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Development of New Formulations of Phosphorus Solubilizing Bacteria Using Spray Drying Technology

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Spray drying technology is often used as an encapsulation technique by the food and microbe mediated industries. Encapsulation of microorganisms in a polymer matrix as immobilized microbial cells would facilitate such novel formulation. An experiment was conducted to investigate the best water-soluble carriers through encapsulation of *Bacillus megaterium* var. *phosphaticum* by spray drying technology. Among the carriers tested malto dextrin and soluble starch produced fine encapsulated powder with high population load of 10^7 CFU.g^{-1} and shelf life up to twelve months. Regarding the solubility factor malto dextrin was found to be fully soluble in water and the cells were easily suspended whereas, soluble starch had slight precipitations at the end. Further, in this study it was observed that at optimal nozzle temperature of 120 °C, the malto dextrose encapsulated product resulted with high population followed by nozzle temperature of 110°C. The best bioformulation that supported the survivability in rhizosphere was spray dried products with higher microbial load than liquid formulations and lignite based carriers.

Key words: Phosphorus solubilizing bacteria, Spray dried formulation, Maltodextrose

Biofertilizer are living microorganisms, unlike chemical fertilizers they themselves are not the source of nutrients, but can help the plants in accessing the nutrient available in crop rhizosphere. Each of the introduced microbe acts like a bio-machine, continuously generating nutrients flow from soil to the plants in a healthy manner resulting with improved growth and productivity. Exploring such microbes would reduce the chemical inputs. However, timely application of bio-fertilizer is crucial for sustainable crop productivity. When a successful inoculum is obtained, the next important step is to investigate an appropriate formulation. Many strains have been isolated, characterized, cultured and genetically improved, but failed to appear on the commercial market, perhaps because of inappropriate formulation. The constraints generally observed are unattractive carrier material, lower shelf-life, contaminations, loss of quality on transportation and improper storage facilities that brings down the product quality (Herrmann and Lesueur, 2013). Research on formulation has proved pathways for the alternate to the bulkier carriers like peat, lignite, vermiculite *etc*., For example lignite based cultures are massive, having inverse population load with its storage period. Recently encapsulation of bio-inoculants has been challenged and used in agriculture by using the production processes like, spray drying, freeze drying, interfacial polymerization, cross-linking *etc*., (Schoebitz, 2013).

Microencapsulation is defined as a process for packing sensitive material like drugs, microbial cells in specialized polymers, which can release their content at favorable conditions. Encapsulated products are easy to produce, store and handle during production processes (Cassidy *et al.,* 1998). This process involves coating and protection of microorganisms by spray drying technology. The principle of immobilization of rhizobacteria by spray drying is to protect the microorganisms introduced in to the soil and ensure a gradual and prolonged release in rhizosphere region (John *et al*., 2011).

Spray drying technology has received a great attention in the recent past in bio-formulation practices for biofertilizers resulting with a micro encapsulated particulate controlled delivery system with precise physical morphologies (Peighambardoust *et al.,* 2011). Spray drying is a method of producing a dry powder from a liquid or slurry by rapidly drying with a hot gas. Coating of particulate materials with any polymer is a fundamental operation widely practiced during spray drying. Starch is a renewable, inexpensive and biodegradable polymer, and its major sources are the cereals (rice, wheat, and maize) and the root vegetables (potatoes and cassava). Maltodextrin, an enzymatically derived polymer from starch, is another polysaccharide that is used for the same purpose.

Microencapsulation with suitable polymers in water helps in easy dissemination of bacteria in targeted environment. This process can limit heat transfer, while spray-drying and minimizing deleterious effects (Teixeira, 1995). Microbial cell survival during the spray-drying procedure is dependent on many factors, including the strain of organism, the age of the culture, growth conditions and the spray-drying conditions (Lievense, 1994).

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Higher microbial populations can be obtained from the resultant product. This compact formulation is very much advantageous for easy dissemination of the bioinoculants and for storing purpose. The encapsulation of selected bacteria has been investigated for use in soil environments in agriculture and bioremediation (Rebah *et al.,* 2007).

The intention of this research is to investigate a novel water carrier for bio inoculants delivery with an aid of cell protection, increased shelf life, cheaper carriers, precise process and delivery technology in a cost effective manner.

Material and Methods

Water soluble bioformulation studies were carried out in the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. Sterilized commercial grade soluble starch and malto dextrin were used as polymers for encapsulation. Standardization of optimum temperature for spray drying, growth phases of the cell, shelf life, storage studies and survivability in rhizosphere were studied and this was compared with the existing formulation like carrier based and liquid formulation. A substance to be encapsulated (the cell load) and an amphipathic carrier (polymer) are homogenized as a suspension in water (makes the slurry). *Bacillus megatherium var. phosphaticum,* a phosphorus solubilizing bacteria was used as test culture. The resultant product was tested by serial dilution and plating technique after completion of each experiment and nutrient agar medium was used for this purpose.

