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INFLUENCE OF SPRAY DRYING TECHNIQUE ON SURVIVAL OF *BRADYRHIZOBIUM* ONTO SODIUM ALGINATE BASED CARRIERS

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Abstract

Application of microbial inoculants to the soil can improve the nutrient uptake by plants and increase the productivity of the crops. Inoculants should ensure a suitable microenvironment and long survival of bacteria in biological fertilizer and after application to seeds. The immobilization of rhizobia onto a carrier can involve the covering and protecting of the microorganisms. Spray drying is a relatively new immobilization technique for the immobilization of microorganisms onto a carrier and it is rarely used for rhizobia. The survival of bacteria during spray drying immobilization onto alginate based carriers was just 8.1 and 6.8% for *Bradyrhizobium* spp. strains 542 and 526. The working temperature during drying process was high (outlet 50°C) and it presents more than temperature limit for rhizobia survival. Accordingly, the obtained results were justifiably low in value. The presence of sucrose in liquid inoculant had a positive influence on the survival of rhizobia during immobilization.

***Key words*:** *Bradyrhizobium* spp., sodium alginate, immobilization, spray dryer, rhizobia survival.

INTRODUCTION

Bradyrhizobium is a soil bacterium that can establish nodules on the root of the legumes, and fixes atmospheric nitrogen (N_2) in symbiosis with the host plant. The inoculation of the legume seed is an efficient and simple way of introducing effective rhizobia to the rhizosphere of the legumes to improve the productivity of the crop. Selected rhizobial strains, which are used as plant growth

promoting biofertilizers, are chosen according to their competitiveness in relation to native rhizobia in soil. All of this is influenced the most by the number of viable rhizobia available for the infection of the legume roots. Presumably, a higher numbers of viable rhizobia per inoculated seed can be accomplished by improving survival during seed inoculation (Alvarez, 2010)

According to this fact, one of the main problems of the biofertilizer industry is how to keep rhizobia viable in large numbers in the inoculants and on pre-inoculated seeds during storage period. Today, the world market offers commercial rhizobia inoculants in solid, liquid or freeze-dried form. However, the survival of the plant growth promoting rhizobia is variable on different carriers in formulation and the carrier has to provide delivery of live bacteria over a long period of storage. Also, it should have several good qualities such as good water holding and aeration, as well as simplicity of handling, sterilizing and production, should be non-toxic, cost-effective, and environmentally friendly. However, the main role of the inoculants formulation is to ensure a suitable microenvironment for rhizobia, and sufficiently long survival of bacteria in biofertilizer and after its application to seeds (Žvagiņa, 2015).

Peat-based inoculants with high numbers of rhizobia are the most common biofertilizers which are used by farmers. These kinds of biofertilizers are preferred because of their advantageous characteristics such as incitement of rhizobia multiplication, high moisture-holding capacity, and to protect rhizobia once they are applied to the seeds. However, the problems with the use of peat, including high sterilization costs and difficulty in large-scale application on the field, as well as inaccessible dumpsites of true peat in certain areas. This led to the development and application of the alternative materials in order to replace peat as a carrier for rhizobia. In the literature there are available researches which were done using materials such as chitosan (Namasivayam, 2014), alginate (Young, 2006), agar, coal, bentonite, vermiculite and perlite (Temprano, 2002). In addition, the advantage in application should be given to carriers which are biodegradable in the soil.

In the present study, the possibility of the technical feasibility of applying a spray dryer for the immobilization of the *Bradyrhizobium* spp. strains onto sodium-alginate based carrier was investigated. The spray dryer technique was used in order to convert liquid inoculants into solids for their further potential application as commercial inoculants in powder formulation.

MATERIAL AND METHODS

Bacteria cultures

Working cultures of rhizobia were prepared by using of two strains of *Bradyrhizobium* spp., 542 and 526. These are two of several nitrogen fixing effective strains which are selected from the Collection of the Institute of Soil Science (ISS WDCM375-Collection of Bacteria, Institute of Soil Science, Department of Microbiology). The bacterial strains were grown in Erlenmeyer flasks containing yeast mannitol broth in a rotary shaker (150 rpm) at 28°C for 5 days (Vincen, 1970).

Preparation of inoculants

Preparation of inoculants was performed in Erlenmeyer flasks containing 6% peat extract and 0 or 1% sucrose. Bacterial cultures (542 and 526) were added in ratio 1:10 (w:w) in different inoculants and incubated in a rotary shaker (150 rpm) at 28°C for 5 (for strain 542) and 7 (for strain 526) days. The number of bacterial cells after incubation has been determined by colony-plating of dilutions.

Immobilization of rhizobia

After incubation, the samples were mixed with sodium alginate solutions of concentrations of 20 g/L and with a solution which contains 15 g/L of sodium alginate and 5 g/L of starch in ratio 1:1 (w:w). These obtained liquid mixtures were placed at room temperature on a rotary shaker (150 rpm) for one hour because of homogenization of mixture. The immobilization was continued by converting the liquid inoculant to solid form using a spray drying technique by Mini Spray Dryer (Büchi Labortechnik AG, Flawil, Switzerland). The instrument was set at an inlet temperature of 120°C and at an outlet temperature of 50°C. Between the drying of the samples and their translation into powder, the whole system was washed with sterile distilled water. The number of bacterial cells after immobilization also has been determined.

