ENCAPSULATION OF PLANT-GROWTH PROMOTING BACTERIA IN POLYMER MATRIX: DEVELOPMENT OF NEW BIOFERTILIZERS

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INTRODUCTION

The need of inoculation

Current agricultural practices have a huge impact on environment as they are highly polluting and are diminishing the biodiversity in ecosystems. Emission of pollutants, especially those derived from fertilizers, pesticides and animal waste decrease the quality of air, ground and surface water. However, these pollution problems are not insurmountable, since they may moderate considering better agricultural practices as the application of biotechnological processes that included bacteria that promote plant health.

Inoculation of seeds is an efficient and convenient way of introducing effective plant-growth-promoting-bacteria (PGPB) to soil and consequently the rhizosphere of plants. However, commercialization of biological agents like biofertilizers still encounters limiting factors, mainly due to poor bacteria survival. Thus, developing formulations that provide high concentrations of microbial inoculant and high survival rates during storage constitute an important step in the development of effective inoculants.

Generation of "improved seeds"

Seed coating is a general technique for inoculation of plants that allows producing "improved seeds" and it is the most reliable way to apply biocontrol/biofertilzer agents in close proximity with germinating seeds.

Layer-by-layer coating is a simple method to coat biopolymers onto biological control agents and was suggested to be a potential technique for the generation of so-called artificial spores (Yang 2012) and consequently "improved seeds" (Figure 1).

Layer-by-layer assembly involves multilayer coatings formation by repeated exposure of colloids (wished encapsulated material) to polyelectrolytes of alternating charges existing of an acidic (e.g. carboxylic, sulfonic) and basic component (e.g. amino function). Thus, living cells can be used as functional elements of polyelectrolyte multilayers which include the incorporation of cells into multilayers and the attachment of multilayers to the surface of the cells (Rubner 2012).

Two different approaches have been applied for bacteria encapsulation in the polyelectrolyte layers (Figure 1). Since bacteria cell membrane is negatively charged, bacteria can be directly entrapped in the polyelectrolyte complex owing to ionic interactions. The other approach is to first entrap the bacteria in inorganic porous carriers, which would help to increase bacteria shelf life in the formulation and ensure their controlled release.

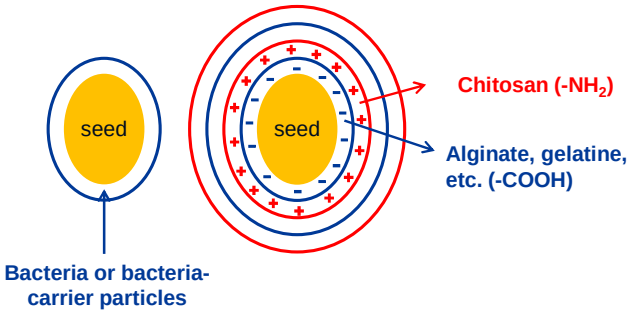

Figure 1.Bacteria encapsulation and seed coating approaches.

Hence the main aim of our work was to create "artificial spores" based on multilayer coatings (Domnanich 2011) with good mechanical stability and selective permeability in order to develop environmentally friendly formulation technologies with increased product shelf life and efficient release and activation of encapsulated biomaterials.

To address this challenge we have developed new surface coating solutions based on polyelectrolyte complexes and inorganic carriers for field application of PGPB such as *Burkolderia phytofirmans* PsJN (Mitter 2013) and *Paenibacillus* sp. WV11.

MATERIALS AND METHODS

The materials tested for seed coating of maize were non-toxic and biodegradable, cost-effective and readily available and included proteins like gelatin and polysaccharides like celluloses, alginate, and xanthan. Depending on the coating adhesive the material concentration was 1% to 10% (w/v). In addition, inorganic carriers containing Si^{+4} in their structure, such as fumed and precipitated silica (ranging from a few nm to 15 µm) or talc were included in the formulations.

Bacteria concentration used for encapsulation was 10^9 cfu/mL of coating agent. Coating was carried out in a SATEC ML2000 seed coater. Single and multilayer coatings were performed.

Coating quality was defined by visual inspection, while cell viability and release kinetics were determined by submerging the seeds in a buffer solution and plating serial dilutions on Luria-Bertani agar. The effect of the coating on seed germination was investigated in agar plates.

RESULTS AND CONCLUSIONS

The coating quality of the different formulations was evaluated according to seed coverage and coating homogeneity. Seeds were described as slightly and moderately covered. The coating was heterogeneous in all the cases.

Results compiled up to now showed that viability of bacteria included in the coating was reduced during the processing step. Since the initial bacteria concentration used for encapsulation was10^9 cfu/mL of coating agent, it was expected to be found in the order of 10^7 cfu/seed, however the maximum amount of cfu/seed found was in the order of 10^5.

Favorable effects in germination were observed, such as germination of maize treated with *Burkolderia phytofirmans* PsJN was enhanced by up to 60% compared with the untreated controls. The germination rate was monitored over storage of one month and no decrease was observed.

Preliminary results from release kinetics show that bacteria are mostly released within the first hour after submersion. Studies on release kinetics and stability testing are in progress. Scanning electron microscopic characterization of the coating layers is also being carried out to further study the quality of the coating and characterize the bacteria-matrix interface.

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