Vol 3 Issue 4 Oct 2013

Impact Factor : 1.2018 (GISI)

ISSN No :2231-5063

Monthly Multidisciplinary Research Journal

GoldenResearch Thoughts

IMPACT FACTOR : 0.2105

Welcome to ISRJ

RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

Indian Streams Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

International Advisory Board

Editorial Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.net

Golden Research Thoughts
Volume-3, Issue-4, Oct-2013
ISSN 2231-5063

Available online at www.aygrt.isrj.net

Studies On Immobilization And Storage Of Plant Growth Promoting Inoculants In Bacterial Alginate Beads

Lakshmipriya V. P. , P. K. Sivakumaar And R. Parthasarathi
Department of Microbiology, Annamalai University, Annamalai Nagar, Tamil Nadu

Abstract: The growth and distribution of few plant growth promoting inoculants individually and as consortium, immobilized within bacterial alginate beads was assessed by viable cell counts on nutrient agar method and Scanning Electron Microscopy (SEM). Both techniques indicated that the inoculants survived and grown within alginate beads. The plant growth promotion was assessed by using tray culture. The recovery of the inoculants was observed after storage up to six months. It was found that the required inoculants cell load was maintained. The observations indicated a constant random distribution of cells within alginate beads for an extended period. This indicated that the spaces within the alginate bead were maximally supported for their survival in high cell densities.

Key words: Bacterial alginate, Immobilization, SEM, Plant Growth Promotion.

INTRODUCTION :

Importance of native strains and ecological specificity while selecting the microbial inoculants for a specific environment is realized (Pandey et al., 1998). Use of microorganisms for plant growth promotion and disease control is well recognized. Isolation of microorganisms, screening for desirable characters and selection of efficient strains and production of inoculums are important steps for making use of this microbe-based technology. For field applications, the inoculum is required in an appropriate formulation. Viability of inoculum in an appropriate formulation for a certain length of time is important for commercialization of the technology (Bashan, 1998).

With a view of developing microbial inoculants using bacterial alginate for use in plant growth promotion, alginate was produced by Azotobacter vinelandii and used for immobilization studies using various agricultural inoculants. The inoculants were prepared as individual as well as consortium for rice plant. The compatibility of the inoculants was analyzed using plate assay method.

MATERIAL AND METHODS

Bacterial alginate and inoculums used in the study

Three selected species of bacteria, Bacillus sp., Pseudomonas fluorescens and Azospirillum sp., isolated from the soil samples collected from Pichavaram mangrove and characterized using biochemical tests, were used as inoculants individually and as consortium. The criterion for screening and selection of the bacterial species was their growth promotion, biocontrol and survival under storage. The cultures of Bacillus, Pseudomonas and Azospirillum sp. were maintained on slants of Tryptone yeast extract agar, Pseudomonas agar and Nfb agar slants respectively at 4°C. The bacterial alginate was produced by Azotobacter vinelandii, isolated from Pichavaram mangroves by the method suggested by Lakshmipriya and Sivakumaar,(2013).

Immobilization technique

The microbial cells (Bacillus sp., Pseudomonas sp. and Azospirillum sp.,) were immobilized as beads according to the procedure of Leung et al., (2000). Two percent bacterial alginate solution is prepared in sterile distilled water. Later 100 ml of the alginate is cooled to room temperature and 10% of the cell culture is added, the optimum condition was also studied as described above. The contents were mixed well by vigorous shaking to get a homogenised mixture. In a separate beaker 100 ml of 0.1 M calcium chloride solution was taken. The bacterial alginate containing cell culture suspension is extruded drop wise through a syringe and allowed to fall in the beaker containing calcium chloride solution. The beads formed are left in the beaker overnight for hardening. Then beads were washed and stored in distilled water at 28 ± 2ºC.

Scanning electron microscopic studies

Scanning electron microscopy (SEM) evaluation of the immobilized alginate beads with and without inoculum was carried out to examine surface morphology. Beads were mounted on metal stubs with carbon tape and the sputtered with a 150 A thick layer of gold in a Bio-Rad apparatus. A SEM (Jeol- Model 6390 Device in 30 kV) was used to evaluate surface characteristics.

