Int.J.Curr.Microbiol.App.Sci (2017) *6*(5): 379-385

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 5 (2017) pp. 379-385**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.605.043

Carrier Based Formulation of Plant Growth Promoting *Bacillus* Species and their Effect on Different Crop Plants

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Keywords

Bacillus, Formulation, Plant growth, Phytopathogen, Charcoal.

Article Info

Accepted:
04 April 2017
Available Online:
10 May 2017

A B S T R A C T

Rhizospheric microbes have immense potentiality to synthesize and release various compounds, that are regulating plant growth as well as physical and chemical texture of the soil. In this small piece of research, we evaluated the plant growth promoting activity of two different carriers such as charcoal and talc based formulation of *Bacillus* species. It was observed that, the bio-inoculants were able to enhance the organic carbon, nitrogen, phosphorous and potassium in soil, there by promoting growth of test crop plants such as mung bean (*Vigna radiata* L.) and rice (*Oryza satiava* L.). Charcoal based formulation depicts higher plant growth promoting activity in comparison with other carrier. Moreover, the *Bacillus* specie showed antagonistic effect against different phytopathogens including *Rahizoctonia solani* (ITCC-186) and *Fusarium oxysporum* (ITCC-578). Thus, the charcoal based formulation of *Bacillus* specie can be used for plant growth promoting activity of various crops. Before field application extensive research is highly indispensable in this regard.

Introduction

With advent of civilization, population explosion has demanded more space for industrialization, urbanization resulting decrease in agricultural land. The present day problem is to produce food grain with the available land without affecting soil health has become a great challenge to scientists. Soil harbors a wide array of microbes, among them several beneficial bacteria are colonizing in the rhizospheric region their by promoting growth of plant. Such type bacteria are generally affiliated as PGPR (Plant Growth-Promoting Rhizobacteria). Plant growth promotion by the PGPR can be either through stimulating plant growth by the production of phytohormones or by the application of bio inoculants to control various plant diseases (Glick, 1995; Bashan and de-Bashan, 2005; Bloemberg and Lugtenberg, 2001; Sivakumar *et al.*, 2014).

In the present scenario development of carrier based formulation of bio-inoculant is an industrial skill to renovate a promising laboratory documented bacteria to a commercial profitable field product (Bashan, 1998). Formulation characteristically should contain active constituent or ingredient in a suitable carrier with additives that will assist in the stabilization and perform as protective shield of the bacterial cells during storage, transportation and at the target region. It is easy to handle, increase the activity of the organism in the field, cost-effective and convenient for field applications. For this bio-agent dependent technology, screening of microbes for desirable traits, selection of potential strains and inoculum development are important steps.

Bashan (1998) reviewed that, viability of inoculum in a suitable formulation for a definite duration is vital for the commercialization of the product. Importance of formulation is to obtain the desired benefit when applied to soil by maintaining the bacterial cell and the active constituent to be in a metabolically and physiologically competent state.

According to Cassidy *et al.,* (1996), immobilization of bacterial cells into polymer matrix has confirmed to be beneficial over direct inoculation to the soil.

A major purpose of bacterial inoculant formulation is to offer more suitable micro-habitat for survival in the soil ecosystem. Moreover, for field applications use of encapsulated cells has several advantages over free cell formulations namely, protection from biotic stresses (Smit *et al.,* 1996) and abiotic stresses such as the inhibitory effect of toxic compounds (Cassidy *et al.,* 1997), enhanced survival and improved physiological activity (Weir *et al.,* 1995), supply of encapsulated nutritional additives (Trevors *et al.,* 1993), increased cell densities and preferential cell growth in various internal aerobic and anaerobic zones of encapsulating gel. In view of this, the small piece of research is focused towards evaluation of biocontrol efficacy of the potential PGPR isolate against different phyto-pathogens and development of carrier based formulation of PGPR isolates and study their effect on growth of Mung bean and Rice plant by pot culture method.

Materials and Methods

Inoculum preparation for green house study

Previously isolated *Bacillus* species (Pradhan *et al.,* 2015) was taken from the glycerol stock and streaked onto nutrient agar. Single colony of the bacteria was inoculated and grown in tryptone yeast extract broth with constant shaking at 150 rpm for 48 h at room temperature. The culture obtained at stationary phase was centrifuged at 6000 rpm for 10 min and bacterial cells re-suspended in phosphate buffer (100 mM, pH. 7.0). The cell concentration was adjusted to 9×10^8cfu/ml. (0.3 OD at 595 nm = 10^8cfu/ml).

