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Research paper

Encapsulation and characterization of slow-release microbial fertilizer from the composites of bentonite and alginate

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ABSTRACT

Raoultella planticola Rs-2 was innovatively encapsulated with sodium bentonite and alginate (NaAlg) composites to develop efficient slow-release biofertilizer formulations and minimize production costs. These microcapsules were spherical in shape and their encapsulation efficiency was nearly 100%. Approximately 88.9% of Rs-2 in dried microcapsules of bentonite–NaAlg survived after 6 months of storage. The NaAlg amount required to produce desirable bentonite-blended Rs-2 microcapsules was significantly lower than that of single NaAlg. Swelling, biodegradability, and release rate increased with increasing NaAlg content and decreased with increasing bentonite content. All release curves of bacteria from the bentonite–NaAlg microcapsules presented an initial burst followed by a gradual increase manner, which mainly implied the release behavior followed a first-order release model. Thus, bentonite–NaAlg composites could be ideal low-cost encapsulated wall materials for slow-release bacterial fertilizers in farmlands.

1. Introduction

Probiotics are considered as being living micro-organisms that, when administered in adequate amounts, can confer a beneficial physiological effect on the host (Rokka and Rantamaki, 2010). Plant growth-promoting bacteria (PGPB) belong to a group of plant probiotics that help maintain soil fertility and plant establishment; therefore, these bacteria serve important functions in sustainable agriculture. In previous screening studies, the indigenous plant growth-promoting rhizobacterial strain *R. planticola* Rs-2 was isolated from saline soil in a cotton field in Xinjiang Province, China (Wu et al., 2012a). The strain can increase the germination rate of cotton seeds and promote the growth of cotton seedlings under salt stress conditions. However, free PGPB cells that are directly inoculated into the soil encounter difficulties in colonizing and surviving around plant roots because these cells are susceptible to soil competitors and environmental stresses (Takei et al., 2008; Covarrubias et al., 2012).

Encapsulated microorganisms are slow-release biofertilizers (Young et al., 2006) that are prepared by coating PGPB with alginate to ensure a slow release of nutrients to the soil by pore-assisted diffusion or erosion and degradation of capsules (Flores et al., 2013). In addition, encapsulated bacteria possess an extended shelf life and can even be stored at room temperatures for relatively long periods (Takei et al., 2008; Schoebitz et al., 2013). Microencapsulation reduces the risk of bacterial transport in water and soil as well as minimizes the spread of hazardous residues in various environments (Wu et al., 2011; Bhattacharyya and Jha, 2012). The process yields a uniform cell distribution close to the targeted site, even on small seeds enhancing the application efficacy (John et al., 2011). Encapsulation methods vary with the nature of applications from diverse fields for miscellaneous objectives. Some methods include emulsion, spray drying, solvent extraction/evaporation, coacervation, ionic gelation, and extrusion (John et al., 2011). Extrusion is a conventional and simple process of creating capsules from hydrocolloids (Krasaekoopt et al., 2003). One advantage of the method is its excellent stability because carbohydrate matrices in glass states possess barrier properties. Consequently, extrusion turns out to be a convenient process for encapsulation and diffusion of cells.

Microcapsule properties mainly depend on the type of polymeric material. Sodium alginate is a biodegradable polymer that is generally regarded as a safe substance because of its nontoxic properties, simplicity of handling, gel-enhancing properties, and biomedical applications (Schoebitz et al., 2012, 2013). Van Elsas et al. (1992) found that encapsulated cells in various bead types could colonize the wheat rhizoplane at high population levels 1 week after inoculation into the soil. Therefore, it is clear that alginate-mediated establishment of inoculants can improve inoculants' effectiveness. Rekha et al. (2007) demonstrated that inoculation of encapsulated bacterial isolates promoted plant growth similar to their respective free cells. However, the high cost of alginate limits its applications; in addition alginate matrices do not possess a high mechanical strength and are easily destroyed in the presence of monovalent cations in field applications. The presence of macrospores in the alginate matrix results in rapid diffusion and release of compounds (Cordoba et al., 2013). Addition of clays in gel-forming alginate polymers produces an excellent matrix to control the release of compounds (Benli, 2013). Bentonite is a kind of sedimentary rock

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that is rich in smectite, which has some excellent properties such as good water absorption, swelling, and drug-carrying capability because of their unique crystal structure (Gunister et al., 2007; Wu et al., 2008). Adding low-cost bentonite to sodium alginates (NaAlg) increases the solid content and solves the drawbacks (e.g., low porosity) of single alginate beads. It also improves the mechanical strength because the clay increases viscosity and improves stability.

Furthermore, adding bentonite as a modifying agent in alginates increases the efficiency of herbicide encapsulation and controls the release profiles of active ingredients (Flores et al., 2013). Alginates and bentonite have been widely utilized to synthesize controlled-release drugs (Liew et al., 2006; Kevadiya et al., 2011) and fertilizers (Xie et al., 2012). For example the use of bentonite as a filler agent in NaAlg regulated the release rate of atrazine and diuron (Flores et al., 2013). Bentonite helped in controlling the release of thiram from bentonite–starch–alginate-based formulation in supplying nutrients to plants. The slower release with increasing clay contents is due to the lower swelling behavior of bentonite (Singh et al., 2009). The less swollen microcapsules resulted in smaller release channels, which slowed down the release. Thus, this study investigates the feasibility of using bentonite and NaAlg composites as alternatives for the microencapsulation of controlled-release biofertilizers (Fig. 1). The aim of this work is to certify that these encapsulated microbial fertilizers could be a novel and feasible technique for agricultural application.

