Biopolymer capsules as carrier for crop-specific microbial consortia

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INTRODUCTION

Agricultural practice involving microorganisms for bio-fertilization and plant protection has been basically recognized as an appropriate approach to feed the world in an environmental friendly and sustainable way. Because of their, compared to chemical-based products, lower stability and consistency the actual use of microbial inoculants in large scale agriculture is still far from being established. On the one hand, the sensitivity of most of the microbes to stress events during production and storage results in reduced cell viability and competitiveness *ad planta* under field conditions. On the other hand, the majority of microbial inoculants currently used for biological plant protection bases on single bacterial or fungal strains and claimed to be effective for a broad spectrum of crops and pathosystems. However, because of the complexity and fluctuations of biotic and abiotic parameters occurring under field conditions the performance of sole isolates is often inconsistent. Variable effects of microbial inoculants used in agriculture may be explained by the narrow eco-physiological optimum under which a microbe effectively acts. In consequence, due to the variability of their performance, microbial products are considered to be unreliable.

Here, we describe two approaches to apply a complementary set of microorganisms in the pathosystems sugar beet – *Rhizoctonia solani* and pumpkin – *Didymella bryoniae*, respectively. Crop-specific sets of synergistically acting microorganisms were compiled and applied as plant protection and growth promotion agents for sugar beet and Styrian oil pumpkin. Each microbial mixture was selected according to the results of a multiphasic isolation and screening process. By combining strains from various taxonomic groups possessing different plant-beneficial traits the performance of the bacterial preparations under field conditions were stabilized.
To control the phytopathogenic fungus *Rhizoctonia solani* causing the late root rot (AG2-2IIIB) in sugar beet a compilation consisting of microorganisms isolated from sugar beet (autochthonous) and different other crops (allochthonous) was composed (Zachow et al. 2008). The consortium of BCAs includes the bacterial strains *Pseudomonas trivialis* RE*1-1-14, *P. fluorescens* L13-6-12 and *Serratia plymuthica* 3Re4-18. In *in vitro* assays, modes of plant growth promotion as well as different antagonistic traits towards the pathogen were studied (Zachow et al. 2010).

During the last few years, substantial yield losses of oil pumpkin were reported in Styria due to fruit rot caused by the fungus *Didymella bryoniae* (black rot) and the bacterial pathogen *Pectobacterium carotovorum* (soft rot). Vegetative plant parts are affected by *Pseudomonas* spp*.*, *Xanthomonas cucurbitae*, *D. bryoniae* and *Sphaerotheca fuliginea* (powdery mildew). Additionally, drought stress events were frequently observed, what will be of increasing importance in the future due to the effects of climate change. In a previous study, bacteria from the spermosphere (seeds), endorhiza (roots), anthosphere (flowers) and carposphere (fruits) were isolated. Out of 2320 endophytic bacteria isolated from oil pumpkin plants, six phylogenetically distant broad-spectrum antagonists were selected according to their *in vitro* antagonism towards the oil pumpkin pathogens (Fürnkranz et al. 2012a). The oil pumpkin-derived bacterial broad-spectrum antagonists *Lysobacter gummosus* L101 (endorhiza), *Paenibacillus polymyxa* PB71 (spermosphere) and *Serratia plymuthica* S13 (anthosphere) were evaluated under field conditions (Fürnkranz et al. 2012b).

Seed treatment is considered as one of the most promising strategies to apply microbial inoculants to plants. To protect and promote plant growth from the moment of germination, seeds of sugar beet and oil pumpkin were inoculated with beneficial microbes simultaneously entrapped in alginate microspheres. Alginate hydrogels were selected as matrix due to the general suitability for formulation purposes and implementability in commercially established seed coating procedures.

