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THE UTILIZATION OF PHOSPHATE SOLUBILIZING LACTIC ACID BACTERIA IN PLANT GROWTH PROMOTION

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ABSTRACT

Lactic acid bacteria (LAB) are a heterogenous group of acid-tolerant, salt-tolerant, phosphate solubilizing microbes being able to produce lactic acid on carbohydrate fermentation. Since the advancements in plant-microbe interactions research have enhanced the significance of microbial communities in promoting soil productivity, therefore, our current study aims to isolate and characterize those LAB strains that possess antimicrobial, phosphate-solubilizing potential and plant growth promoting properties *in vitro*.

Isolation of LAB was done from milk, meat, rice, fruits, vegetables and soil and the isolated strains were identified up to genus level by catalase test and Gram staining. The isolated strains were screened for antibacterial potential against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus faecalis*. Antifungal activity was determined against predominant phytopathogens, *Aspergillus niger*, *Fusarium oxysporum*, *Macrophomina phaseolina* and specie belonging to genus *Penicillium*. Phosphatase activity of the promising strains was demonstrated on National Botanical Research Institute's phosphate growth medium (NBRIP). The most promising isolate *Lactobacillus* MBV41 was grown in the presence of 5% NaCl to assess its salt tolerance capabilities. Finally, fresh tomato seeds were coated with the most promising strain MBV41 to analyze the potential of LAB in plant growth promotion. Twenty-five strains were successfully isolated among which 21% species of *Lactococci* and 27% *Lactobacilli* species showed significant antibacterial activity against *Pseudomonas aeruginosa* and *Streptococcus faecalis* and effective antifungal activity was also observed against the tested phytopathogens. Significant phosphatase production in vitro was demonstrated by 12% Lactobacilli species giving a maximum halo of 30mm. Our isolated strain, MBV41 exhibited strong tolerance to 5% NaCl and was able to increase the root and shoot length of the tomato plant giving a germination percentage of 51% and seedling vigor index of 558.4.

Our findings justify the use of the LAB strains as plant growth promoters. Also, the strong antagonism of our LAB strains towards phytopathogens makes them effective against preventing plant diseases which could minimize the use of hazardous pesticides. Still elaborate studies are needed until they can be utilized to enhance the soil fertility and plant growth.

Key-words: Lactic acid bacteria, antimicrobial activity, phosphate solubilizing bacteria, plant growth promotion

INTRODUCTION

Lactic acid bacteria (LAB) are traditionally rated as universal and diverse group of microorganisms that can ferment a range of nutrients mainly into lactic acid (Brooijmans *et al*., 2009). They are usually detected in carbohydrate rich sources including food and feed, also in human and animal cavities and in sewage and plant matter *(*Kandler and Weiss, 1986). It is a heterogeneous group of Gram positive and non-spore forming facultative anaerobic bacteria (Angelova and Beshkova, 2015). Lactic acid bacteria (LAB) may appear as cocci or bacilli and forms white, small and pinpointed colonies on de Man, Rogosa and Sharpe (MRS) agar plates (Ikeda *et al*., 2013).

The antagonistic activity of LAB towards Gram positive pathogens is observed because of bactericidal effect of protease sensitive bacteriocins (Jack *et al*., 1995). On the other hand, the inhibitory activity against Gram negative pathogens is associated with the production of organic acids and hydrogen peroxide (Ito *et al*., 2003). Whereas, the antagonism against fungi is a result of the production of a collection of antifungal compounds including organic acid, fatty acids, proteinaceous compounds, cyclic dipeptides, phenolic compounds and hydrogen peroxide. Many of the studies have reported the isolation of antifungal compounds from *Lactobacillus* species due to which they are being used for biocontrol of variety of microbial phytopathogens to boost agriculture and for biopreservation in various foods as majority of LAB are generally recognized as safe (Gajbhiye and Kapadnis, 2016).

Nowadays, organic farming has been established as one of the best approach that not only promotes food safety but also enhances the biodiversity of soil (Megali *et al*., 2013) and prolongs the shelf life of crop without affecting the environment (Bhardwaj *et al*., 2014). When plant growth promoting microbes are coated on seeds or

