

Original Research Article

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## Utilization of Lactic Acid and Phosphate Solubilizing Bacterial Consortia for Healthy Spinach (*Spinacia oleracea*) Cultivation

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### ABSTRACT

An attempt was made to isolate, screen and evaluate lactic acid bacteria for its phosphate solubilization potentiality both under *in vitro* and *in vivo* conditions. In the course of investigation as many as five lactic acid bacterial isolates were obtained from milk and milk products and all the five isolates were characterized as *Lactobacillus sp.* based on morphological and biochemical studies. Further, when all the lactic acid bacterial isolates tested for the phosphate solubilization ability, the isolate LAB – 5 (*Lactobacillus sp.*) showed maximum phosphate solubilization ability on the Sperber's media. Hence, LAB -5 was selected for further field evaluation studies along with standard phosphate solubilizing bacterial isolate (*Bacillus megaterium*). The effective LAB – 5 (*Lactobacillus sp.*) and phosphate solubilizing bacterial (*Bacillus megaterium*) consortia were evaluated on growth and yield of Spinach under field condition. Out of five treatments imposed, the treatment T5 = LAB - 5 (*Lactobacillus sp.*) + phosphate solubilizing bacteria (*Bacillus megaterium*) + compost + RDF recorded highest germination percentage (100%), more number of leaves (23 no./plant) and high chlorophyll content of 3.81 mg/g of tissue at the end of 45 days. Similarly, the highest fresh and dry weight of 40 and 6.2g/plant respectively were observed in the same treatment. Finally, the treatment 5 also showed the maximum NPK content in both plant and in soil. Scale of studies and further required to evaluate the effective LAB-5 (*Lactobacillus sp.*) and phosphate solubilizing *Bacillus megaterium* in multi-location trials using different crops.

#### Keywords

Lactic acid bacteria,  
Microbial consortia,  
Phosphate  
solubilization,

#### Article Info

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### Introduction

Biofertilizers are the compounds that enrich the nutrient quality of soil by the use of microorganisms, having symbiotic relationship with the plants. The commercial history of biofertilizers began with the launch of Nitragin by Nobbe and Hiltner, a laboratory culture of *Rhizobia* in 1895, followed by the

discovery of *Azotobacter* and then the blue green algae and most of other microorganisms. Through the use of biofertilizers, healthy plants can be grown, while enhancing the sustainability of the health of the soil. Now days, the application of these biofertilizers in the vegetables production is gaining lot of importance because of their cost effectiveness and eco

friendliness. Different microorganisms like *Azospirillum*, *Azotobacter*, PSB like *Bacillus megaterium* and other biocontrol agents like *Trichoderma harzianum*, *Pseudomonas fluorescens* are used in the leafy vegetables production for increasing the yield and to reduce the disease incidence.

Among different leafy vegetables, the Spinach (*Spinacia oleracea*) belongs to the family of *Amaranthaceae* is one of the ancient and popular leafy vegetable which is grown in South East Asian Countries. Presently, it is grown throughout the tropical regions of Asia, Africa, and America *etc.*, and it has reached Europe by 8<sup>th</sup> century. Spinach has a high nutritional value and is extremely rich in antioxidants, especially when freshly steamed or quickly boiled. It is also a rich source of Vitamin A, Vitamin C, folic acid, dietary fiber *etc.*, Because of all these nutrients, the dietitians and nutritionists recommended this Spinach to the diabetic and heart patient.

Lactic Acid Bacteria (LAB) organisms are important in dairy products. One of the most important groups of acid producing bacteria in the food industry is the Lactic Acid Bacteria (LAB) which is used in making starter culture for dairy products. Lactic acid bacteria are among the most powerful prokaryotes, and also these were first described as milk souring organisms due to the sour milk that arose from their production of lactic acid. They are a relatively diverse group of bacteria, but related by a number of typical metabolic and physiological features. Generally, the group consists of gram positive bacteria, cocci or rods and produce lactic acid as the major end product during fermentation of carbohydrates.