Single bentonite, NaAlg composites, and bentonite–NaAlg (B-NaAlg) beads were analyzed by Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) to determine the interactions between bentonite and NaAlg. The particle size, swelling properties, entrapment efficiency, and biodegradability of the microcapsules, as well as the release kinetics and survival of bacteria, were investigated to evaluate the feasibility of using bentonite and alginate composites as efficient slow-release formulations for Rs-2 and to provide a theoretical foundation for the potential use of encapsulated PGPB in agriculture.

2. Materials and methods

2.1. Bacterial strains and culture medium

The strain Rs-2 used was previously isolated from a saline soil in the Xinjiang province of China (Wu et al., 2012b). Prior to immobilization, free Rs-2 cells were propagated in nutrient agar liquid medium consisting 10 g/l of tryptone (supplied by Beijing AOBOX Biotechnology Co. Ltd), 5 g/l of beef extract (supplied by Beijing AOBOX Biotechnology Co., Ltd), and 5 g/l of NaCl (supplied by Tianjin Hengxing Chemical Reagent Co. Ltd) while shaking at 200 rpm at 30 °C for 48 h (Wu et al., 2012a). At its early stationary phase the cells were harvested and determined as 3.1×10^{13} cfu/ml of concentration in the broth by counting the colony-forming units (cfu) on nutrient agar (NA) agar plates after overnight incubation at 30 °C.

2.2. Preparation of the microcapsules

The raw bentonite samples were collected from XiaZiJie deposits in Xinjiang Uyghur Autonomous Region of China. The bentonite was purified by soaking dried grounded raw bentonite 1000 g in 5000 ml of water for 24 h; then, the bentonite suspension was stirred for 40 min to disperse montmorillonite in water and was allowed to stand for a period of 4 h. Finally, the bentonite suspension was centrifuged at 6000 rpm for removing impurity minerals then dried at 85 °C. The bentonite used was purified using the method of Sun et al. (2007). The resultant bentonite has a composition (%, by mass) of $A1_2O_3$ 13.06, SiO_2 64.62, Na_2O 2.66, K_2O 2.43, CaO 1.92, MgO 2.38, Fe_2O_3 4.93, TiO_2 0.59, MnO 0.26, and P_2O_5 0.18, and an ignition loss of 6.20. The montmorillonite content was 93.0 g based on 100 g bentonite, the cation exchange capacity was 98.4 mmol based on 100 g bentonite and the swelling index was 89.5 ml g^{-1}. Solutions of bentonite–NaAlg (B–NaAlg) with various concentrations were prepared in triplicate (Table 1), and the composite solutions were mixed evenly with Rs-2 broth at a 2:1 proportional ratio (60 ml/30 ml). These mixtures containing Rs-2 cells were drop-wise added with an injection needle into the $CaCl_2$ (2%) crosslinking agent solution (150 ml). After the bead-forming reaction for about 2 h, the microcapsules obtained were cleaned 2 or 3 times with sterile water. The excess water of the wet beads was wiped away with tissue paper and its quantity was measured immediately.

2.3. Drying of the microcapsules

All the wet microcapsules were collected and dried in Intelligent Ovens (Equipment Co., Ltd. Shanghai Jing Mai) at 40 °C until constant weight was obtained. Then the dried microcapsules containing Rs-2 obtained were placed in a sterile EP tube and sealed for further experimentation.

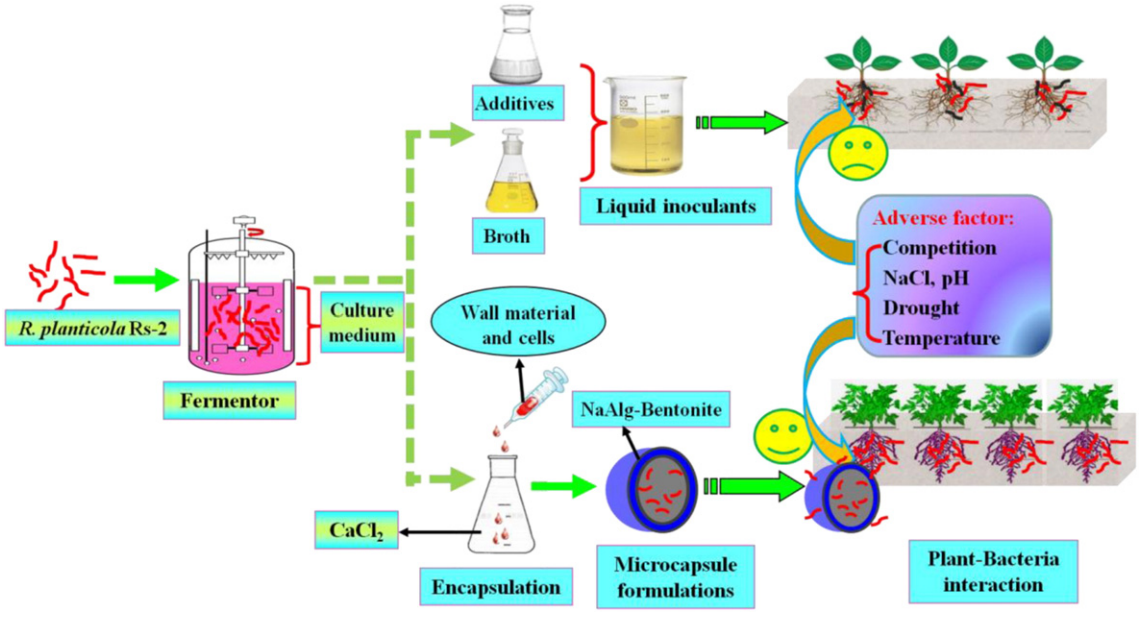

Fig. 1. The comparison of free and encapsulation bacterial cells in the application of plant–bacteria interaction.