incorporated in soil, they proliferate and contribute in nutrient cycling and increase crop yield (Singh *et al*., 2011). The phosphate solubilizing potential makes LAB eligible to be used as an alternative to agrochemicals. Phosphorus is considered to be the second most important constituent in plant development after nitrogen (Sharma *et al*., 2013) as it participates in primarily all important metabolic processes in plant including photosynthesis, energy transfer, signal transduction, biosynthesis of macromolecules, respiration (Khan *et al*., 2010) and nitrogen fixation in legumes (Hussain, 2017). Even though soil is rich in phosphorus in both inorganic and organic forms, it becomes unavailable in the form required for plant root uptake. The recurrent use of chemical fertilizers tends to the accumulation of inorganic phosphorus in the form of insoluble mineral complexes which are not absorbed by the plants (Rengel and Marschner, 2005). Therefore Phosphate solubilizing microbes such as LAB are employed in soil where they transform the insoluble phosphorus into soluble form thus increasing the availability of phosphorus for plant absorption (Zhu *et al*., 2011). Phosphate solubilizing microorganisms (PSM) exhibit combined effect on the growth and productivity of crops. Along with solubilization of phosphorus, many PSM also play a prominent role as bio control agents exhibiting antagonistic effect towards various phytopathogens (Tallapragada and Gudimi, 2011; Alori *et al*., 2017).

The purpose of our study was to assess the potential of Lactic acid bacteria to inhibit phytopathogens and their ability to make phosphorous available in the soil for plant growth promotion.

MATERIALS AND METHODS

Isolation

Twenty-five strains of LAB were successfully isolated from various sources including milk, grapes (*Vitis vinifera*), oranges (*Citrus sinensis*), strawberry *(Fragaria ananassa*), onion (*Allium cepa*), tomato (*Lycopersicon esculentum*), rice (*Oryza sativa*), date plant soil (*Phoenix dactylifera*) and meat. Isolation was done on de Man Rogosa Sharpe (MRS) agar by Standard spread plate method by serially diluting the samples in phosphate buffer saline (Thatcher and Clark, 1986; Liu *et al*, 2012). The LAB isolates were characterized preliminarily by Gram and Catalase reaction.

Bacterial and Fungal strains

The pure cultures of *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis* and *Pseudomonas aeruginosa* were obtained from the culture collection and maintenance unit, Department of Microbiology, University of Karachi. The fungal cultures of *Aspergillus niger*, *Fusarium oxysporum*, *Macrophomina phaseolina* and specie belonging to genus *Penicillium* were collected from Department of Botany, University of Karachi.

Antibacterial activity

The supernatants of LAB isolates were divided in two aliquots, one used in its crude form as cell free supernatant (CFS) and the other as neutralized cell free supernatant (NCFS). The suspensions of freshly grown bacterial indicators matched with standard 0.5 McFarland index were inoculated in semisolid nutrient agar and then overlaid onto prepared nutrient agar plates. Wells (7mm in diameter) were made and 200µL of LAB supernatents (CFS and NCFS) were added to their respective wells. The plates were placed at 4ºC for 45 minutes and results were recorded after 24 h of incubation at 37ºC.The results were observed in terms of the measurement of zones of inhibition in mm (Vankedesin and Sumathi, 2015).

Antifungal activity

The size of inoculum of fungi was adjusted between 1.0×10^6 to 5.0×10^6 spores/mL with the help of hematocytometer (Kivanc *et al*., 2014). Antifungal activity was determined as described by (Magnusson and Schnurer, 2001) with slight modifications. A lawn of fungal indicator was made on the prepared Sabouraud dextrose agar plates instead of overlaying the fungal cultures. The rest of the assay was conducted following the same protocol as that of antibacterial activity.

ASSESSMENT OF LAB IN PLANT GROWTH PROMOTION

Phosphate solubilization Assay

The pre-incubated National Botanical Research Institute Phosphate Growth medium (NBRIP) plates were stabbed with 24 h old cultures of LAB. The plates were kept at 30ºC for 24 h and the amount of inorganic phosphate solubilized by phosphatase enzyme produced by LAB was recorded in terms of the measurement of total diameter of the halo and the colony diameter as described by Nautiyal (1999) by using the following formula, Phosphate solubilization = Total diameter of Halo – Colony diameter.

Salt Tolerance Assay

Broth de Man Rogosa Sharpe (MRS) supplemented with 5% NaCl was inoculated with 10^6 cells as described by Huang *et al*, (2014). This experiment was run in triplicate and the standard error was calculated. The results were recorded in terms of cfu/mL and absorbance (at 650 nm) after 24, 48, 72, 96 and 144 h of incubation. Numbers of colonies were determined by Miles and Misra technique (Miles *et al*, 1938).

***IN VIVO* ASSESSMENT OF LAB AS A BIOFERTILIZER**

Seed treatment and germination of tomato seedlings

Fresh tomato seeds were soaked for overnight in 5 mL MRS broth inoculated with 10^6cfu/mL of MBV41. The seeds were then placed in pots supplemented with garden soil to determine the effect of LAB on seed germination.