The use of lactic acid bacteria along with agriculturally important microorganisms like *Trichoderma*, *Rhizobium*, *Pseudomonas etc.*, are practiced to harness the combined effect of both the organisms for effective crop

production. A variety of lactic acid bacteria, isolated from plant surfaces and plant-associated products were found to be antagonistic to test strains of the phytopathogens *Xanthomonas campestris*, *Erwinia carotovora*, and *Pseudomonas syringae*. Effective "*in vitro*" inhibition was found both on agar plates and in broth cultures. In pot trials, treatment of bean plants with a *Lactobacillus plantarum* strain before inoculation with *P. syringae* caused a significant reduction of the disease incidence (Visser, 1986). In view of greater need for developing microbial fertilizers using lactic acid bacteria and phosphate solubilizing bacterial consortia for increased yield of Spinach, the present investigation undertaken to isolate, characterize and evaluate the Lactic Acid Bacteria (LAB) for its phosphate solubilization potentiality using Spinach as the test crop.

## **Materials and Methods**

### **Isolation and characterization of Lactic Acid Bacteria**

The fresh milk and milk products were collected from canteen and hostel of Agriculture College Shivamogga for isolation of lactic acid bacteria. Further, the collected samples were serially diluted and plated on MRS media and incubated at 37<sup>0</sup>C for three days to isolate lactic acid bacterial isolates. Further all the bacterial isolates growing on the MRS media were examined for colony morphology, cell shape and biochemical characters Anon (1957) and Barthalomew and Mittewer (1950).

### **Collection of standard culture**

Pure cultures of phosphate solubilizing bacteria (*Bacillus megatherium*) were collected from Department of Agricultural Microbiology, College of Agriculture, Navile,

Shimoga. This was sub cultured by using Sperber's agar media and incubated at 37°C for 24-48 hours.

### **In vitro Screening of lactic acid bacterial isolates for phosphorus solubilization**

All the lactic acid bacterial isolates were spotted on Sperber's media for analyzing the phosphate solubilization potentiality of each isolates. Based on the zone of solubilization of phosphorus on the media the phosphate solubilizing potentiality of the lactic acid bacterial isolates was interpreted (Gaur, 1990).

### **Chemical method**

Isolates of the Lactic acid bacteria (10 ml of the overnight culture were inoculated to 100 ml of Pikovskaya's broth in 250 ml flask with equal number of uninoculated controls. The flasks were incubated on a mechanical shaker at 28<sup>o</sup> C for 10 days. The amount of pi released in the broth in flasks was estimated at 10 days after inoculation. The broth cultures of bacteria were centrifuged at 9000 rpm for 20 minutes in a centrifuge to separate the supernatant from the cell growth and insoluble phosphate. The available pi content in the supernatant/filtrate was estimated by phosphomolybdic blue colour method (Jackson, 1973).

### **Development and evaluation of efficient lactic acid and phosphate solubilizing bacterial consortia on growth of spinach**

The efficient lactic acid bacteria and standard phosphate solubilizing bacteria (*Bacillus megaterium*) were purified and streaked on the Nutrient agar for testing their compatibility. Based on the compatibility results the talc based formulation were prepared and evaluated for its influence on plant growth under field condition using Spinach as the test crop and the inoculations were made as single, dual and triple combination (Plate -1).

### **Treatment details of the field experiment**

T1 = Absolute control

T2 = Control (Compost + RDF)

T3 = LAB-5 (*Lactobacillus sp.*) + Compost + RDF

T4 = PSB (*Bacillus megaterium sp.*) + Compost + RDF

T5 = LAB-5(*Lactobacillus sp.*) + PSB (*Bacillus megaterium*) + Compost + RDF

### **Results and Discussion**

#### **Isolation and characterization of lactic acid bacteria**

Out of five different milk and milk products collected for the studies, as many as 5 lactic acid bacterial isolates were obtained and further they named as LAB-1, LAB- 2, LAB-3, LAB-4, and LAB-5.

#### **Characterization of lactic acid bacteria colony morphology**

The determination of the strain was performed according to their morphological, and biochemical characteristics as per the procedures described in the Bergey's Manual.

