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알긴산 폴리머 하이드로젤내의 세균의 인캡슐레이션 및 생존률에 관한 연구

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Encapsulation of Serratia plymuthica in alginate hydrogel microspheres and structural factors on its high survival rate

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실험목적

Hydrogel materials are widely used in the fields of biomedical remediation, optical lenses, sanitation, construction industries, bacterial or enzyme immobilization, etc. Recently biodegradable hydrogel materials has been beeing applied for increasing the productivity of crop plants by minimizing any probable damages from droughness. So, we tried to encapsulate the two agriculturally useful bacteria *Serratia plymuthica* A21-4 and *Pseudomonas fluorescens* B16, known to kill plant pathogens as well as to serve as elicitor, into alginate based gel beads.

재료 및 방법

We developed the technique based on the understanding of intrinsic material properties of natural polymers. The resulting technique employed novel environmentally friendly physical crosslinking of natural polymers instead of chemical crosslinking process. Therefore, we selected alginate as the base material because it has the saccharide unit of guluronic acid and mannuronic acid which has a carboxyl group on the unit. Thus Ca2+ can physically link carboxyl groups on the different polymer chains. The physical crosslink is not so strong enough and thus the process needed an additional stabilization process. We employed layer-by-layer process using polycation for the stabilization of bacteria loaded alginate gels. Historically, expensive poly-L-lysine has been being used as a stabilizer in biomedical hydrogel science. In this study we found that chitosan, much cheaper than poly-L-lysine, can successfully substitute for the stabilizer. *Serratia plymuthica* A21-4 loaded alginate gels (two different sizes of beads 3 ~ 5 ㎜ beads and several hundreds micron beads were successfully developed) exhibited high viability of the loaded bacterium up to 3 months after freeze-drying, comparable to the viability of the control zero-time wet cells. We believe that the unexpected long-term high viability of freeze-dried cells could be ascribed to the very high content (almost twice the volume of a dried alginate bead) of "bound water" in alginate hydrogels.

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결과 및 고찰 (Results and Discussion)

1. Empty 3 to 5 ㎜ in dia. alginate shell beads were prepared by alginate-Ca2+ crosslinking/polycation layering/silica coating, utilized for encapsulating *Serratia plymuthica* A21-4 and *Pseudomonas fluorescens* B16. Especially, alginate gel was stabilized successfully using cheaper chitosan instead of very expensive poly-L-lysine having been used.
2. Encapsulated 3 ~ 5㎜ alginate beads loaded with *S. plymuthica* A21-4 was round forms on the whole and their interiors was highly porous and the pores was connected each other. Excellent loadability of alginate beads against *S. plymuthica* A21-4 could be attributed to this layered porous structure.
3. Chitosan-based *S. plymuthica* A21-4 loaded alginate gel beads were lyophilized and kept at room temperature for 3 months. And after this period, the viable cells was counted as 1.1x107 CFU/ml, proving that the alginate gel matrix offered excellent habitat for survival during lyophilization and storage.
4. The state of water molecules in alginate gel beads was analyzed by differential scanning calorimetry. The content of "bound water" was approximately one and a half times of the bead volume. This bound water is believed to play an important role in the survival of *S. plymuthica* A21-4 during freeze-drying process.