Talc-based formulation of *Bacillus* species

The talc-based formulation was prepared by following the method described by Vidhyasekaran and Muthamilan (1995). A loopful bacterial culture was inoculated into the tryptone yeast extract broth and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (25 ± 2^0C). One kg of sterilized talc powder was taken in a metal tray and its pH was adjusted to neutral by adding $CaCO_3$ at the rate of 15 g/kg. 10 gm of CMC was added to 1 kg of talc powder and mixed well. This mixture was autoclaved for 30 min on each of two consecutive days. The 400 ml of 48 h grown bacterial suspension containing 9×10^8cfu/ml was mixed with carrier-CMC mixture under aseptic conditions. After drying overnight in laminar air flow hood, it was packed in polypropylene bag, sealed and stored at room temperature (25 ± 2^0C).

Charcoal-based formulation of *Bacillus* species

Charcoal-based formulation was developed as described by Trivedi *et al.,* 2005. The bacterial culture was grown in on tryptone yeast extract (TYE) broth at 28 ± 2ºC for 24–48 h rising the final concentration of 9×10^9cfu/ml. 150 gm of sterile charcoal was mixed with 150 ml bacterial suspension and 10 gm of gur (local sugar) was added. The slurry was mixed properly under aseptic conditions and air dried at 28 ± 2ºC overnight in a laminar flow hood.

Greenhouse study

The seeds of Mung bean (OUM-11-5) and Rice (Lalat) were obtained from the Department of Agronomy, OUAT and these seeds were soaked overnight in water, surface sterilized with 0.2% $HgCl_2$ solution for 2-3 min and air dried for 15 min. The seeds were soaked in double volume of sterile distilled water containing different formulation (10 gm/L) (Salaheddin *et al.*, 2010) and the treated seeds were shade dried for 30 min. Total 4 kg of sterilized soil was taken in each pot and the holes of the pots were closed to prevent of drainage of water. The bacteria treated seeds were showed in soil (diameter 0.25 m; height 0.3 m) at the rate of 7 seeds per pot and un-inoculated seeds were served as control. After 45 days the total chlorophyll content in leaf was measured by using the method stated by Arnon (1949) and growth parameters such as root length, shoot length and Biomass were recorded after harvesting. Physico-chemical parameters such as organic carbon, pH, Electrical conductivity (ds/m), available N (kg/ha), P_2O_5 and K_2O (kg/ha) of the soil and total bacterial population in each pot were also studied in regular interval.

Testing of *in vitro* antagonism

The antagonistic effect of *Bacillus* sp. was tested for by duel culture method against two common plant pathogen *Rahizoctonia solani* (ITCC-186) and *Fusarium oxysporum* (ITCC-578). Spores of fungal cultures grown on patato dextrose agar medium (PDA). A 5mm diameter mycelial agar disc was cut from the margin of 7-day-old fungus culture and placed on one side of a 9 cm Petri dish containing PDA medium and test bacteria was streaked on the other end of the Petri dish. Plates were incubated at 28^0C±2^0C for 5 to 8 days. Dishes inoculated only with test pathogens served as controls. The percent of inhibition of each fungus was measured using the formula (Vincent, 1927): Inhibition percentage (%) = (R1-R2) / R1 X 100 where R1 is radial growth of mycelia in control and R2 is radial growth of mycelia in treatment.

Statistical analysis

All the experiment was done in triplicate and the data was analyzed statistically by one way ANOVA at $p<0.05$ significant level.

Results and Discussion

Carrier based formulation protect the bacteria against many environmental stress; release to the soil, slowly but in large quantities. In the present study it was found that, talc based and charcoal based formulations of *Bacillus* species effectively increase the growth of Mung bean and rice when it was applied as seed treatment. Increased root and shoot elongation was apparent in PGPR treated seeds compared to control. Several strains of *B. subtilis* have proven to be efficient in plant growth promotion (Bai *et al.*, 2003). In case mung bean and rice, the highest root elongation, shoot elongation and increase in total biomass in respect to the control observed when the seeds were treated with different carrier based formulation (Tables 1 and 2). Highest root (22.83 cm) and shoot elongation (43.53 cm) was recorded in case of mung bean and 22.67 cm root length and 75.97 cm shoot length was observed in case of rice when seeds were pre-treated with Charcol-based formulation. The total chlorophyll content of rice and mung bean of different treatment were also recorded (Table 5).