The colony characteristics of all lactic acid bacterial isolates are yellowish cream and round colonies. The cell morphology of all lactic acid bacterial isolates was Gram positive cocci, non-spore forming, non-motile and based on the biochemical test all lactic acid bacterial isolates were tentatively identified as *Lactobacillus sp.* (Table 1). The results are in agreement with the findings of Kacemet *al.*, (2004) who isolated *Lactobacillus plantarum* from fermented olives. Similarly, Chen *et al.*, (2010); Vijai pal *et al.*, (2006) also isolated

and characterized lactic acid bacteria isolated from ripe mulberries in Taiwan. As reported by Ibrahim and Anwar (2016) that, the milk and milk products are the good source for isolation of the lactic acid bacteria, the present study were concentrated to isolate the lactic acid bacteria from milk and milk products.

### ***In vitro* screening of lactic acid bacteria for phosphate solubilization potentiality**

The results were obtained phosphate solubilization potentiality of lactic acid bacterial isolates are furnished in Fig.1. All the 5 Lactic Acid Bacterial strains were plated on Sperber's media for evaluating phosphate solubilization potentiality. Out of five bacteria tested, the LAB-5 showed highest phosphate solubilization potentiality of 5.2 mm zone of solubilization of phosphorus on the Sperber's media whereas LAB-2, LAB-3, LAB-4 showed 3.5, 3.93, 4.0 mm zone of solubilization of phosphorus. On the other hand the LAB-1 did not produce any zone of hydrolysis on the Sperber's media (Plate 2). Further all isolates were inoculated to the Sperber's broth to know inorganic phosphate (Pi) released into the liquid medium. The highest Pi released was observed in LAB-5 on 10<sup>th</sup> day (5.8%) followed by LAB-4 (4.9%). However the lowest Pi released was found in LAB- 1, LAB-2 and LAB-3 (2.2, 3.1, and 3.3%) respectively. Similarly, Gaiind and Gaur, (1981) isolated and screened *Bacillus megatherium*, *B.brevis*, *B. cerculiance*, *Bacillus subtilis* from rhizosphere of Oat and Arhar.

### **Influence of efficient lactic acid and phosphate solubilizing bacterial consortia on growth of Spinach**

#### **Germination percentage and number of leaves**

The efficient lactic acid and phosphate solubilizing bacterial consortia in single,

double and triple inoculation combinations were evaluated to know their effect on germination percentage and number of leaves of Spinach. Statistically, highest germination percentage of 100 % was observed in the combined application of both LAB - 5 and PSB (*Bacillus megaterium*) along with recommended dose of fertilizers and compost followed by treatment 3 (LAB-5+Compost+RDF). Whereas low germination percentage was observed in absolute control (Table-2).

The perusal of table 2 and plate 3 clearly indicates that, the combined application of lactic acid bacteria and PSB will enhance the germination percent and number of leaves of spinach compared to individual application. Similar findings were reported by Adesemoye *et al.*, (2008) who evaluated different plant growth promoting *Pseudomonas* and *Bacillus* on growth and yield of Tomato, Okra and Amaranthus and concluded their influence on the germination percentage and number of leaves is more due to combined application of *Pseudomonas* and *Bacillus*.

#### **Total chlorophyll, fresh and dry weight**

Statistically the highest chlorophyll content of 3.81 mg/g of tissue was observed in the combined application of both LAB - 5 and PSB (*Bacillus megaterium*) along with recommended dose of fertilizers and compost followed by treatment 3 (LAB-5+Compost+RDF) followed by treatment 4 (2.85 mg/g of tissue) which showed the influence of the microbial isolates on the total chlorophyll. With respect to fresh and dry weight, the treatment receiving the combined inoculation of LAB - 5 and phosphate solubilizing bacteria along with the recommended compost and fertilizers performed well and recorded 40 and 6.20 g/plant of fresh and dry weight respectively.

