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J. Sci. Trans. Environ. Technov. 2015, 8(4) : 173-177

Scientific Transactions in Environment and Technovation

Carrier-based tripartite bacterial consortia promote growth of *Lycopersicon esculentum* L.

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Abstract

An appropriate formulation of bacterial inoculum is required to enhance the productivity of plants in field. Sawdust, sugarcane-bagasse and charcoal were used as solid carriers to enhance the shelf-life of bioinoculants. Plant growth promoting rhizobacteria (PGPR) screened from tomato rhizosphere were characterised and identified on the basis of carbon source utilization by comparing with standard bacterial cultures and UPGMA cluster analysis using MVSP software version 3.2. *Pseudomonas* sp. LEP17, *Azotobacter* sp. LEP21 and *Bacillus* sp. LEP25 showed synergistic effect with each other. Three bacterial consortia showed the maximum viability on sawdust followed by charcoal and sugarcane-bagasse at 360th day of inoculation. Seed germination and seedling emergence got increased with treatment of bacterial isolates individually as well as its consortia. The above bioformulation enhanced the growth of *Lycopersicon esculentum* in field trials. The tripartite consortium of *Pseudomonas* sp. LEP17, *Azotobacter* sp. LEP21 and *Bacillus* sp. LEP25 resulted in better growth followed by twin bacterial consortium and individual isolates.

Keywords: PGPR, *Pseudomonas, Azotobacter, Bacillus*, Consortium, Carrier, *Lycopesicon esculentum.*

INTRODUCTION

Tomato (*Lycopersicon esculentum*L.) is one of the most economical important vegetable crops in the world which belongs to the family Solanaceae. India is the third largest tomato producing country. Tomato consists of several nutrients *viz.*, organic acids, antioxidants, lycopene and â-carotene contents. Lycopene is the principal red pigment of tomatoes (Schofield and Pliyath, 2005) which also acts as a natural antioxidant (Gupta *et al*., 2010). World increasing population demand high production and good quality of vegetables. Several chemical fertilizers are used to overcome this problem but these chemicals harm tomato plants and imbalance the soil nutrients. In recent years efforts are being made to use PGPR inoculants as an alternative to these xenobiotics. PGPR results in the increased crop productivity in an efficient and eco-friendly manner (Dubey*et al*., 2014; Maheshwari*et al*., 2010). However, it is difficult to maintain suitable number of bio-inoculant in field for a long time. Beneficial bacteria can be applied through seed or soil inoculation methods in the form of carrier-based inoculants (Maheshwari*et al*., 2015). Inoculum formulations using carrier material have widely been used to facilitate the introduction of high cell number and increased survival of microorganisms in soil (Kumar *et al*., 2006). In this communication an attempt was made to evaluate the effect of different carrier-based bacterial inoculants on growth of *L. esculentum.*

MATERIAL AND METHODS

Mature and healthy plants of tomato were randomly collected from Roshnabad, Haridwar (Uttarakhand) from a growing field. The plant samples were uprooted gently, placed in sterile plastic bags and brought to laboratory for further study.

Isolation and characterization of plant growth promoting rhizobacteria (PGPR)

A total of thirty one bacterial isolates were screened from the rhizosphere of *L. esculentum* following dilution plate method as described by Dubey and Maheshwari (2010). Isolates were identified biochemically following the standard tests as mentioned in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Further, these isolates were screened on the basis of their PGP activities (Kumar *et al*., 2012). Three PGPR isolates screened as above were identified by carbon source utilization method based on HimediaCarbokit™ which is a standardized colorimetric identification system based on the principle of pH change after substrate utilization. Each bacterium was inoculated on HimediaCarbokit™ aseptically and incubated at 37^{o}C for 4-5 h. Standard bacterial cultures viz., *Pseudomonas aeruginosa*MTCC1934, *Azotobactervinelandii*MTCC124 and *Bacillus subtilis*MTCC441 were procured from IMTECH (Chandigarh) and above three screened cultures (LEP17, LEP21 and LEP25) were compared with the standard cultures and characterized.