Table.1 Effect of different bacterial formulation on shoot length, root length and Biomass of mung bean

Sample	Root length(cm)	Shoot length(cm)	Biomass(gm)
Control	17.37±0.56	35.73±0.45	22.17±0.47
Charcoal based formulation	22.83±0.52	43.53±0.67	31.58±0.89
Talc based formulation	20.66±0.47	42.04±0.14	26.62±0.75

Values represents mean ±SE and highly significant at $p < 0.05$

Table.2 Effect of different bacterial formulation on shoot length, root length and biomass of rice plant

Sample	Root length(cm)	Shoot length(cm)	Biomass (gm)
Control	15.36±0.3	37.06±0.5	19.88±0.31
Charcoal based formulation	22.67±0.52	75.97±0.44	25.90±0.34
Talc formulation	20.97±0.84	71.11±0.31	21.66±0.35

Values represents mean ±SE and highly significant at $p < 0.05$

Table.3 Effect of formulated bacteria on soil physico-chemical parameter of Mung bean

Soil parameter	pH		E.C(ds/m)		O.C(%)		N(kg/ha)		P_2O_5(kg/ha)		K_2O (kg/ha)	
	BS	AH	BS	AH	BS	AH	BS	AH	BS	AH	BS	AH
Control	6.81 ± 0.01	6.81 ± 0.01	0.011 ± 0.01	0.011 ± 0.01	0.211 ± 0.01	0.223 ± 0.01	155.5 ± 0.32	158.75 ± 0.74	83.9 ± 0.31	84.26 ± 0.45	197.4 ± 0.42	198.8 ± 0.25
Charcoal formulation	6.81 ± 0.01	6.85 ± 0.01	0.011 ± 0.01	0.014 ± 0.01	0.211 ± 0.01	0.529 ± 0.01	155.5 ± 0.32	277.2 ± 0.66	83.9 ± 0.31	87.97 ± 1.4	197.4 ± 0.42	199.61 ± 0.38
Talc formulation	6.81 ± 0.01	6.82 ± 0.01	0.012 ± 0.01	0.011 ± 0.01	0.211 ± 0.01	0.423 ± 0.01	155.5 ± 0.32	251.58 ± 1.45	83.9 ± 0.31	85.17 ± 0.35	197.4 ± 0.42	199.03 ± 0.34

Values represents mean ±SE and highly significant at $p < 0.05$

Table.4 Effect of formulated bacteria on soil physico-chemical parameter of rice

Soil parameter	pH		E.C(ds/m)		O.C(%)		N(kg/ha)		P_2O_5(kg/ha)		K_2O (kg/ha)	
	BS	AH	BS	AH	BS	AH	BS	AH	BS	AH	BS	AH
Control	6.81 ± 0.01	6.81 ± 0.01	0.011 ± 0.01	0.011 ± 0.01	0.211 ± 0.01	0.218 ± 0.01	155.5 ± 0.32	156.64 ± 1.86	83.9 ± 0.31	89.55 ± 0.32	197.4 ± 0.42	197.71 ± 0.20
Charcoal formulation	6.81 ± 0.01	6.85 ± 0.01	0.011 ± 0.01	0.014 ± 0.01	0.211 ± 0.01	0.538 ± 0.01	155.5 ± 0.32	274.76 ± 0.35	83.9 ± 0.31	89.37 ± 1.15	197.4 ± 0.42	199.77 ± 1.07
Talc formulation	6.81 ± 0.01	6.82 ± 0.01	0.012 ± 0.01	0.011 ± 0.01	0.211 ± 0.01	0.422 ± 0.01	155.5 ± 0.32	243.69 ± 1.81	83.9 ± 0.31	85.56 ± 0.35	197.4 ± 0.42	197.69 ± 0.23

Values represents mean ±SE and highly significant at $p < 0.05$; BS= Before Sowing Ah= After harvest

Table.5 Estimation of total chlorophyll content in Rice and Mung bean plant

Sample	Control (mg/g)	Talc formulation (mg/g)	Charcoal formulation (mg/g)
Mung bean	3.37±0.04	4.65±0.04	4.76±0.11
Rice	1.65±0.23	3.63± 0.10	4.18± 0.30