**Table.1** Morphological and biochemical characteristic of isolates of lactic acid bacteria

Isolates	Morphological Test		Biochemical Test							
	Colony morphology	Grams reaction, and cell shape	Gelatin liquefaction	Starch hydrolysis	Casein hydrolysis	Catalase hydrolysis	Acid	Gas	H <sub>2</sub> S production	Probable genus
<b>LAB-1</b>	Medium round creamy to yellowish	+ ve rods	+	-	+	-	+	+	-	<i>Lactobacillus sp.</i>
<b>LAB-2</b>	Medium round creamy to yellowish	+ ve rods	+	+	+	-	+	+	-	<i>Lactobacillus sp.</i>
<b>LAB-3</b>	Medium round creamy to yellowish	+ ve rods	+	+	-	-	+	+	-	<i>Lactobacillus sp.</i>
<b>LAB-4</b>	Medium round creamy to yellowish	+ ve rods	+	+	+	-	+	+	-	<i>Lactobacillus sp.</i>
<b>LAB-5</b>	Medium round creamy to yellowish	+ ve rods	+	+	+	-	+	+	-	<i>Lactobacillus sp.</i>

Note: + = Positive to test, - = Negative to test

**Table 2.**Effect of lactic acid bacterial and phosphate solubilizing bacterial consortia on germination (%) and No. of leaves of spinach under field condition.

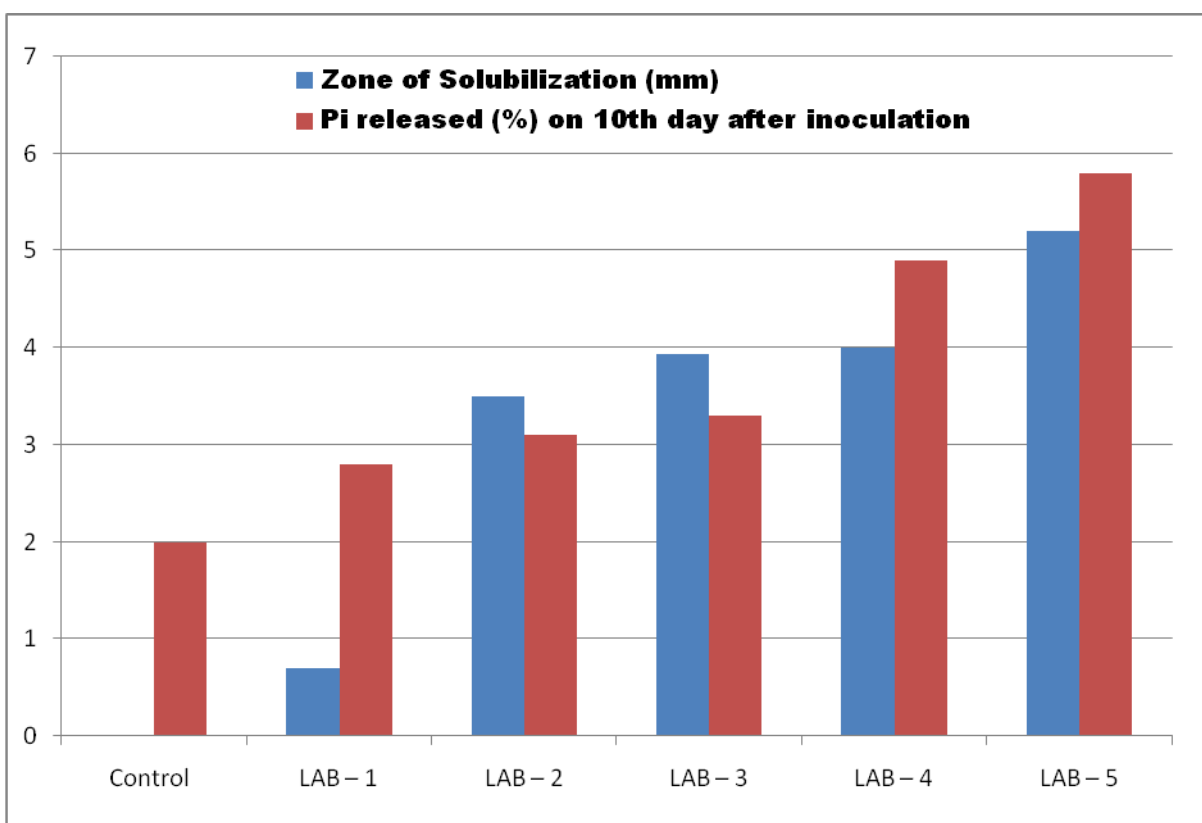
Sl. No.	Treatments	Germination (%)	No. of leaves per hill		
			15days	30days	45days
<b>1</b>	Absolute control	70.00 <sup>(c)</sup>	9.00 <sup>(d)</sup>	11 <sup>(e)</sup>	14 <sup>(d)</sup>
<b>2</b>	Control + compost + RDF	87.24 <sup>(d)</sup>	10.00 <sup>(c)</sup>	12 <sup>(d)</sup>	16 <sup>(b)</sup>
<b>3</b>	<i>LAB-5(Lactobacillus sp.)</i> + Compost + RDF	96.32 <sup>(b)</sup>	11.00 <sup>(b)</sup>	14 <sup>(b)</sup>	15 <sup>(c)</sup>
<b>4</b>	PSB( <i>Bacillus megaterium</i> sp) + Compost + RDF	94.00 <sup>(c)</sup>	10.00 <sup>(c)</sup>	13 <sup>(bc)</sup>	16 <sup>(b)</sup>
<b>5</b>	<i>LAB-5(Lactobacillus sp.)</i> + PSB ( <i>Bacillus megaterium</i> ) + Compost + RDF	100.00 <sup>(a)</sup>	12.00 <sup>(a)</sup>	17 <sup>(a)</sup>	23 <sup>(a)</sup>
<b>SEm ±</b>		0.46	2.00	3.02	3.10
<b>CD at 5%</b>		1.28	5.80	8.91	8.85

