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Viability evaluation of alginate-encapsulated *Pseudomonas putida* Rs-198 under simulated salt-stress conditions and its effect on cotton growth

Yanhui He [a], Zhansheng Wu [a, *], Bang-Ce Ye [a], Jun Wang [b], Xinyuan Guan [b], Jinghao Zhang [a]

[a] School of Chemistry and Chemical Engineering, Shihezi University, Shihezi, 832003, PR China
[b] Agricultural Techniques Extension Center, Xinjiang Agricultural Reclamation Academy of Sciences, Shihezi, 832000, PR China

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ABSTRACT

This study was analyzed the survival and colonization efficiency of encapsulated *Pseudomonas putida* Rs-198 prepared with alginate sodium, bentonite, and starch on cotton root under saline conditions. The FTIR and XRD findings in this study showed that absence of chemical reactions and good mixing performance were detected with alginate, starch, and bentonite. The survival rates of *P. putida* Rs-198 were 81.07%, 89.67% in free and encapsulated bacteria, respectively, after 50 days of storage. The amount of colonization by encapsulated *P. putida* Rs-198 was less than that of free *P. putida* Rs-198 at days 7–21 and was significantly higher than that of free *P. putida* Rs-198 after day 35. This level was sustained for up to day 63. At day 49, the population size of the encapsulated *P. putida* Rs-198 significantly increased by 2.48% and 2.44% compared with free *P. putida* Rs-198 at day 49 under 0% and 2% salt stress, respectively. The cotton biomass was significantly increased by the encapsulated *P. putida* Rs-198 strain under salt stress. This finding may be attributed to the increase in the number of bacteria and the high level of indole-3-acetic acid and gibberellin production. Thus, microcapsule bio-inoculants are potential alternatives for sustainable agriculture.

1. Introduction

Saline soils and saline irrigation constitute a serious problem in agriculture because they suppress plant growth and yield worldwide. Various studies have documented the increased of health and productivity of different plant species by applying plant growth-promoting rhizobacteria (PGPR) under both normal and salt stressed conditions [1,2]. Inoculation with PGPR, which can establish relationships with plants to assist in the uptake of mineral nutrients and increase tolerance to environmental salt stresses directly, is a sustainable alternative for agro-ecosystems [3,4]. This microorganism may supply additional phytohormones to plants, stimulate root growth, and reverse the growth-inhibiting effect of salt stress to a certain extent in both shoots and roots (Fig. 1). Indole-3-acetic acid (IAA) and gibberellins (GA) are phytohormones from bacteria that play key role in plant growth promotion [5]. IAA is considered the most abundant naturally occurring auxin produced by bacteria, which play an important role in the symbiotic relationship between bacteria and plants [6]. GAs are a large group of phytohormones that are important for developmental processes of plant growth, including seed germination, stem elongation, flowering, and fruit setting [7]. In our previous studies, the *Pseudomonas putida* Rs-198 isolated from alkaline soil in Xinjiang was determined that has the capacity to produce IAA and GAs, and improve the resistance of salt-stressed cotton [8].

Several reports have demonstrated that the beneficial activities of PGPRs are directly related to the ability to colonize on roots [7,9]. However, regardless of the mechanism of plant growth promotion, to be effective in the rhizosphere, the candidate strains need to be able to establish and maintain a sufficient population in the host plant for a longer time period [10]. Microencapsulation is a new and efficient technique to release cells into the target soil in a slow and to increase the survival rates of cells in soil after inoculation [4,11].

Abbreviation: CaAlg-B-S, CaAlg-bentonite-starch; SEM, scanning electron microscopy; FTIR, Fourier transform infrared; XRD, X-ray diffraction; IAA, indole-3-acetic acid; GA, gibberellins; PGPR, plant growth-promoting rhizobacteria; NA, nutrient agar; LSD, least significant difference.

* Corresponding author.
E-mail address: wuzhans@126.com (Z. Wu).

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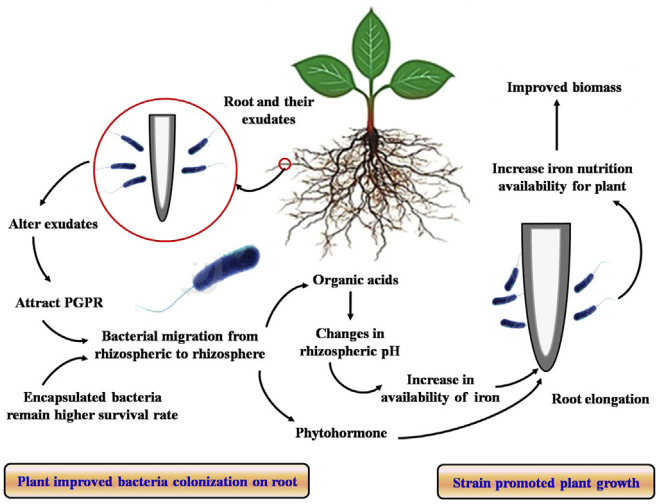

Fig. 1. The interactions of plants and *P. putida* Rs-198.