MATERIAL AND METHODS

For simultaneous encapsulation of up to four bacterial strains in alginate microspheres, 200 mL of a 1.5 % (w/v) solution of alginate (Fluka, St. Louis, USA) in autoclaved, deionized water were prepared and filtrated through a filter membrane with a pore diameter of 45 µm. Log_{10} 10.0 cells of respective strain suspended in 10 ml 0.9% NaCl solution were added and alginate beads were prepared by dropping the alginate solution with compressed air through a syringe needle into a stirred 1000 mL glass beaker filled with 300 mL of 0.1 M $CaCl_2$. After 15 min, alginate beads were sieved out and washed with deionized and autoclaved water. The alginate beads were directly air-dried at room temperature under laminar flow and ground to a final particle size of less than 300 µm.

Sugar beet or pumpkin seeds were inoculated by adding the respective bacteria-loaded alginate microspheres to the coating material talcum. Seeds were wetted with 1.5% methyl cellulose solution. Talcum supplemented by the microbial inoculum (1.0% w/w) was added until the seeds were completely covered. Several field trials using a random block design were conducted at sites where the respective pathogens were whether artificially introduced or naturally present. The parameters measured quantitatively for both crops throughout the vegetation period were germination rate, disease incidence and yield.

RESULTS AND DISCUSSION

The effect of the sugar beet-specific microbial inoculant was determined by performing eight field trials between 2007 and 2011. It was demonstrated that plants from inoculated seeds showed generally a lower disease index and better growth in comparison to the untreated control. Results from single strain treatments and greenhouse experiments reveal strain-specific plant-beneficial traits (Table 1). Whereas Serratia plymuthica 3Re4-18 strongly promote seed germination and reduced symptoms of seed-borne diseases, both Pseudomonads suppressed late symptoms of Rhizoctonia solani.

Table 1: List of strains included in crop-specific microbial consortia and their effects on host plant

Crop/Strains	Beneficial effects
<u>Sugar beet</u> • *Pseudomonas poae* RE*1-1-14 • *Pseudomonas fluorescens* L13-6-12 • *Serratia plymuthica* 3Re4-18	• Suppression of seed- and soil-borne diseases • Improved germination and seedling development
<u>Styrian oil pumkin</u> • *Paenibacillus polymyxa* PB71 • *Lysobacter gummosus* L101 • *Serratia plymuthica* S13	• Suppression of seed-borne and foliar diseases • Enhanced drought tolerance • Improved germination and seedling development

Four strains with a broad-spectrum antagonistic potential against oil pumpkin pathogens were selected and applied to the seeds. Effects of the bacterial inoculants, with and without additional chemical seed treatment, on plant growth and health were evaluated during field trials in two consecutive years (2010 and 2011). The treatments showed different effects against fungal diseases: no effect on fruit rot was observed, whereas powdery mildew could be significantly suppressed by Paenibacillus polymyxa PB71 and Lysobacter gummosus L101. In addition, both strains increased harvest yield in all field trials. S. plymuthica S13 led to an increased seedling emergence rate by up to 109% in comparison to the control treatment in 2011. Furthermore, positive effects on drought tolerance and 100-corn weight were observed in respect to particular treatments (Table 1).

CONCLUSIONS

Inoculating seeds with microorganisms is a suitable method to establish beneficial communities in the plant rhizosphere and to grow crops sustainably. Successful concepts will be achieved by interrelating microbial ecology with commercial and technological demands. Accordingly, a multi-species based application is ecologically worthwhile compared to single-strain products. However, the apparent advantages of the application of microbial mixtures are faced with technological and mainly legal problems. Whereas the production of a multi-strain preparation is feasible, hurdles of the registration process appear still impeding. Technological challenges include the necessity of fermentation and, particularly, formulation protocols specifically adapted to the respective strains. Alginate hydrogels are suitable to encapsulate and stabilize most of bacterial species. Moreover, the polymer matrix provides a maximum of protection for the immobilized cells from stress conditions occurring during the seed treatment process.

In order to commercialize the microbial cocktails as biological control product applied as seed treatment, the promising bacterial inoculants presented here are continued to be evaluated under practical conditions.

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