RESULTS AND DISCUSSIONS

Rhizobial bacteria are usually grown in the yeast mannitol broth. However, in this study the growth of *Bradyrhizobium* spp. strains in the peat extract medium with and without adding sucrose, was investigated. The usual number of rhizobia which are grown in yeast mannitol broth are reaching approximately 10^9 cell/mL (Vincen, 1970). The growth of rhizobia in the 6% peat extract, which was prepared in water, was higher than growth in medium with peat extract and 1% sucrose (Table

1). The 542 strain had about 5 times more bacteria in the peat extract than in medium with sucrose, while the difference in the number of the 526 strain was smaller and it was 2.5 times higher in peat extract. Comparison of growth with applied growing medium, in approximately, the bacterial number in the peat extract was similar for both used strains, and it was 1.28 x 10^8 and 1.37 x 10^8 cell/mL for strains 542 and 526, respectively. On the other hand, the presence of sucrose in the growing medium did not stimulate to the growth of rhizobia. The number of the 526 strain was two times higher than the 542 strain and it was about 5.60 x 10^7 cell/mL. Therefore, the addition of sucrose did not prove to be a good supplement in the growing media, but its purpose was potential protection of bacteria cells during the drying process. In any case, the peat extract growing medium can be consider acceptable for the growth of soybean strains 542 and 526, even if their success was 10 times less compared to the typical yeast mannitol broth.

A microencapsulation technique by using the spray dryer is useful when an active material is dissolved or suspended in polymer solution. During the drying process, active material of interest becomes trapped in the dried particles (Bansode, 2010). The main reason why in this research spray dryer was chosen is due to its ability to handle labile materials. Its advantage is the short contact time in the spray dryer, and also can be adjusted to a temperature below 85-90°C, even if the temperature in the drying chamber is much greater. The operation is economical due to the lower operation temperature and storage and transport product cost reduction. In addition, the liquid which are a solution, an emulsion or a suspension can be dried (viscosity up to 300 mPa.s) (Bansode, 2010; Estevinho, 2013).

The results for obtained dry matter of powder from liquids during the drying process were different, but they still showed some orderliness. The achieved dry matter of the powder, when the sodium alginate was used as carrier for rhizobia, was between 0.64 and 0.85 g/100 mL (Table 1). These values are similar to each other, like when the sodium alginate and starch were used as encapsulating agent, and values ranged between 0.68 and 0.95 g/100 mL (Table 2). However, if that result is calculated based on used start dry matter values, which are 1 g/100 mL (with 0% of sucrose) and 1.5 g/100 mL (with 1% of sucrose), the maximum yield of dry matter during drying process was 73% and 63%, respectively.

Table 1. Dry matter content and bacterial survival using of sodium alginate as carrier for the rhizobia immobilization

Strain*	**Liquid inoculant content**			
	Peat extract + sodium alginate		*Peat extract + sucrose + sodium alginate*	
	Dry matter (g/100 mL)	CFU**/g of carrier	Dry matter (g/100 mL)	CFU/g of carrier
542	0.73	2.50×10^6	0.85	9.14×10^7
526	0.64	1.81×10^8	0.81	2.35×10^8

**Bradyrhizobium* spp. strain
**CFU- colony forming unit

Table 2. Dry matter content and bacterial survival using mixture of sodium alginate and starch as carrier for the rhizobia immobilization

Strain*	**Liquid inoculant content**			
	*Peat extract + MIX****		*Peat extract + sucrose + MIX****	
	Dry matter (g/100 mL)	CFU**/g of carrier	Dry matter (g/100 mL)	CFU/g of carrier
542	0.71	1.25×10^6	0.95	1.15×10^8
526	0.68	1.60×10^8	0.89	1.32×10^6

**Bradyrhizobium* spp. strain
**CFU- colony forming unit
*** MIX- sodium alginate + starch

In this research, alginate based carriers, sodium alginate and mixture of sodium alginate and starch, were chosen because the alginate matrix protects the cells from mechanical stress and limits their mortality during prolonged storage (Schoebitz, 2013). The bacterial survival for the strain 542 onto used carriers was from 2.50×10^6 to 1.15×10^8 CFU/g. The most suitable carrier for the strain 542 was a mixture of sodium alginate and starch, but with addition of sucrose in the liquid bacterial culture. Presence of sucrose did not have a positive influence on bacterial growth, but during the drying process it probably has protected the bacteria, which contributed in increasing their survival. In previous researches in literature, starch application has shown good advantages in immobilization of rhizobacteria, as is the case with this study (Schoebitz, 2012; O'Riordan, 2001a).