The encapsulation of rhizobacteria can enhance cell survival during storage, and microencapsulated cells could be released into the target medium in a slow and controllable manner, which increase long-term effectiveness [12]. Furthermore, after long period of storage, rhizobacteria do not lose their ability to stimulate plant growth [13]. A suitable formulation, encapsulating PGPR with alginate (NaAlg), low-cost starch (S), and bentonite (B), to mediate salt stress in cotton plants will be important and effective in sustainable agriculture. The process of root colonization is significantly influenced by various parameters such as bacterial traits, root exudates, and several other biotic or abiotic factors [14]. Plants also can attract PGPRs to their rhizosphere or even root surfaces by secreting root exudates that can influence motility in some PGPRs [7,10,15]. The improved plant growth and yield by bacterial species have been reported by several authors [16,17]. However, there are few studies available on the biological interactions of the opportunistic bacterium associated with plant roots, and their adaptation and survival under hostile environmental conditions. In this study, we investigated the GA and IAA production capabilities of encapsulated *P. putida* Rs-198 under salinity stress, as well as their colonization amount in the rhizosphere of cotton and the influences of encapsulated *P. putida* Rs-198 on cotton seedling growth. Immobilized forms of bacteria in sodium alginate (NaAlg), bentonite (B), and starch (S) have the potential to be developed as bio-inoculation methods for maintaining high crop productivity.

2. Materials and methods

2.1. Materials

Common cottonseeds were used for the pot experiments. Strain *P. putida* Rs-198 was previously isolated from saline soil in Xinjiang, China [8]. *P. putida* Rs-198 was cultured in nutrient agar (NA) liquid medium with shaking at 200 rpm and at 30 °C for 48 h. The cell concentration in the broth was determined to be 10^{13} colony forming unit (cfu)/ml using a serial dilution for counting colony-forming units on NA agar plates after an overnight incubation at 30 °C.

2.2. Methods

2.2.1. Preparation of microcapsules

The encapsulation of *P. putida* Rs-198 cells with the composites of NaAlg, starch, and bentonite (purified using the method of Sun et al. [18]) was carried out by adopting the method described by Wu et al. [19,20]. Solutions having different compositions were prepared using NaAlg (1.5%), bentonite (4%), and starch (3%) contents, and the composite solutions were mixed evenly with *P. putida* Rs-198 broth at a 2:1 ratio. The mixtures containing *P. putida* Rs-198 cells were added drop-wise with an injection needle (needle size 0.9 mm) into the $CaCl_2$ (2%) crosslinking solution (150 ml). After the bead-formation reaction, which took about 2 h, the microcapsules obtained were cleaned with sterile water two or three times. All of the wet microcapsules were collected and dried in Intelligent ovens at 40 °C until a constant weight obtained. The dried microcapsules containing *P. putida* Rs-198 were placed in sterile Eppendorf tubes and sealed for further experimentation.

2.2.2. SEM

CaAlg-B-S microcapsules and root tissue were sputtered with gold to form a uniform coating with a thickness of 10 μm and for conduction. The microcapsules were placed on a copper stub. SEM (JSM-6700F; JEOL, Tokyo, Japan) was performed to scan the samples.

2.2.3. FTIR

Pure NaAlg, bentonite, starch and CaAlg-B-S microcapsules samples were ground and mixed well with potassium bromide powder to form pellets under a hydraulic pressure of 400–450 kg/cm^2. All of the samples were scanned to confirm the incorporation of NaAlg with bentonite and starch using Nicolet Avatar 360 FTIR spectrophotometer in the wavelength range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and a number of scans at 50 cm^{-1}.

2.2.4. XRD

A D8 Advance (Bruker) X-ray diffractometer was used to examine the solid-state morphology of the pure NaAlg, bentonite, starch and CaAlg-B-S microcapsules. X-rays of 1.5406 Å wavelengths were generated by a Cu Kα source. The equipment was operated at a slow scan of 0.5 s per step, and a scan rate of 0.3°/s. The angle of diffraction (2θ) varied from 15° to 65° to identify any changes in the crystalline domains.

2.2.5. Survival of bacterial strains in the microcapsules

Dried microcapsules (0.2 g) were obtained and immersed in 5 ml of sterile phosphate buffer (pH 7.0) at 30 °C for 1 h. The microcapsules were then ground into the solution, resulting in the release of bacteria entrapped or covered in the capsules. All of the viable bacteria were counted for colony counting after serial dilution.

$$\text{Survival rate } (\%) = N_u/N_0 \times 100$$

where N_0 represents the initial colony-forming unit of *P. putida* Rs-198 per gram microcapsules. N_u represents the viable bacteria amount of *P. putida* Rs-198 per gram after 50 days of storage at 4 °C.

2.3. Pot experiment

Cotton seeds were sterilized by immersion in 70% ethanol for 5 min and subsequently in 0.1% $HgCl_2$ for 10 min, washed several times with sterile water, and allowed to natural drying. The seeds were then placed in plastic bottle (15 cm diameter; 40 cm depth) that contained 500 g of sterilized vermiculite (diameter 0.5 mm). To assess the potential of the *P. putida* Rs-198 applications for cotton, experiments were conducted using either free or encapsulated *P. putida* Rs-198 microcapsules, with or without salt stress. The treatments were as follows: F-0 with 10 ml free *P. putida* Rs-198 and