PRODUCTION AND VIABILITY OF ENCAPSULATED BACTERIAL-FUNGAL CONSORTIA FOR DELIVERY IN SOIL

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ABSTRACT

In this study, we present a novel type of microfluidics-based formulation for the delivery of microbial control agents (mBCAs) in soil, where bacterial cells and fungal spores are directly combined into millimeter-sized alginate capsules. This process resulted in the reproducible production of 500 µm sized beads in a good throughput for this type of biofluid (up to 48 beads/min). A subsequent chitosan coating (1-2 µm-thick) provided a good storage stability at 3 months, with 100% microcapsules containing viable fungal biomass and 60-80% with viable bacterial cells depending on the species. This novel formulation was tested both in the greenhouse and on-farm with lettuces, and encapsulated microbial biomass had a more prominent effect on crop growth and yield, as compared to their delivery as a liquid suspension.

KEYWORDS: Microcapsules, Fungal Highways, Biopesticides, Droplet Generation, Jellification, Microbial Biocontrol Agents

INTRODUCTION

As an alternative to synthetic pesticides, biocontrol agents (BCAs) are powerful tools in the field of sustainable agriculture. BCAs based on microorganisms usually consist of single strains, however combining different species into a microbial consortium leads to a more efficient and robust biocontrol [1]. This is due to both functional redundancy and synergistic effects among the consortium microorganisms. In addition to this, consortia consisting of a filamentous fungus combined to bacteria also promote BCAs dispersion in the soil matrix through fungal highway dispersal [2]. Droplet microfluidics constitutes a powerful tool set that enables chemical and biological experiments to be performed at high speed and with enhanced efficiency when compared to conventional instrumentation [3]. Bio- and food-compatible gels such as alginate have been successfully used in microfluidic to produce microspheres [4]. In this study, we used microfluidics to process a sodium alginate suspension containing

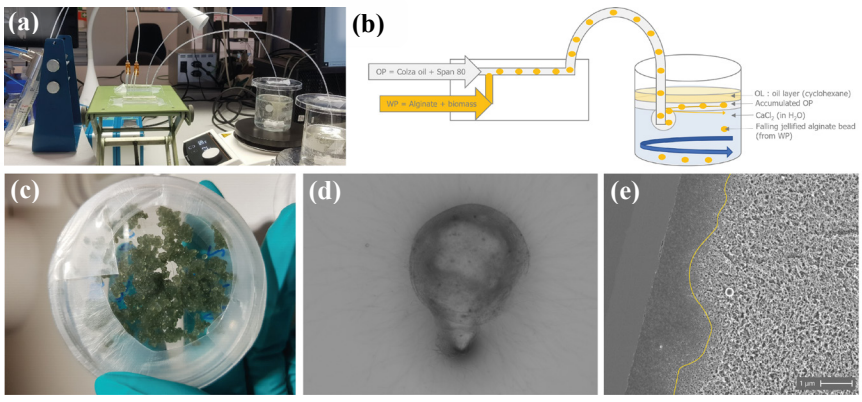

Figure 1: ***(a)*** *Picture of the experimental setup: flow control is done by a pump from Fluigent.* ***(b)*** *Schematic of the bead gelation experimental setup: The WP is introduced in the OP to individualize alginate droplets. The outlet tube ends into a Ca^{2+} reservoir where the droplets will fall in to from the jellified microbeads. We added the OL to facilitate the passage of the droplets through the interface formed by the OP. (OP: oil phase – colza oil + 0.4% Span80; WP: water phase containing biomass and sodium-alginate; OL: oil layer - cyclohexane);* ***(c-e)*** *Pictures of beads at different scales; Bead diameter: 500 µm.* ***(c)*** *Microcapsules after jellification and coating with chitosan. The microbial consortium consists in two commercially available products from Andermatt Biocontrol AG: T-gro (Trichoderma asperellum) and Amylo-X (Bacillus amyloliquefaciens).* ***(d)*** *fungal hyphae emerging from the microcapsule, note that bacteria cannot be seen due to the magnification.* ***(e)*** *Cryo-Scanning Electron Microscopy image showing the detail of the chitosan layer and the alginate matrix. The yellow line indicates the limit between alginate (right-hand side) and chitosan (left-hand side).*

fungal spores and bacterial cells to produce millimeter-sized capsules. We then evaluated microbial viability within these capsules up to 3-months shelf-life and tested their efficacy in promoting lettuce growth.

EXPERIMENTAL

We constructed a microfluidic setup to trap a bacterial-fungal consortium in sodium alginate (10^9 bacterial cells/mL and 10^7 fungal spores/mL) following the general idea reported in [4]. Our PDMS microchannels were molded on 3D printed Veroblue (Stratasys Object30prime) material. The microfluidic chip consists of a PDMS T-channel (1mm width, 1mm depth, and 32mm length) bonded on a PDMS bottom layer by oxygen plasma. Figure 1a-b summarizes the experimental setup and conditions for the generation of the alginate-biomass microbeads. These beads were then covered in a further step with a 1-2 µm-thick layer of chitosan to protect the alginate microbeads from desiccation, allowing to obtain microcapsules, as pictured in Figure 1c. The biomass viability was regularly tested by placing a few microcapsules on a growth medium (nutrient agar and malt-extract agar).

RESULTS AND DISCUSSION

Our process allowed the generation of 500 µm diameter jellified microbeads from a sodium alginate suspension containing fungal spores and bacterial cells. By running two T-channels in parallel, we achieved production rates from 20 to 48 beads/min depending on the microbial strains encapsulated. CryoSEM observations showed that the final microcapsules consisted of a low microbial cell density, thereby avoiding competition inside the capsules. The chitosan layer was about 1-2 µm in thickness, with a smooth transition between the alginate and the chitosan matrix. Our results showed that fungal spores already started germinating within the alginate matrix but did not break the chitosan layer before being delivered in a growth-promoting environment. Upon inoculation in a favorable medium, fungal hyphae emerged out of the beads within 10 hours and bacteria effectively colonized the medium using fungal hyphae to disperse (fungal highways). In terms of viability, after 3 months, 100% of the microcapsules contained active fungal biomass capable of colonization. On the other hand, the viability of bacteria was lower, with 60-80% viability depending on the bacterial strain that was encapsulated. Thus, one aspect to investigate in the future will be to understand how to improve the viability of bacteria during the storage of microcapsules. As a proof-of-concept, we carried out experiments in the greenhouse and on-farm with lettuces. Preliminary results show that our encapsulated microbial consortia effectively colonized lettuce roots and had a positive effect on plant growth that is slightly higher than BCAs formulation already available on the market.

CONCLUSION

In this study, we successfully encapsulated microbial consortia through a microfluidics process. Further steps are required to optimize this new type of formulation: 1) improve capsule production throughput; 2) increase bacterial viability; and 3) carry out further field trials in different climatic contexts.

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