Note: 1. Absolute control = only soil without compost or fertilizer treatment  
 2. RDF = Recommended Dose of Fertilizer  
 3. Means followed by the same letters do not differ significantly

**Table.3** Influence of microbial inoculants on chlorophyll content at the time of harvest

Sl.No	Treatments	Chlorophyll content mg/g of tissue	Fresh weight (g/plant)	Dry weight (g/plant)
1	Absolute control	1.2 <sup>(e)</sup>	27 <sup>(e)</sup>	2.64 <sup>(d)</sup>
2	Control + Compost + RDF	2.57 <sup>(c)</sup>	30 <sup>(d)</sup>	4.12 <sup>(bc)</sup>
3	LAB-5( <i>Lactobacillus sp.</i> + Compost+ RDF	2.18 <sup>(d)</sup>	32 <sup>(c)</sup>	4.34 <sup>(c)</sup>
4	PSB + Compost +RDF	2.85 <sup>(b)</sup>	36 <sup>(b)</sup>	5.96 <sup>(b)</sup>
5	LAB-5( <i>Lactobacillus sp.</i> )+ PSB( <i>Bacillus megaterium</i> )+ Compost +RDF	3.81 <sup>(a)</sup>	40 <sup>(a)</sup>	6.28 <sup>(a)</sup>
	SEm ±	0.04	2.41	0.01
	CD at 5 %	0.13	5.16	0.08