Synergism among the bacterial isolates

The bacterial isolates LEP17, LEP21 and LEP25 were tested for their synergistic behaviour with each other following the method of Pierson and Weller (1994). 5µl log phase culture of each isolate was spotted together on NAM plate. The growth characteristics and synergism were recorded after 24 h of incubation.

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Table 1.In vitro interaction among the PGPR isolates used for the preparation of bacterial consortia.

Strain	LEP17	LEP21	LEP25
LEP17	+	+	+
LEP21	+	+	+
LEP25	**+**	**+**	**+**

Table 2.Physical properties of different carriers.

Carriers material	pH	Moisture content (%)	Water holding capacity (%)	Inherent moisture content (%)
Sawdust	6.8	5.7	74	14
Charcoal	7.4	3.3	72.5	7.3
Sugarcane-baggasse	7.2	4.6	70	8.5

Table 3. Effect of *Pseudomonas sp.* LEP17, *Azotobacter sp.* LEP21, *Bacillus sp.* LEP25 and their consortia on growth of *L. esculentum* after 120 days of sowing.

Treatment	Germination (%)	120 DAS							
		Length (cm)		Fresh weight (g)		Dry weight (g)		No. of leaf/ Plant	No. of fruits/ Plant
		Shoot	Root	Shoot	Root	Shoot	Root		
Pseudomonas sp.LEP17	87.00	57.58	7.28	176.03	37.33	59.74	13.07	95.00	12.00
*Azotobacter*sp. LEP21	79.00	59.02	7.44	178.56	38.78	60.06	14.48	100.33	13.00
Bacillus sp. LEP25	89.00	59.88	7.82	180.39	40.03	60.40	16.07	102.66	14.00
Consortium 1	90.00	60.43	8.67	183.78	40.89	62.01	17.91	106.00	14.33
Consortium 2	92.00	61.78	9.06	187.95	42.11	63.19	17.84	109.00	14.66
Consortium 3	96.00	64.63	11.55	194.59	47.61	70.66	23.07	125.33	16.00
Control	64.00	54.74	7.50	161.45	33.78	54.41	10.39	88.66	9.00
SEM	0.825	0.646	0.458	0.827	0.668	0.581	0.474	1.856	0.3411
CD at 1%	3.565	2.789	2.023	3.573	2.883	2.508	2.047	8.015	1.472
CD at 5%	2.546	1.990	1.444	2.550	2.058	1.790	1.461	5.719	1.051

Estimation of carrier properties

Sugarcane bagasse, charcoal powder and sawdust were procured from different sources. Electrical conductivity of selected carriers was measured by using digital conductivity meter. The pH of carriers was determined by using a digital pH meter. Water holding capacity was determined on dry weight measurement basis. Distilled water was added to oven-dried carrier material until it formed slurry and became saturated. The slurry was transferred in a measuring cylinder with a sieve (0.25 mm) covering the drain hole at its bottom. Water was drained out overnight from carrier under normal conditions. The carrier material left in cylinder was measured and water holding capacity (%) was determined after drying. Inherent moisture content of different carriers was determined by weighing 10 g of solid carrier and placing into an oven at 70°C for 24 hours. The weight of carriers was determined with the help of digital balance and again placed in an oven for 24 hours to determine end point of moisture loss. Moisture content was estimated by using the formula: $M = [(w_1-w_2)/w_2] \times 100$, where M= moisture content (%), w_1= weight of carrier material before drying, w_2= weight of carrier after drying.

Preparation of bacterial inoculants

Bacterial isolates were grown in nutrient broth for 24 hours in an orbital shaker (150 rpm) at room temperature. Broth having 10^9/ml cfu was further taken for inoculant preparation (Somasegaran and Hoben, 1994). The carrier materials *viz.*, sawdust, sugarcane-bagasse and charcoal powder were separately powdered and air-dried properly. These carrier materials were double sterilized and autoclaved at 121°C for 20 minutes. Thereafter, 500 g of each sterile carrier material was packed in the sterile

Figure1. UPGMA cluster of bacterial isolates on the basis of carbon source utilization by using Multi Variate Statistical Package (MVSP) software version 3.2.