Simultaneously, another set of test was also run in which fresh tomato seeds were surface sterilized with ethanol and $HgCl_2$ respectively and rinsed with autoclaved distilled water (Caetano-Anolles *et al*., 1990).The sterilized seeds were also sown in garden soil in triplicate to determine the efficacy of LAB after the removal of contaminating microbes.

After three weeks, the germination percentage and seedling vigor index was calculated by the following formula (Ashwini and Giri, 2014):

Germination % = Number of germinated seeds / Number of total seeds x 100.
Seedling vigor index = [Mean root length (cm) + mean shoot length (cm)] × germination percentage.

RESULTS

Isolation

A total of twenty-five LAB strains were successfully isolated from various traditional and nontraditional sources. From milk (36%), rice (*Oryza sativa*) 16%, meat (16%), tomato (*Lycopersicon esculentum*) 8%, strawberry (*Fragaria ananassa*) 8%, grapes (*Vitis vinifera*) 4%, orange (*Citrus sinensis*) 4%, onion (*Allium cepa*) 4%, and date plant soil (*Phoenix dactylifera*) 4% LAB strains were isolated. All isolates were found to be catalase negative and Gram-positive with varying morphologies and arrangements. Among the isolated strains, 52% were cocci while 48% were found to be bacilli (Fig. **1**).

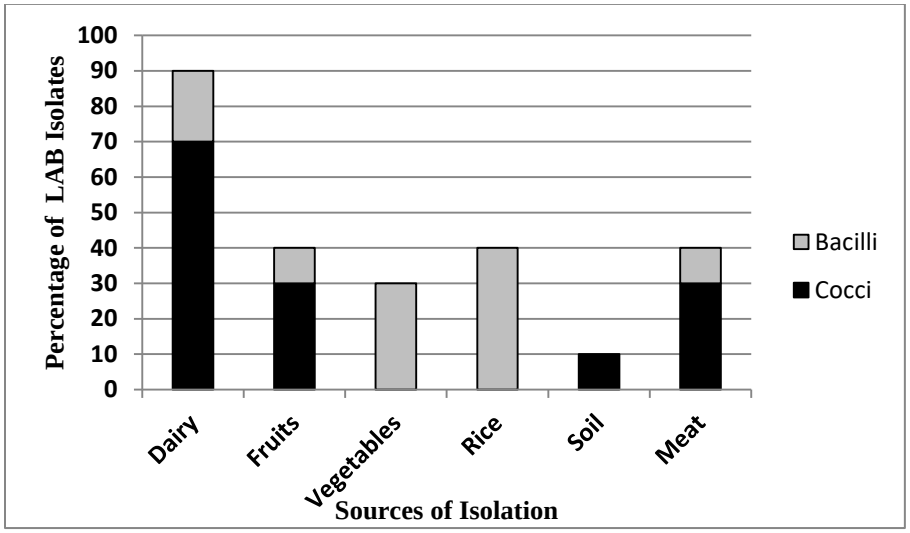

Fig. 1. Sources of isolation of Lactic acid bacteria (LAB) and their morphology.

Screening for antibacterial activity of LAB

Out of 25 LAB isolates, 96% strains showed inhibition towards the tested bacterial pathogens. Broad spectrum of antagonism against all the tested bacterial indicators was demonstrated by 20% isolates while 12% exhibited inhibition against *Staphlococcus aureus, Streptococcus faecalis* and *Bacillus subtilis,*16% demonstrated inhibitory action against *Pseudomonas aeruginosa* (Table. **1**).

Table 1. Antibacterial activity of LAB isolates.