**Figure.1** *In vitro* evaluation of lactic acid bacteria for phosphate solubilization



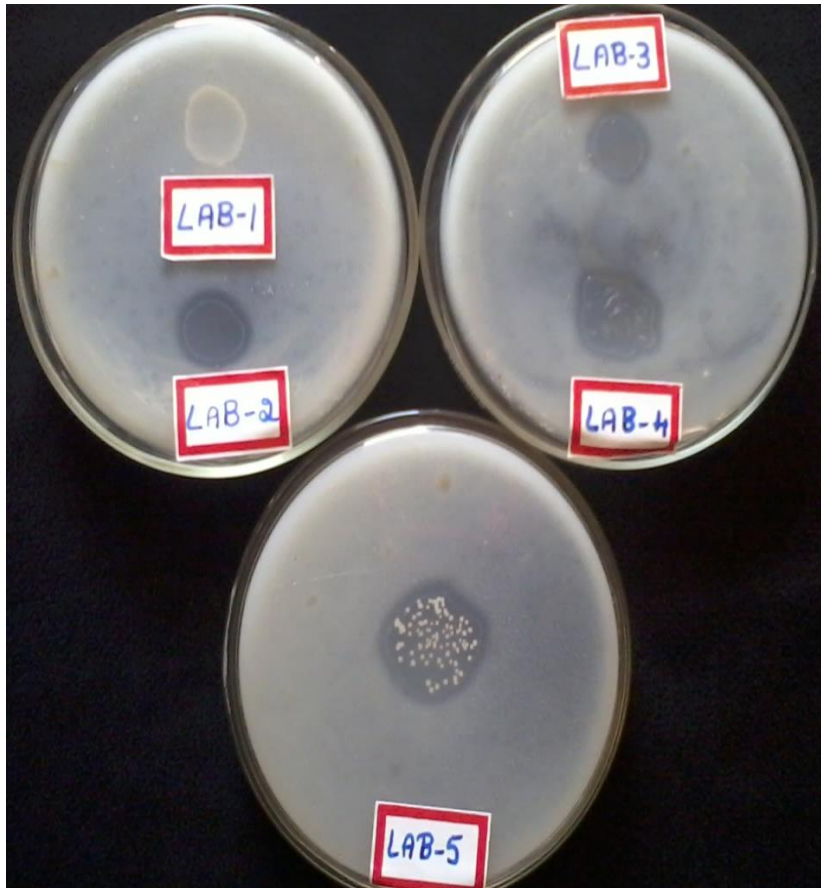


**Table.4** Influence of microbial inoculants on NPK content of spinach (mg/plant) at the time of harvest

Sl. No.	Treatments	Plant NPK			Soil NPK		
		Nutrient (mg/plant)			Nutrient (kg/ha)		
		N	P	K	N	P	K
1.	Absolute control	152.33 <sup>(e)</sup>	145.33 <sup>(d)</sup>	138.33 <sup>(e)</sup>	140.33 <sup>(e)</sup>	16.00 <sup>(e)</sup>	130.67 <sup>(d)</sup>
2.	Control + Compost + RDF	251.33 <sup>(c)</sup>	254.66 <sup>(c)</sup>	162.67 <sup>(bc)</sup>	198.00 <sup>(a)</sup>	22.57 <sup>(d)</sup>	142.67 <sup>(c)</sup>
3.	LAB-5( <i>Lactobacillus sp.</i> + Compost+ RDF	265.00 <sup>(b)</sup>	260.00 <sup>(c)</sup>	153.33 <sup>(d)</sup>	184.00 <sup>(c)</sup>	24.83 <sup>(c)</sup>	150.33 <sup>(b)</sup>
4.	PSB + Compost +RDF	234.00 <sup>(d)</sup>	265.00 <sup>(b)</sup>	160.33 <sup>(b)</sup>	180.00 <sup>(d)</sup>	26.67 <sup>(b)</sup>	152.67 <sup>(a)</sup>
5.	LAB-5( <i>Lactobacillus sp.</i> )+ PSB ( <i>Bacillus megaterium</i> ) + Compost +RDF	278.00 <sup>(a)</sup>	298.00 <sup>(a)</sup>	172.33 <sup>(a)</sup>	190.00 <sup>(b)</sup>	28.84 <sup>(a)</sup>	153.67 <sup>(a)</sup>
	SEm ±	6.48	6.46	5.48	12.58	9.15	18.62
	CD (5 %)	1.98	1.96	1.90	2.68	2.21	3.18

**Plate 1.**Over view of field experiment





**Plate 2.** Phosphate solubilization of potentiality of Lactic acid bacterial isolates



**Plate.3** Comparative view of best treatments



However, the treatment 4 where only phosphate solubilizing bacteria and recommended dose of compost and fertilizers as used showed the statistically on par results (36 and 5.96 g/plant) with the treatment 5 (Table 3). The results of the present study was strongly supported by the findings of Mathaura et al., (2010) who evaluated effective microbial formulations on Amaranthus and concluded that the leaf area, leaf fresh and dry weight and chlorophyll content is influenced by microbial formulation applications in the field studies.