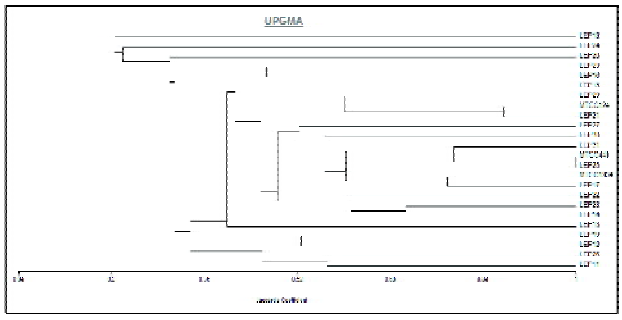


Figure 2.Shelf-life of *Pseudomonas sp*. LEP17 in different carrier.

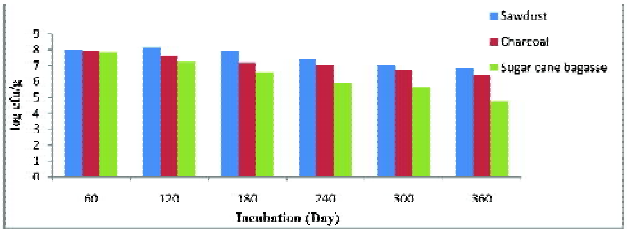


Figure 3.Shelf-life of *Azotobacter sp*. LEP21 in different carrier.

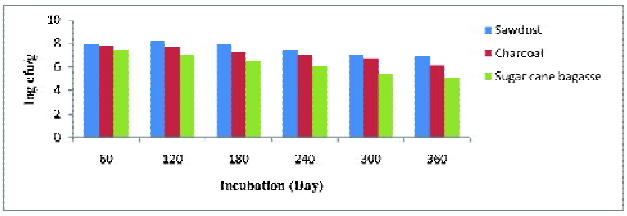


Figure 4.Shelf-life of *Bacillus sp*. LEP25 in different carrier.

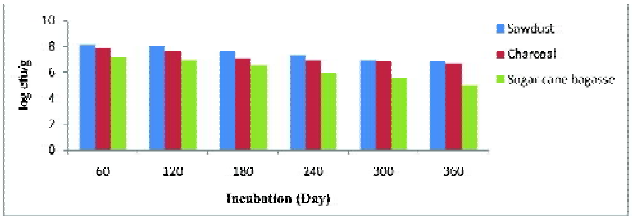
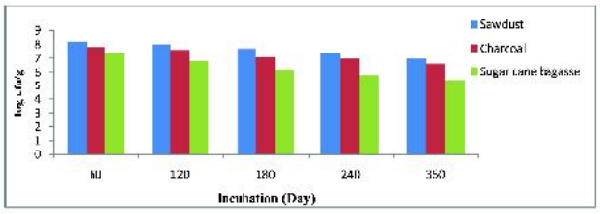


Figure 5.Shelf-life of bacterial consortium in different carriers.



polythene bags (50-75 mm thick) and sealed properly leaving about 75% space vacant. Low density and flexible sheet of polythene prevents the loss of moisture from packing. The bags were surface sterilized by 70% ethanol and suspension of bacterial strain was introduced aseptically with the help of a sterile clinical syringe followed by immediately sealing with an adhesive tape to prevent moisture loss and contamination. The contents of the polythene bags were properly mixed by shaking and kneading the packet between fingers. The packets were incubated at 25°C for 2 days so as to obtain the desired number of population of bacteria. The polythene bags containing inoculum were kept at room temperature in dark and incubated for 360 days.

Study of shelf-life

Carrier-based bacterial sample (1g) was taken out aseptically from each bag on at every 60 day interval for 360th days separately and transferred into test tubes containing 9 ml of sterile distilled water to prepare suspension followed by serial dilution of samples. Culture suspension (100 µl) from each dilution was spread over NAM plates in triplicate and incubated at 30°C for 48 h. The colonies were counted and log CFU/g was determined at each interval (Acea*et al.*, 1988).

