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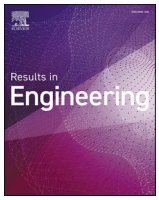

Freeze-drying of *Beauveria bassiana* suspended in Hydroxyethyl cellulose based hydrogel as possible method for storage: Evaluation of survival, growth and stability of conidial concentration before and after processing

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ABSTRACT

Beauveria bassiana (*Bb*) is an entomopathogenic fungus considered as a valid alternative to chemical pesticides. However, Bb use is still limited due to short storage period and persistence in field conditions. Storage can be extended by reducing water content in products but an excessive drying can cause damage during rehydration. Persistence is promoted by using conidia into pellets or granules formulation. Integration of these two aspects can be challenging and costly. In this work, we present how utilizing a hydrogel containing Bb as growth substrate could potentially overcome both issues.

1. Introduction

Beauveria bassiana (Agostino Bassi's 1835, *Bb*) is an entomopathogenic fungus. Bb's spores (conidia) are actually employed as bio-insecticide against a large number of pest in dry or liquid (aqueous or oily) formulations or after encapsulation in granules, pellets etc. [1]. Conidial formulations can be a valid alternative to chemical pesticides because of their comparable lethality to chemical insecticides, lower toxicity (to human and environment) and lower risk of resistance phenomena in insects [2,3]. Nevertheless, Bb use is still limited due to storage period and limited survival after field dispersion. Conidia do not reach the minimum storage period (two years in WHO/FAO regulations) without loss in efficacy unless drug-like very expensive storage conditions are used (<5 °C and controlled humidity). Even trace of moisture in the conidia result in foreshortened shelf life. Nevertheless, conidia can be dehydrated to a moisture content in a range of 5–9%w/w because extreme desiccation can lead to a significant loss of vitality at rehydration. On the other hand, conidia lethality and persistence (lethality in time) is strongly influenced by environmental conditions (desiccation, UV light etc.) then pellets or granules can improve insecticidal effect by protecting spores from environment as in seed coating [4]. Methods usually employed in other fields such as biomedical engineering (e.g. freeze-drying or other scaffolding and/or cell encapsulation technique [5–12]) can be exploited to protect spores and conidia. Usually, Bb based productive steps involve conidia mass production through solid substrate fermentation (e.g. on rice), drying and separation from substrate, reprocessing to produce commercial forms and final dehydration for storage. In the present work, a Bb/Gel system was produced by adding a gelling biopolymer directly to conidial suspension. In this study, we evaluated fungal vitality after rehydration of freeze-dried samples and the stability of conidial concentration during storage as dried system.

2. Material and methods

2.1. Conidial suspension preparation

Beauveria bassiana, *Bb* (CD1123) was obtained and cultured as described in Cafarchia et al. [12], then maintained on Potato Dextrose Agar (PDA, Liofilchem) at 4 °C. A *Bb* conidial suspensions (CS) was obtained by culturing 15 strains on PDA for 3 weeks at 25 ± 1 °C and adding them to a sterile standard liquid growth media (1% pepton water, 2% in weight of chitin). The amount of conidia in CS at starting time t_0 was evaluated by quantitative plate counts of colony forming unit/ml (CFU/ml) on PDA plates.

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Table 1
Bb/Gel system produced.

Hydrated Bb/Gel		Freeze dried-rehydrated Bb/Gel		Freeze dried- stored Bb/Gel	
HEC16	HEC30	HEC16-Lio	HEC30-Lio	HEC16-Lio-Stored	HEC30-Lio-Stored

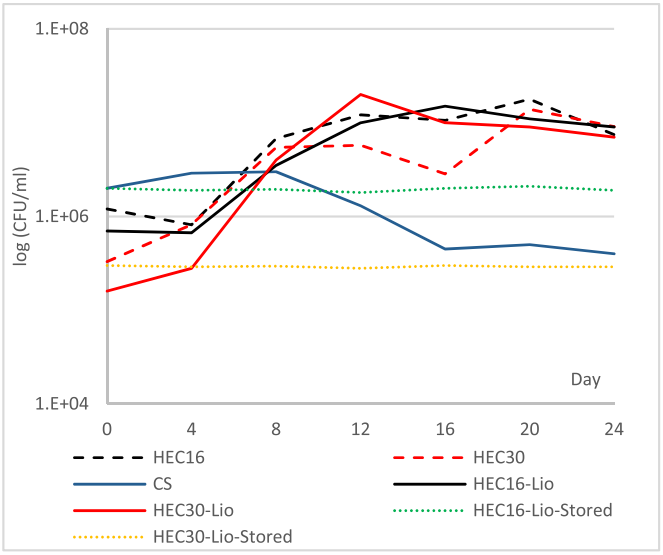

Fig. 1. Curves of growth of Bb before (HEC16 and HEC30) and after freeze-drying (HEC16-Lio and HEC30-Lio-dotted lines) and Conidial level of stored freeze-dried samples (points line).

2.2. Preparation and freeze drying of Beauveria bassiana/Gel system

Beauveria bassiana/Gel systems (Bb/Gel system) were prepared by adding 2-Hydroxyethyl cellulose sterile powder (HEC, Aldrich Chemistry, average Mw 720000) to CS and stirring it at room temperature for 30 min. Two different Bb/Gel systems containing 16% and 30% in weight of HEC (HEC16 and HEC30) were arranged. Part of HEC16 and HEC30 was separately collected, frozen at −15° and then freeze-dried for 24h. A part of freeze-dried system was immediately rehydrated to restore initial HEC concentration and tested in growth assay (HEC16-Lio and HEC30-Lio). The remaining part was stored as dried samples in a closed plastic container to evaluate conidial concentration stability (HEC16-Lio-Stored, HEC30-Lio-Stored). Conidial concentration was evaluated at t_0 through PDA plate count in each sample. Samples produced are reported in Table 1.

2.3. Beauveria bassiana growth evaluation and conidial concentration stability

All the samples in Table 1 were kept at 25 ± 1 °C and 90% RH inside a dark Binder Climatic Chamber. *Beauveria bassiana* growth rate and conidial concentration stability were evaluated every 4 days for 24 days. Growth and conidial stability were evaluated respectively on hydrated HEC16 and HEC30 and rehydrated HEC16-Lio and HEC30-Lio. Growth and conidial concentration were assessed through quantitative plate count on PDA plates (CFU/ml). CS was used as positive control. Results are expressed as Log_{10} of CFU/ml and reported in Fig. 1.

2.4. Experimental results

From the assay emerged (Fig. 1) as HEC was not toxic to Bb and promoted its growth (in terms of growth rate) and survival (extension of growth period) compared to control. These effects could be related both to the presence of a "solid" substrate (already effective in solid fermentation method) and to the ability of the gel to provide a growth sutable microenvironment (presence of water and nutrients). Furthermore, it emerged no difference in growth curves before and after the exposure to freeze-drying process and to successively rehydration (e.g. comparison between HEC16 and HEC16-Lio). Additionally, no loss of conidial concentration emerged from freeze-dried samples monitored during storage (constant conidial level, e.g. HEC16-Lio-Stored). Further experiments will explore lower HEC concentrations to evaluate the change in growth and protection during the freeze-drying.

3. Conclusions

This research, even though in early stages, positively assessed the survival of Bb in a system (hydrogel) that can be used as growth substrate. The system can be directly freeze-dried (without conidia separation) then stored for long period (due to the absence of water and the protective action of the gel matrix during rehydration) and grinded or pelleted to obtain an easily rehydratable commercial product in a ready to use form, reducing the number of passages usually needed and then costs.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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