Lakshmipriya V. P. , P. K. Sivakumaar And R. Parthasarathi , "Studies On Immobilization And Storage Of Plant Growth Promoting Inoculants In Bacterial Alginate Beads" Golden Research Thoughts Vol-3, Issue-4 (Oct 2013): Online & Print

Bioassay for evaluation of growth promotion

The bioassay was conducted using rice as a test species by the method of Trivedi et al., 2005. Rice seeds were grown in trays (32×32×10 cm) each containing sixteen cups. A total number of five treatments were taken; three inoculation treatments with each bacterial strain (Bacillus sp., Pseudomonas sp., and Azospirillum sp.), one consortium (Bacillus sp. +Pseudomonas sp. +Azospirillum sp) and one control (without any inoculation). For each treatment three trays were used. One plant was raised in each cup filled with local soil. Inoculations were carried out by adding 1 g of preparation of bacterial alginate. In the control treatment, seeds were sown without any inoculation. For evaluation of growth twenty five randomly selected plants from each treatment and control after 42 days of inoculation were uprooted and observations were recorded for increment in length and dry weight of root and shoot. Dry weight was determined by placing the roots and shoots, separately into small, pre-weighed brown paper bags and oven drying at 80°C for 48 h.

Recovery of plant growth promoting bacteria from bacterial alginate beads

The method of Pankaj Trivedi and Anita Pandey(2007) was followed for the recovery process. The viability of bacteria in alginate beads was observed by dissolving 1.0g of alginate beads in 1.0 ml of respective broth containing 9.0 ml of potassium phosphate buffer (0.25 M, pH 6.8) in a test tube for 16- 24 h at 28 ± 2ºC. Further the growth of the PGPR was observed by pour plate technique. The viability test was carried out following serial dilution technique with freshly prepared alginate beads and as well as alginate beads stored at 4ºC at an interval of 1 month up to a period of 6 months of study.

RESULTS AND DISCUSSION

The surface morphology of the prepared beads was studied by scanning electron microscopy (SEM). SEM photographs of the blank beads (Figure 1) compared with inoculums loaded beads show a difference in surface morphology. Smoothness increased when inoculum was loaded in the beads. The SEM photographs of the inoculum loaded beads (Figure 2) show that the culture is dispersed in the polymeric matrix, which further confirms that this system is a polymeric matrix system for beads prepared.

In terms of growth, both viable cell counts and visual observations indicated that the culture cells did survive and grow within alginate beads. Growth is referred to as change in total population, rather than an increase in the size or mass of an individual microorganism (Pelczar et al., 1986). Unlike Wada et al.(1980) and Shinmyo et al. (1982), who reported growth to be limited to the outer layer of the bead, our observations indicated that growth occurred in the cavities all over the bead showing no remarkable preferences between the outer layer and the central part. Cells were observed in the central part of the alginate beads even two weeks after incubation.