Production of cell concentrates and spray drying

The stationary phase test culture was harvested by centrifugation (7000 g for 15 min at 5°C), washed twice with sterile distilled water. A cell pellet from the culture was re-suspended in a sterile solution of 10 % (w/v) skim milk. This basal medium was aseptically supplemented with 15 % (w/v) of test powders *viz*., sterilized starch or malto dextrin separately at constant flow rate: 2.5 mL. min^{-1} with standard air temperatures. The powder was collected in a single-cyclone separator (Boza *et al*., 2004). The resultant products were homogenized and stored for further studies.

Effect of nozzle temperature during spray drying

To study the optimal temperature, the nozzle temperature varied outlet air drying temperature *viz*., 90, 100, 110, 120, 130, 140 and 150°C in the spray drier were adjusted for each experiment and the products were spray dried. The spray dried samples were collected and enumerated for standardizing the optimal spray drying temperature.

Viability assays

One gram of spray dried product was re-hydrated in 10 mL of sterile solution of skim milk (10%w/v) at 25°C and placed in a shaker (100 rpm) for 24 h. Cell suspension (1 mL) was then subjected to serial dilutions by transferring onto NA agar plates and Incubated for 36 h. The total viable cell number was expressed as colony forming units per gram (CFU.g^{-1}) of dry powder (Morgan *et al*., 2006).

Enumeration of encapsulated products in soil rhizosphere region

A pot culture experiment was designed to test verify the survivability of the encapsulated bacterial culture when inoculated in soil. The encapsulated formulations of phosphobacteria tested *viz*., spray dried malto dextrose, spray dried soluble starch, lignite based carrier and liquid formulation were considered as each treatment. The product having higher shelf life was used in this soil survivability experiment. One gram of the product having 109 CFU was inoculated to 7 kg of sterile soil in a pot planted with 2 sugarcane setts. The shelf life was studied by drawing samples at monthly intervals for four months period.

Results and Discussion

In the selection of the optimal nozzle temperature experiment, 90 and 100°C had no powder formation.

Table 1. Enumeration of *Bacillus sp.* instarch and maltodextrin before and after spray drying

Treatments	No. of viable cells (CFU. g^{-1})		Solubility in water
	Before drying	After drying	
Starch	2.3 $x10^{11}$	5.3 $x10^{9}$	Slightly soluble-forms precipates
Malto dextrin	3.6 $x10^{11}$	4.3 $x10^{9}$	Soluble

The resulted product had slurry consistency. Fine powders were obtained in drying chamber only at 110°C. When the spray dried product was subjected for its microbial load at 120°C, the malto dextrose encapsulated product resulted with 43.30 $x10^{9}$ CFU mL^{-1} followed by the temperature at 110°C (34.72 $x10^{9}$ CFU mL^{-1}). Soluble starch encapsulated product recorded 33.12 $x10^{9}$ CFU mL^{-1} and 30.50 $x10^{9}$ CFU mL^{-1} at 120°C and 110°C, respectively (Table 2). At higher temperatures the microbial load was found to decline. Silva *et al. (*2005) has reported that cells at stationary phase are significantly more resistant to spray drying than cells at exponential phase. Similar results were obtained with *L. bulgaricus* in skim milk at different temperatures (Teixeira *et al.* 1995).

The resulted spray dried products were experimented for population load before and after spray drying. Before spray drying, the starch infused product produced a population load of 2.3 x 10^{11} CFU. g^{-1} and the same after spray drying produced 5.3 x 10^{9} CFU.g^{-1}. The malto dextrose product produced 3.6 $x10^{11}$ CFU.g^{-1} and after spray drying the same treatment was found to produce 4.3$x10^{9}$ CFU.g^{-1} (Table 1). Sugars such as trehalose and sucrose was reported to exhibit enhanced desiccation tolerance in numerous organisms during spray drying (Rudolph and Crowe, 1985). In shelf life studies, at 150 DAS soluble starch encapsulated product had 39.7 x 10^{8} CFU. g^{-1}. At the end of 12^{th} month the

microbial load in soluble starch encapsulations was 28.0 x 10^9 CFU. g^{-1} and this was more or less nearer to liquid formulation (27.7 x10^9 CFU. mL^{-1}). In the solubility test, when the two products were dissolved in water, maltodextrose had higher solubility than starch encapsulated product.