Values represents mean ±SE and highly significant at $p < 0.05$

Table.6 Total bacterial populationof Mung bean soil in different time interval

Soil Parameter	Initial day	30 Days (CFU/gm)	60 Days (CFU/gm)	90 Days (CFU/gm)	120 Days (CFU/gm)
Control	$3.4 \pm 0.02 \times 10^5$	$3.47 \pm 0.14 \times 10^5$	$3.47 \pm 0.20 \times 10^5$	$3.53 \pm 0.24 \times 10^5$	$3.63 \pm 0.24 \times 10^5$
Charcoal formulation	$3.4 \pm 0.02 \times 10^5$	$5.87 \pm 0.12 \times 10^5$	$6.23 \pm 0.24 \times 10^5$	$6.47 \pm 0.2 \times 10^5$	$6.63 \pm 0.29 \times 10^5$
Talc formulation	$3.4 \pm 0.02 \times 10^5$	$5.43 \pm 0.20 \times 10^5$	$5.9 \pm 0.13 \times 10^5$	$6.1 \pm 0.2 \times 10^5$	$6.37 \pm 0.18 \times 10^5$

Values represents mean ±SE and highly significant at $p < 0.05$

Table.7 Total bacterial population of rice soil in different time interval

Soil parameter	Initial	15 days (CFU/gm)	30 days (CFU/gm)	45 days (CFU/gm)	60 days (CFU/gm)
Control	$3.4 \pm 0.02 \times 10^5$	$3.42 \pm 0.03 \times 10^5$	$3.43 \pm 0.03 \times 10^5$	$3.43 \pm 0.04 \times 10^5$	$3.58 \pm 0.15 \times 10^5$
Charcoal formulation	$3.4 \pm 0.02 \times 10^5$	$4.0 \pm 0.11 \times 10^5$	$4.3 \pm 0.12 \times 10^5$	$4.44 \pm 0.18 \times 10^5$	$4.6 \pm 0.11 \times 10^5$
Talc formulation	$3.4 \pm 0.02 \times 10^5$	$3.75 \pm 0.17 \times 10^5$	$4.13 \pm 0.08 \times 10^5$	$4.23 \pm 0.12 \times 10^5$	$4.37 \pm 0.14 \times 10^5$

Values represents mean ±SE and highly significant at $p < 0.05$

Table.8 *In vitro* antagonistic effect of *Bacillus* sp. on mycellial growth of different plant pathogens

Isolate No.	Inhibition %	
	Rhizoctonia solani	***Fusarium oxysporum***
Bacillus species	61.49 ± 0.69	59.33 ± 0.48

Values represents mean ±SE and highly significant at $p < 0.05$

It was also found that, the bio-inoculants were able to increase the organic carbon, nitrogen, phosphorous and potassium in soil, there by promoting growth of mung bean and rice (Tables 3 and 4) in respect to control. It was well established fact thatthe microbial members of soil communities are the most sensitive and rapid indicators for soil quality evaluation (Zelles, 1999; Zornoza *et al.*, 2009). The pH of soil is one of the most important physicochemical parameter, which influence the mineral nutrient of soil quality and microorganism activity (Saseeswari *et al.*, 2015). In the present investigation it was observed that the bacterial population in all the treatments was increased in respect to the control (Tables 6 and 7). The potential *Bacillus* species was tested for *in vitro* antagonism against *Rahizoctonia solani* and *Fusarium oxysporum* and showed positive result (Table 8). It was already proved that plant growth promoting rhizobacteria can protect the plant from different types of plat pathogens (Raupach and Kloepper, 1998). Result of the current study showed the positive impacts of *Bacillus* specie. on growth of mung bean and rice plant compared to the compared to control. So as a simple and safe method, bacterization of seeds could be a promising technique for improvement of plant growth efficiency. Thus, the potential bacteria *Bacillus* sp. further investigated to increase productivity under field condition and use of PGPR as inoculants bio fertilizers is a novel approach to replace chemical fertilizers and pesticides for sustainable agriculture in India.

Acknowledgement

The authors are thankful to Dr. D.P. Samantaray, Assistant Professor, Department of Microbiology, OUAT and Staff of the Department of Vegetable science, OUAT for providing laboratory facilities during the period of study. The authors have no conflict of interest to declare.

References

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. *Plant Physiol.,* 24: 1-15.

Bai, Y., Zhou, X. and Smith, D.L. 2003. Enhanced soybean plant growth resulting from co-inoculation of *Bacillus* strains with *Bradyrhizobiumjaponicum*. *Crop Sci*., 43: 1774 -1781.