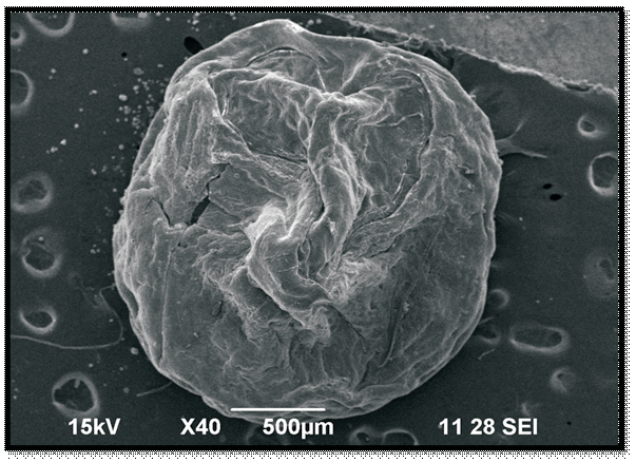

Figure 1: SEM micrograph of bacterial alginate bead without inoculum

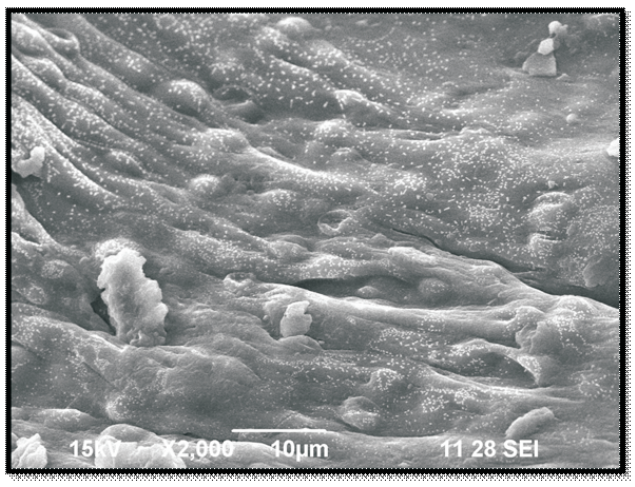

Figure 2: SEM micrograph of bacterial alginate bead with inoculum

The effect of bacterial inoculations under different formulations on growth of rice showed varied results. The response of inoculations varied with different formulations as well as bacterial species used. The inoculations resulted in significant increase in most of the growth parameters with respect to control. There are reports of plant growth promotion ability of both the bacteria used in this study (Ryder et al. 1999; Pandey et al. 2000). Due to the limitations of direct inoculation and the use of various solid-phase bacterial inoculants, several polymer- based formulations, such as alginate beads, wet and dry alginate micro beads and gum-arabic preparations of bacterial species like Azospirillum brasilense Cd, Pseudomonas fluorescens, and Rhizobium sp. have been evaluated (Bashan 1998; Forestier et al. 2001; Bashan et al. 2002).

Alginate beads supported the required population levels of PGPR isolates up to six months (Table 1). The population of the microbes increased in both consortium and single inoculants during the first month of storage and thereafter reduced with increase in the period of storage up to

six months. The surviving populations were 76.06 × 108 cfu g-1 for Bacillus (Bs), 77.88 × 108 cfu g-1 for Pseudomonas (Ps), 76.44 × 108 cfu g-1 for Azospirillum (Azp) in single inoculants preparation after one month storage.

The surviving populations were 78.66× 108 cfu g-1 in consortium after one month storage. However it was found that the required inoculants cell load was maintained up to six months. The surviving populations in the sixth month were 52.33 × 108 cfu g-1 for Bacillus (Bs), 55.33 × 108 cfu g-1 for Pseudomonas (Ps), 49.00 × 108 cfu g-1 for Azospirillum (Azp) in single inoculants preparation and 60.00× 108 cfu g-1 in consortium.

The observations in this study indicated a constant random distribution of immobilized cells within alginate beads for an extended period. This indicated that the space within the alginate bead was maximally used for growth to obtain high cell densities. The results also suggested the distribution and outer layer preference depending on the bead size and the aeration conditions.

Slow release of the entrapped bacteria and the protective environment created by encapsulation on alginate (Fravel, 1985; Bashan, 1986), may be the reason for the initially low and subsequently higher populations of bacteria in the rhizosphere in case of alginate-based formulations. Greater ability of survival and colonization of rhizosphere by immobilized form of inoculants in comparison to free forms have been reported by (Hammad and El-Mohandes, 1999). For commercialization, viability of bioinoculant in a prescribed formulation for a certain period with preservation of strain characteristics is desirable (Fages, 1992; Smith, 1992).

Table 1: Survival of individual Bacillus sp. , Pseudomonas sp., Azospirillum and consortium (Bs+ Ps+ Azp) in Alginate beads

Storage period in months	Number of cfu× 10^8 g^{-1} Alginate beads			
	Bs	Ps	Azp	Consortium (Bs+ Ps+ Azp)
0	73.00(9.85)	74.00(9.88)	72.33(9.85)	75.33(9.88)
1	76.06(9.88)	77.88(9.89)	76.44(9.88)	78.66(9.89)
2	70.33(9.85)	73.33(9.87)	70.66(9.85)	74.66(9.87)
3	65.66(9.82)	67.66(9.82)	64.88(9.81)	69.66(9.84)
4	60.70(9.78)	62.00(9.79)	58.22(9.77)	64.69(9.81)
5	56.66(9.75)	58.33(9.76)	54.66(9.73)	62.66(9.80)
6	52.33(9.71)	55.33(9.74)	49.00(9.69)	60.00(9.87)

Values in parenthesis are log10 transformed values

The immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems (Holan and Volesky, 1998). Also, immobilized beads are hard enough to withstand the application, pressures, water retention capacity, porous, transparent to metal ion sorbate species and have high and fast sorption uptake even after repeated regeneration cycles. In addition because of immobilization, the biosorbents will have better shelf life and offer easy and convenient usage compared to free biomass, which is easily biodegradable (Volesky and May- Phillips, 2000).

