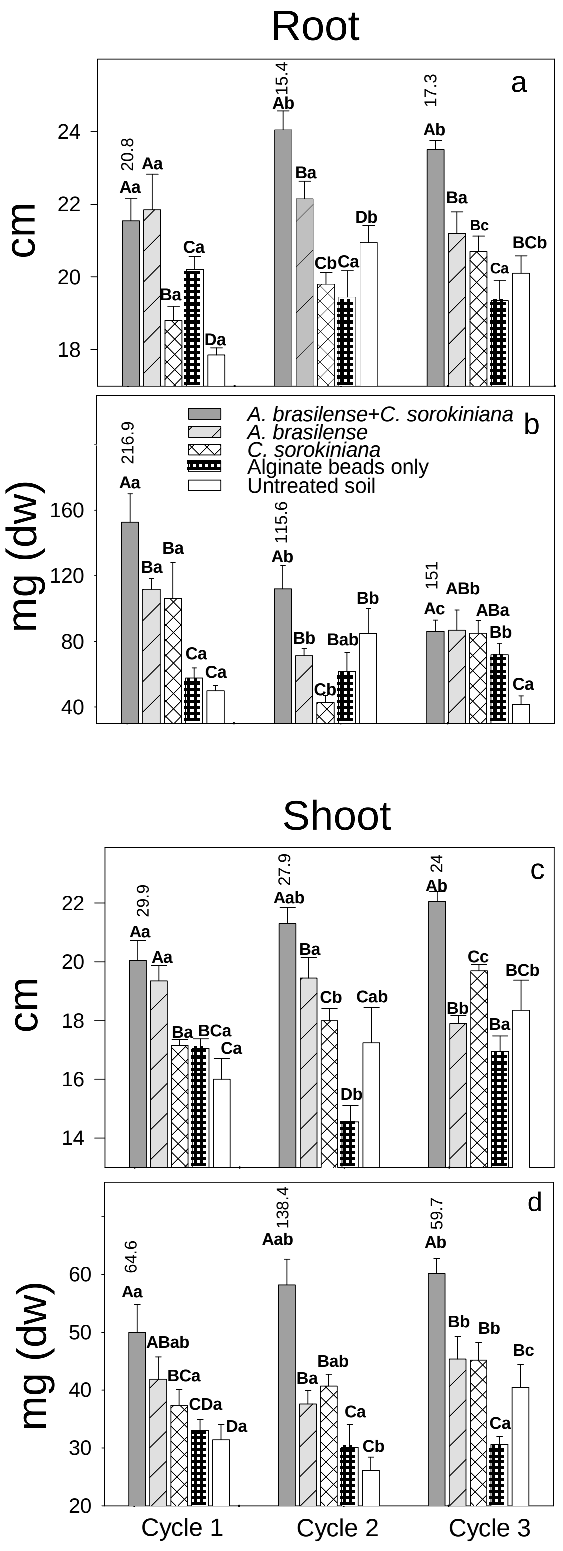

Figure 2: Effects on root (a,b) and shoot (c,d) growth of sorghum plants after three 20-d cycles of growth following addition of alginate beads with and without microorganisms to eroded soil. In each subfigure, and for each treatment, separately, columns denoted with different lower case letter differ significantly at $P \leq 0.05$ by one-way ANOVA and Tukey's analyses. Columns denoted with different capital letter for each cycle of growth (groups of 6 columns) differ significantly at $P \leq 0.05$ by one-way ANOVA and Tukey's analyses. Bars represent standard error (SE). Vertical numbers above the joint immobilization treatment indicate relative increase, in percentage, over untreated plants.

Study 3 — Improvement of alginate inoculants for agriculture and the environment.

Two culture media for mass cultivation of the PGPB *Azospirillum* sp. were developed. They are based on substitution of glucose in a previous mass production tryptic-yeast extract-glucose medium by Na-glucanate or glycerol. This modification significantly increased the population to a level that allowed production of alginate inoculants without any need for further growth of the PGPB in the inoculants, as was necessary in previous cases of culture media used by the agricultural inoculant industry.

CONCLUSIONS

Alginate inoculants are a powerful tool to introduce PGPB into different environments. They can be easily produced and used for unlimited type of applications with no apparent limits save some increase in cost over traditional inoculants.

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