Bashan, Y. 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. *Biotechnol. Adv*., 16: 729–770.

Bashan, Y., and de-Bashan, L.E. 2005. Bacteria/plant growth-promotion. In: Hillel D, editor. Encyclopedia of soils in the environment. Oxford, UK: Elsevier. Vol. 1, pp. 103- 115.

Bloemberg, G.V., and Lugtenberg, B.J.J. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr. Opin. Plant Biol*., 4: 343 350.

Cassidy, M.B., Lee, H. and Trevors, J.T. 1996. Environmental applications of immobilized microbial cells: A review. *J. Ind. Microbiol*., 16:17- 101.

Cassidy, M.B., Lee, H. and Trevors, J.T. 1997. Survival and activity of lac-lux marked *Pseudomonas aeruginosa* UG2Lr cells in encapsulated kcarageenan over 4 years at 48C. *J. Microbiol. Meth*., 30: 167 -170.

Glick, B.R. 1995. The enhancement of plant growth by free living bacteria. *Canad. J. Microbiol*., 41: 109–117.

Pradhan, A., and Mishra, B.B. 2015. Effect of plant growth promoting rhizobacteria on germination and growth of rice (*Oryza Sativa* L.). *The Ecoscan*, 9(1&2**)**: 213-216.

Raupach, G.S. and Kloepper, J.W. 1998. Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathol*., 88: 1158– 1164.

Salaheddin, K., Valluvaparidasan, V., Ladhalakshmi, D. and Velazhahan, R. 2010. Management of bacterial blight of cotton using a mixture of *Pseudomonas fluorescens* and *Bacillus subtilis. Plant Protect. Sci*., 46: 41-50.

Saseeswari, A., Kanimozhi, G. and Panneerselvam, A. 2015. Studies on physicochemical parameter and bacterial populations in sediment soil at Karankadu Mangrove forest, Ramanathapuram (Dt), Tamilnadu. *Int. J. Sci*., 4: 886 – 895.

Sivakumar, P.K., Parthasarthi, R. and Lakshmipriya, V.P. 2014. Encapsulation of plant growth promoting inoculant in bacterial alginate beads enriched with humic acid. *Int. J. Curr. Microbiol. App. Sci.*, 3: 415-422.

Smit, E., Wolters, A.C., Lee, H., Trevors, J.T. and van Elsas, J.D. 1996. Interaction between a genetically marked

Pseudomonas fluorescence strain and bacteriophage øR2f in soil: Effects of nutrients, alginate encapsulation, and the wheat rhizosphere. *Microb. Ecol.*, 31: 125 -140.

Trevors, J.T., van Elsas, J.D., Lee, H. and Wolters, A.C. 1993. Survival of alginate encapsulated *Pseudomonas fluorescence* cells in soil. *Appl. Microbiol. Biotechnol*., 39: 637- 643.

Trivedi, P., Pandey, A. and Palni, S. 2005. Carrier-based preparations of plant growth-promoting bacterial inoculants suitable for use in cooler regions. *Worl. J. Microbiol. Biotechnol.*, 21: 941–945.

Vidhyasekaran, P., and Muthamilan, M. 1995. Development of a formulation of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis*., 79: 782–786.

Vincent, J.M. 1927. Distortionn of fungal hyphae in presence of certation inhibitors. *Nature*, 59: 850.

Weir, S.C., Dupuis, S.P., Providenti, M.A., Lee, H. and Trevors, J.T. 1995. Nutrient enhanced survival of and phenanthrene mineralization by alginate encapsulated and free *Pseudomonas* sp. UG14Lr cells in creosote contaminated soil slurries. *Appl. Microbiol. Biotechnol*., 43: 946-951.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. *Biol. Fertil. Soils*, 29:111-129.

Zornoza, R., Guerrero, C., Mataix-Solera, J., Scow, K.M., Arcenegui, V. and Mataix-Beneyto, J. 2009. Changes in soil microbial community structure following the abandonment of agricultural terraces in mountainous areas of Eastern Spain. *Appl. Soil Ecol*., 42: 315-323.

How to cite this article:

Pahari, A., A. Pradhan, S. Maity and Mishra, B.B. 2017. Carrier Based Formulation of Plant Growth Promoting Bacillus species and Their Effect on Different Crop Plants. *Int.J.Curr.Microbiol.App.Sci.* 6(5): 379-385. doi: http://dx.doi.org/10.20546/ijcmas.2017.605.043