Field application

Field trials were conducted at Roshnabad village (Haridwar) from August to November, 2010. The field experiments were conducted in a randomize plot design with replicates of each treatment. The plot size was 12 m^2 in which bacterized seeds were shown at an equidistance of 30 cm. Seeds were sown in experimental plot in seven sets of treatments *viz.*, T1- seeds bacterized with *Pseudomonas* sp. LEP17, T2- seed bacterized with *Azotobacter* sp. LEP21, T3- seed bacterized with *Bacillus* sp. LEP25, T4- seeds+*Pseudomonas*sp. LEP17+*Azotobacter* sp. LEP21, T5- seeds+*Pseudomonas*sp. LEP17+*Bacillus* sp. LEP25, T6- seeds+*Pseudomonas*sp. LEP17*Azotobacter*sp. LEP21+*Bacillus* sp. LEP25+ and T7- unbacterized seeds as control. The plots were irrigated with water when required. Seed germination was observed on 15th day of sowing and germination (%) was calculated. Seedling growth *viz.*, root length, shoot length, fresh weight and dry weight was recorded on 30, 60, 90 and 120th days after sowing. Three plants from each plot were randomly selected for recording the data.

RESULTS

Isolation and characterization of bacterial isolates

Out of thirty three bacterial isolates LEP17, LEP21 and LEP25 showed PGP activities. These isolates were compared with standard bacterial strains (*viz.*, *Pseudomonas aeruginosa*MTCC1934, *Azotobactervinelandii*MTCC124 and *Bacillus subtilis*MTCC441) and identified on the basis of their carbon source utilization. The phylogenetic relatedness

among all the isolates was assessed by UPGMA cluster using Multi Variate Statistical Package (MVSP) software version 3.2. Node-1 showed 78.8% similarity between LEP17 and *P. aeruginosa* MTCC1934 and node-2 87.5% similarity between LEP21 and *A. vinelandii* MTCC124, node-3 displayed 100% similarity between LEP25 and *B. subtilis* MTCC441. The isolates LEP17, LEP21 and LEP25 were named as *Pseudomonas* sp. LEP17, *Azotobacter* sp. LEP21 and *Bacillus* sp. LEP25, respectively (Fig.1).

Synergism among bacterial isolates

All the three bacterial isolates did not inhibit the growth of each other (Table.1). On the basis of their synergistic behaviour the three bacterial consortia were prepared *viz.*, consortium-1 (*Pseudomonas* sp.LEP17 + *Azotobacter* sp. LEP21), consortium- 2 (*Pseudomonas* sp.LEP17 + *Bacillus* sp.LEP25) and consortium-3 (*Pseudomonas* sp.LEP17 + *Azotobacter* sp. LEP21 + *Bacillus* sp. LEP25). These consortia were used in field trials.

Properties of the carriers

The physiochemical properties of sawdust, charcoal powder and sugarcane-bagasse varied with each other. Sawdust had 74% water holding capacity, charcoal 72.5% and sugarcane-bagasse 70%. Initially the mass cultures (10^8 cells/ml) of the isolates were properly mixed with separate carriers. The pH of all the three carriers was monitored and found within the optimum range of 6.8 to 7.2 (Table 3).

Shelf-life of bacterial isolates in different carriers

The three individual isolates as well as their consortium (*Pseudomonas* sp. LEP17 + *Azotobacter* sp. LEP21 + *Bacillus* sp. LEP25) showed the maximum viability on sawdust ($7.1x10^8$ log cfu/g) followed by charcoal ($6.5x10^8$ log cfu/g) and sugarcane-bagasse ($5.2x10^8$ log cfu/g) even after 360 days of inoculation.