On the other hand, the most suitable carrier for the strain 526 was sodium alginate with addition of sucrose during rhizobia growing. The number of bacteria of 2.35×10^8 CFU/g was achieved. Presence of starch also had positive effect on the rhizobia survival. However, in combinations when bacteria have been growing in presence of sucrose, it did not have good influence on bacterial survival. Also, it should be noted that the initial bacterial culture was diluted

two times when it was mixed with carriers. According to this, the strain 542 has higher survival than the strain 542 and it was 8.1%.

Such a low survival can be explained by the high applied operating temperature, especially at the output temperature of 50°C. The optimal growth temperature for these strains, 542 and 526, is 28°C. This applied output temperature is definitely the upper limit for their survival. For example, O'Riordan et al, in their research, were encapsulated the *Bifidobacterium* cells with gelatinized modified starch as a coating material at a outlet temperature of 45°C. The success was 30% at optimal air aspiration conditions (O'Riordan2001b). Therefore, it should be studied lower temperature conditions below 50°C and optimize working condition for increase the survival of bacteria. On the other hand, the lower temperature can lead to the formation of wet powder and sticking and accumulation of product in the cyclone (Schoebitz, 2013).

Nevertheless, there are some reports which indicate that the spray-drying technology is not considered as a good immobilization technique for rhizobia due to a high mortality during drying. Mortality of bacteria is the result of simultaneous dehydration and microorganism inactivation by high temperature (John, 2011; Picot, 2003).

CONCLUSIONS

Although, the spray dryer is an economical and effective technology for protecting immobilized bacterial cells onto carrier, it is rarely considered for rhizobia immobilization because of the high mortality of these microorganisms. In this study, the using of spray dryer for this purpose, was not enough efficient for *Bradyrhizobium* spp. strains 542 and 526. The survival of strains 542 and 526 was low (8.1 and 6.8%, respectively). The study also showed that the mixture of sodium alginate and starch was a better carrier for the immobilization of these rhizobial strains. In a further study, use of lower inlet and outlet working temperatures during drying process should be considered.

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REFERENCES

Alvarez GS, Pieckenstain FL, Desimone MF, Estrella MJ, Ruiz OA, Díaz LE. 2010: Evaluation of sol-gel silica matrices as inoculant carriers for Mesorhizobium spp. Cells. *In*: A. Mendez-Vilas (Eds). Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. *FORMATEX* Badajoz, 160 p.

Bansode SS, Banarjee SK, Gaikwad DD, Jadhav SL, Thorat RM. 2010: Microencapsulation: a review. *International Journal of Pharmaceutical Sciences Review and Research* 1(2):38-43.

Estevinho BN, Rocha F, Santos L, Alves A. 2013: Microencapsulation with chitosan by spray drying for industry applications – A review. *Trends in Food Science & Technology* 31(2):138-155.

John RP, Tyagi RD, Brar SK, Surampalli RY, Prévost D. 2011. Bio-encapsulation of microbial cells for targeted agricultural delivery. *Critical Reviews in Biotechnology* 31:211–226.

Namasivayam SKR, Saikia SL, Bharani ARS. 2014: Evaluation of Persistence and Plant Growth Promoting Effect of bioencapsulated Formulation of Suitable Bacterial Biofertilizers. *Biosciences Biotechnology Research Asia* 11(2):407-415.

O'Riordan K, Muljadi N, Conway P. 2001a. Characterization of factors affecting attachment of *Bifidobacterium* species to amylomaize starch granules. *Journal of Applied Microbiology* 90:749–754.

O'Riordan K, Andrews D, Buckle K, Conway P. 2001b. Evaluation of microencapsulation of a *Bifidobacterium* strain with starch as an approach to prolonging viability during storage. *Journal of Applied Microbiology* 91:1059–1066.

Picot A, Lacroix C. 2003: Production of multiphase water-insoluble microcapsules for cell microencapsulation using an emulsification/spray-drying technology. J Food Sci 68:2693–2700.

Schoebitz M, López MD, Roldán A. 2013: Bioencapsulation of microbial inoculants for better soil–plant fertilization. A review. *Agronomy for Sustainable Development* 33(4):751–765.

Schoebitz M, Simonin H, Poncelet D. 2012 Starch filler and osmoprotectants improve the survival of rhizobacteria in dried alginate beads. *Journal of Microencapsulation* 29:532–538.

Temprano FJ, Albareda M, Camacho M, Daza A, Santamaría C. Rodríguez-Navarro N. 2002: Survival of several *Rhizobium/Bradyrhizobium* strains on different inoculant formulation and inoculated seed. *International Microbiology* 5(2):81-86.

Vincent JM (Eds.).1970: A manual for the practical study of the root nodule bacteria. IBP Handbook no. 15. Oxford: Blackwell Scientific Publications, 3 p.

Young CC, Rekha PD, Lai WA, Arun AB. 2006: Encapsulation of Plant Growth-Promoting Bacteria in Alginate Beads Enriched with Humic Acid. *Biotechnology and Bioengineering* 95(1):76-83.

Žvagiņa S, Petriņa Z, Nikolajeva V, Lielpētere A. 2015: Immobilization and Survival of Root Nodule Bacterium *Rhizobium leguminosarum* Biovar *viciae*. *Materials Science and Applied Chemistry* 32(1):75-79.