In conclusion, the bacterial alginate bead-based formulation of various inoculants individual as well as consortium in accordance with their storage was found to be the best carrier-based preparation for improving plant growth. The present investigation is a step towards field application and commercialization of the bacterial inoculants selected for their use in the rice fields. The large scale production of the bacterial inoculants in bacterial alginate bead form is recommended for field application and commercialization.

ACKNOWLEDGEMENTS

The authors wish to thank the University Grants Commission (UGC reference No. F 40-122/2011 (SR), dated 04-07-2011), Delhi for its financial support.

REFERENCES

I.Bashan, Y. 1986 Alginate beads as synthetic inoculant carriers for slow release of bacteria that effect plant growth. Applied and Environmental Microbiol., 51: 1098-1098.

II.Bashan, Y. 1998 Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnology Advances. 16: 729–770.

III.Bashan, Y., Hernandez, J.P., Leyva, L.A. & Bacilio, M. 2002 Alginate microbeads as inoculants carriers for plant growth-promoting bacteria. Biology and Fertility of Soils. 35: 359–368.

IV.Fages, J. 1992. An industrial view of Azospirillum inoculants: formulation and application technology. Symbiosis. 13: 14-22.

V.Fravel, D.R., Marois, J.J., Lumsden, R.D and Connick, W.J. Jr. 1985. Encapsulation of potential biocontrol agents in an alginate-clay matrix. Phytopathol., 75: 774-777.

VI.Hammad, A.M.M. and El-Mohandes, M.A.O. 1999. Controlling fusarium wilt disease of cucumber plants via antagonistic microorganisms in free and immobilized states. Microbiological Research. 154: 113–117.

VII.Holan, Z.R and Volesky. B. 1998. Biosorption of heavy metals. Biotechnol., 11: 235- 250.

VIII.Lakshmipriya, V.P and Sivakumaar P.K. 2013. Optimization of certain growth parameters for the production of exopolysaccharides from Azotobacter species isolated from mangrove ecosystem. Research J. of Biological Sci., 5(1): 27-33.

IX.Leung, W.C., M.F. Wong and C.K. Leung. 2000. Removal and recovery of heavy metals by bacteria isolated from activated sludge treating industrial effluents and municipal waste water. Water Sci. Technol., 12: 233-240.

X.Pandey, A., Sharma, E. & Palni, L.M.S. 1998 Influence of bacterial inoculation on maize in upland farming systems of the Sikkim Himalaya. Soil Biology and Biochemistry 30, 379–384.

XI.Pankaj Trivedi and Anita Pandey. 2007. Performance of different carrier materials. Biofertilizers Newsletter. 4(2): 7-9.

XII.Pelczar, M.J., E.C.S. Chan and N.R. Krieg. 1986. Microbiology. McGraw Hill, New York.

XIII.Shinmyo, A., H. Kimura and H. Okada. 1982. Physiology of - amylase production by immobilized Bacillus amyloliquefaciens. Eur. J. Appl. Microbiol. Biotechnol., 14: 7-12.
XIV.Smith, R.S. 1992. Legume inoculant formulation and application. Can. J. of Microbiol., 38: 485-492.
XV.Trivedi, P., A. Pandey and L.M.S. Palni. 2005. Carrier-based preparations of plant growth- promoting bacterial inoculants suitable for use in cooler regions. World J. of Microbiol. And Biotechnol., 21: 941-945.
XVI.Volesky , M and May- Phillips, S. 2000. Immobilization of heavy metals from contaminated sediments using microbial Bacillus sp. J. of Environmental quality. 167(7): 269- 280.
XVII.Wada, M., J. Kato and I. Chibata. 1980. Continous production of ethanol using immobilized growing yeast cells. Eur. J. Appl. Microbiol. Biotechnol., 10: 275-287.

Lakshmipriya V. P.
Department of Microbiology, Annamalai University,
Annamalai Nagar, Tamil Nadu