Field trial

The certified seeds of *L. esculentum* were procured from IARI, New Delhi. Healthy seeds were selected and bacterized with *Pseudomonas* sp. LEP17, *Azotobacter* sp. LEP21, *Bacillus* sp. LEP25 individually as well as their consortia. Seed germination and seedling emergence got increased after treatment with the three bacterial isolates individually as well as its consortia. Consortium-3 resulted in the maximum seed germination (96%) followed by consortium-2 (92%) and consortium-1 (90%). The experimental plot had 61.25% sand, 28.44% silt, 11.35% clay and 37.5% water holding capacity. Shoot length, root length, fresh and dry weight of shoot got enhanced as compared to control. All the vegetative growth parameters (root length, shoot length, fresh and dry weight of root as well as shoot and number of leaf/plant) were significantly (1% of LSD) enhanced after 120 DAS in comparison to control (Table 3).

DISCUSSION

Characterization of bacterial isolates following Bergey's Manual of Determinative Bacteriology and carbon utilization by using HimediaCarbokit™ has also been reported by several researchers (Garland and Mills, 1991; Bouzar, *et al*., 1993). The characteristics features of bacterium are that whether it shows synergism or antagonism. Presence of synergism is prerequisite of consortium formation for bioinoculants. In the present study all the three isolates exhibited good synergistic effect on each other. Good synergistic effect between *Pseudomonas* sp. and *Bacillus* sp. was also reported by Rajkumar *et al*. (2008). Suitable carriers are essential for good survival of individual bio-inoculant as well as their consortia. Sugarcane-bagasse is lignocellulosic material which is obtained after juice extraction of sugarcane and the charcoal is a commonly used carrier material, while sawdust was obtained commonly from saw machines. Out of them sawdust is cheaper than the other two carriers that showed the best results for the survival of individual isolates and their consortia due to of its sufficient water holing capacity, significant inherent moisture content, non- toxic nature and pH optima. Keyser *et al*. (1992) stated that a good inoculant carrier should have : *(i)* water- holding capacity, *(ii)* be non-toxic to inoculant, *(iii)* be easily sterilisable to avoid contamination, *(iv)* have pH buffering capacity, *(v)* contain anion exchange capacities, *(vi)* be free from lump-forming materials, *(vii)* be easily processed, *(viii)* be non-toxic and biodegradable, *(ix)* provide nutrients for the microorganisms and favours their survival, both during storage and inoculation and *(x)* be cost effective.

Consortium-3 (LEP17+LEP21+LEP25) showed better shelf-life ($<10^8$) during storage at room temperature for a period of 360 days. Bureau of Indian Standards (BIS) (2001) recommended that the inoculant should contain a minimum of 10^7 viable cells per g. of the carrier on dry mass basis till 6 months expiry period from the date of manufacturing. The characteristics of a good carrier also ensure the capacity to deliver the right number of viable cells in good conditions at a particular site (Smith, 1985; Bashan, 1998; Arora *et al*., 2001). On the other hand, the viability of cells in bio-formulation prolonged storage is a serious problem due to physiochemical nature of carrier and sufficient nutrient availability (Smith, 1985). But in this study sawdust supported the significant number of viable cells at 360 days. Pandey *et al*. (2007) also reported sawdust as a good carrier to support the survival of a larger population of microbial cells.

Consortium-3 (*Pseudomonas* sp. LEP17 + *Azotobacter* sp. LEP21 + *Bacillus* sp. LEP25) resulted in 96% seed germination as compared to consortium-2 (92%), consortium-1 (90%), individual strains and control (64%). The consortium-3 also enhanced the length of shoot (64.63%) and root (11.55%) as compared to the other consortia and individual strains. It also enhanced

the number of leaves and fruits per plant more than the control. Maheshwari *et al*. (2015) also reported the enhancement in the vegetative growth and yield parameters of *B. campastris* by co-inoculation of *Pseudomonas* and *Bacillus*. *Bacillus subtilis* has been reported as a good PGPR by Dubey *et al*. (2014).

In this study the consortium-3 showed better plant growth promoting effects on tomato in experimental field than that of consortium-2, consortium-1 and individual treatments. However, the best growth of consortium-3 treated seedling of tomato may be explained to the presence of sufficient number of viable cells of isolates LEP17, LEP21 and LEP25 in the rhizosphere. Thus, the consortium of all the three isolates in saw dust seems to be the best suitable for use as a better plant growth promoter for *L. esculentum*.

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