### **Influence of efficient lactic acid and phosphate solubilizing bacterial consortia on NPK content of plant and soil**

With reference to NPK levels in the plants, all the treatments showed the accumulation of all the three major nutrients. Among five treatment imposed the treatment receiving combined formulation of LAB – 5 and PSB showed significantly highest NPK content of 278, 298 and 172.33 mg/plant in the economic part of the Spinach.

Followed by the single inoculation of PSB formulation in combination with compost and RDF. However when the soil nutrient status was analyzed chemically, similar results were obtained in combined application (190, 28.84 and 153.67 NPK respectively) and the least was observed in absolute control (Table 4). The result of the study confirm the work of Ishque *et al.*, (2009) and Vasanthkumar (2003) who concluded the maximum accumulation of residual nitrogen and phosphorus is more due to the combined application of N fixers and P solubilizers were used. Similarly, Park et al., (2003) also proved the bacterial inoculations could improve P and K availability in the soils by producing organic acid and other chemicals by stimulating growth and mineral uptake.

### **References**

- Adesemoye, A.O., 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Can.J. Microbiol.* 54:876-886.
- Anonymous, 1957, Manual of microbiological method. McGraw Hill Book Company Inc., New York, p.127.
- Bartholomew, J.W. and Mittewer J. 1950. A simplified bacterial strain. *Stain Tech.* 25: 153.
- Chen Yi-sheng, Hui-chung Wu, and Fujitoshi Yanagida. D, 2010. Isolation and Characteristics of Lactic Acid Bacteria Isolated from ripe Mulberries in Taiwan. *Brazilian J. Microbiol.*, 41: 916-921.
- Gaind, S. and Gaur, A. C., 1991. Thermotolerant phosphate solubilising microorganisms and their interaction with mung bean. *Plant and Soil*, 133: 141-149.
- Gaur, A.C, 1990. *Phosphate Solubilizing Microorganisms as Biofertilizer*, Omega Scientific Publishers, New Delhi, pp. 50-55.
- Ishque, Muhammad Iqbal, Muhammad SaleenJilani and KashifWaseem, 2009. Effect of nitrogen levels on lettuce growth and yield, *J.Agric.Res*, 47: 405-412.
- Ibrahim Khalil and Anwar Nural, 2016, Isolation, Identification and Characterization of Lactic Acid Bacteria from Milk and Yoghurts. *Research & Reviews: Journal of Food and Dairy Technology.* 4:17 – 26.
- Jackson, M.L. 1973, *Soil Chemical Analysis*, Prentice Hall of India (P.) Ltd., New Delhi.
- KacemMourad, ZadiKaram Halima and KaramNour-Eddine, 2004. Isolation of lactic acid bacteria for its possible use

- in the fermentation of green algerian olives. *Grasas Y Aceites*, 55:385-393.
- Muthaura, C. Musyimi, D. M. Ogur, J.A. Okello, S.V., 2010, Effective microorganisms and their influence on growth and yield of pigweed (*Amaranthus dubians*). *J. of Agril. Biol. Sci.* 5:17-22
- Park M. Singvilay D. Seok Y, Chung J, Ahn K and Sat. 2003. Effect of phosphate solubilizing fungi on 'P' uptake and growth of tobacco in rock phosphate. *Appl. Soil Korean J. Soil. Sci. Fertil.*, 36: 233-238.
- Vijai Pal, Marilingappa Jamuna and Kadirvelu Jeevaratnam. 2006. Isolation and Characterization of bacteriocin producing Lactic Acid Bacteria from south Indian special dosa (Appam) batter. *Journal of Culture Collections*, 4: 53-60.
- Vasanthakumar, S. K., 2003. Studies on beneficial endorhizosphere bacteria on solenaceous crop plants. *M.Sc. (Agri.) Thesis, Univ. of Agril.Sci., Dharwad (India)*.
- Visser Ronel, Wilhelm H. Holzapfel, Johannes J. Bezuidenhout, T and Johann M. Kotze, 1986, Antagonism of Lactic Acid Bacteria against Phytopathogenic Bacteria. *Appl. Environ. Microbiol.*, 52: 552-555.

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