Table 2. Effect of nozzle temperature during spraying drying process of *Bacillus megaterium varphosphaticum.*

Treatments	Nozzle temperature (°C)						
	90	100	110	120	130	140	150
No. of CFU. mL^{-1}	x10^9	x10^9	x10^9	x 10^9	x 10^8	x 10^8	x 10^7
Malto dextrose	No powder formation	No powder formation	34.72 (10.54)	43.30 (10.64)	24.75 (10.17)	20.33 (8.09)	9.60 (7.98)
Soluble starch	No powder formation	No powder formation	30.50 (10.48)	33.12 (10.52)	25.60 (10.19)	18.80 (7.94)	3.63 (7.65)
SEd			0.56	2.33	1.63	1.98	0.53
CD (p=0.05)			0.98	5.53	2.75	3.87	1.78

Minimal contamination was registered in spray dried formulation. Similar results were reported by Mary *et al.* (1986), where significantly higher cellviability was achieved from stationary phase rhizobia and higher survival rates of *Sinorhizobium* and *Bradyrhizobium* were produced when sampled

Table 3. Survivability of phosphobacteria in spray dried encapsulations / standard formulation

Formulation	Days after Inoculation (CFU.g^{-1} / CFU.mL^{-1})												Contamination (%) at 150 DAI
	30		60		90		120		150		365		
	x10^9	OD	x10^9	OD	x10^8	OD	x10^8	OD	x10^8	OD	x10^7	**OD**	
Malto dextrose	87.00 (9.93)	0.77	56.33 (9.75)	0.485	30.33 (8.48)	0.257	3.8	0.223	23.21 (8.48)	0.18	12.5 (7.01)	0.133	2.5
Soluble starch	89.33 (9.90)	0.93	52.67 (9.42)	0.489	59.33 (8.47)	0.485	**3.2**	0.401	39.74 (8.22)	0.21	28.0 (7.22)	0.198	**3.0**
Lignitebased carrier	42.23 (9.12)	0.46	39.55 (9.24)	0.362	53.10 (8.33)	0.415	26.61 (8.18)	0.216	8.80 (8.39)	0.19	07.23 (7.12)	-	15.2
Liquid formulation	85.31 (9.33)	0.77	40.60 (9.17)	0.417	50.88 (8.51)	0.409	43.18 (8.77)	0.425	35.15 (8.42)	0.21	27.7 (7.33)	-	3.1
CD	0.109		0.099		0.069		0.093		0.076		0.071		

in the lag phase of growth (Boumahdi *et al.,* 1999). Further this was supported by Carvalho *et al*.(2004), who showed that supplementation of the drying medium with four different carbohydrates had enhanced the desiccation protection during storage. All formulations tested were found to survive in rhizosphere region at initial period and the maximum population was recorded in spray dried malto dextrose (42.00 x 10^7 CFU. g^{-1}) (Table 4).

Table 4. Enumeration of phosphobacteria in rhizosphere soil

Formulation	Days after Inoculation (CFU.g^{-1} / CFU.mL^{-1})			
	30	60	90	120
	x10^7	x10^6	x10^6	x10^6
Spray dried maltodextrose	42.00 (8.08)	25.50 (7.19)	18.80 (7.27)	11.16 (5.20)
Spray dried soluble starch	34.50 (8.16)	37.20 (7.24)	21.23 (7.33)	12.19 (5.28)
Lignite based carrier	15.00 (8.18)	20.8 (7.30)	15.40 (7.35)	02.62 (5.32)
Liquid formulation	24.00 (8.15)	27.88 (7.25)	20.4 (7.31)	09.19 (5.28)
CD	1.89	2.17	2.40	1.64

As days increased there was a reduction in population load and at 4th month, the result was 11.16 x10^6 CFU. g^{-1} of dry soil. But slight increase was noticed in spray dried soluble starch formulations at 90 DAI and 120 DAI, which registered 21.23 x 10^6 CFU. g^{-1} and 12.19 x 10^6 CFU. g^{-1}, respectively. Liquid formulation produced 09.19 x 10^6 CFU. mL^{-1} at 120 DAI. The least colonization was found in lignite based formulations at 120 DAI which had 02.62 x 10^5 CFU. g^{-1} of dry soil.

Conclusion

From this experiment it is concluded that the best suited out let temperature in the spray drier could be 120°C for both soluble starch and malto dextrose encapsulations. Shelf life studies, indicated hat soluble starch encapsulated formulation could support the survival of population up to 12 months, with a minimum percentage of contamination. Moreover, the product was very compact and concentrated with high cell load and good solubility. If the cell concentrates is increased upto10^{15} CFU. g^{-1} and further, before spraying, more bacterial population will result in the end product with higher survivability percentage in rhizosphere region. Hence, this novel formulation could be recommended as an efficient and flourishing

delivery system for bioinoculants that are currently in use. This finding could also help in eco-friendly crop production.

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