S.NO	STRAIN	Zone of Inhibition (mm)							
		B. subtilis		*P. aeruginosa*		*S. aureus*		*S. faecalis*	
		C.F.S	N.C.F.S	C.F.S	N.C.F.S	C.F.S	N.C.F.S	C.F.S	N.C.F.S
1	MCM13	24 ± 0.10	0 ± 0.0	22 ± 0.15	0 ± 0.0	23 ± 0.02	0 ± 0.0	23 ± 0.11	20 ± 0.08
2	MB014	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	22 ± 0.05	0 ± 0.0	22 ± 0.08	0 ± 0.0
3	MCB16	0±0.0	0±0.0	20±0.03	0±0.0	0±0.0	0±0.0	18±0.10	0±0.0
4	MCS17	0±0.0	0±0.0	17±0.15	0±0.0	24±0.10	0±0.0	22±0.05	0±0.0
5	MCS18	0±0.0	0±0.0	17±0.09	0±0.0	23±0.06	0±0.0	20±0.05	0±0.0
6	MBK19	0±0.0	0±0.0	0±0.0	0±0.0	21±0.03	0±0.0	19±0.03	0±0.0
7	MBR23	19±0.06	0±0.0	23±0.06	19±0.08	22±0.10	0±0.0	20±0.15	18±0.11
8	MBR24	19±0.08	0±0.0	21±0.08	17±0.15	20±0.08	0±0.0	19±0.09	17±0.04
9	MBR25	21±0.04	0±0.0	24±0.04	17±0.10	20±0.15	0±0.0	22±0.17	20±0.03
10	MBR26	0±0.0	0±0.0	18±0.02	0±0.0	23±0.09	0±0.0	21±0.04	19±0.09
11	MCC38	0±0.0	0±0.0	17±0.10	0±0.0	20±0.08	0±0.0	25±0.05	24±0.15
12	MCC39	21±0.02	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	25±0.11	24±0.10
13	MCC40	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	25±0.06	20±0.07
14	MBV41	18±0.05	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	25±0.03	20±0.05
15	MBV42	18±0.04	0±0.0	16±0.17	16±0.09	0±0.0	0±0.0	24±0.04	0±0.0
16	MCM3	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	21±0.15	0±0.0
17	MCM8	24±0.03	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	20±0.17	0±0.0
18	MCM11	0±0.0	0±0.0	21±0.08	0±0.0	22±0.05	0±0.0	20±0.08	0±0.0
19	MCM29	0±0.0	0±0.0	0±0.0	0±0.0	20±0.04	0±0.0	15±0.05	0±0.0
20	LDSC 2	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	15±0.02	0±0.0
21	LGPC 5	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	17±0.11	0±0.0
22	LMCB10	18±0.015	0±0.0	20±0.05	0±0.0	22±0.08	0±0.0	20±0.05	0±0.0
23	LMC13	19±0.08	0±0.0	22±0.05	0±0.0	0±0.0	0±0.0	18±0.07	0±0.0
24	LMCB14	22±0.10	0±0.0	19±0.13	0±0.0	0±0.0	0±0.0	21±0.05	0±0.0
25	LMC16	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0

*= zone of inhibition including borer diameter (7mm); SE ±

Screening for antifungal activity of LAB isolates

Amongst the isolated LAB strains, 92% showed antagonism against the tested fungal phytopathogens. Out of 25 LAB strains, 20% demonstrated antagonism against *Fusarium oxysporum, Macrophomina phaseolina* and *Penicillium* sp., 16% exhibited antifungal against *Aspergillus niger*. Lactobacilli MBR24 and MBV41 showed significant antagonism against all the fungal phytopathogens (Table. **2**).

Solubilization of phosphate by LAB isolates

The isolated LAB strain Lactobacilli MBR25 and MCC40 demonstrated a visible halo zone of 30mm and 20mm respectively indicating maximum phosphate solubilization and ultimate phosphatase enzyme production as compared to other isolates (Fig. **2** and **3**).

Table 2. Antifungal activity of LAB isolates.

S.NO	STRAIN	Zone Of Inhibition (mm)							
		A. niger		*F. oxysporum*		*M. phaseolina*		*Penicillium* sp.	
		C.F.S	N.C.F.S	C.F.S	N.C.F.S	C.F.S	N.C.F.S	C.F.S	N.C.F.S
1	MCM13	0±0.0	0±0.0	0±0.0	0±0.0	24±0.15	22±0.06	19±0.08	18±0.13
2	MB014	20 ± 0.02	18 ± 0.04	0±0.0	0±0.0	18 ± 0.04	20 ± 0.04	20 ± 0.13	18 ± 0.09
3	MCB16	22±0.10	17±0.05	0±0.0	0±0.0	0±0.0	0±0.0	20±0.05	19±0.02
4	MCS17	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
5	MCS18	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
6	MBK19	19±0.11	0±0.0	0±0.0	0±0.0	17±0.09	15±0.03	24±0.02	20±0.04
7	MBR23	0±0.0	0±0.0	22±0.11	0±0.0	20±0.11	18±0.15	16±0.03	15±0.05
8	MBR24	20±0.05	19±0.15	21±0.07	20±0.09	25±0.05	20±0.05	21±0.04	16±0.13
9	MBR25	0±0.0	0±0.0	23±0.05	17±0.04	23±0.15	22±0.07	17±0.08	15±0.11
10	MBR26	0±0.0	0±0.0	21±0.03	18±0.05	22±0.07	21±0.03	18±0.05	17±0.07
11	MCC38	0±0.0	0±0.0	23±0.07	20±0.11	23±0.13	20±0.04	20±0.13	0±0.0
12	MCC39	18±0.03	16±0.04	21±0.09	18±0.07	21±0.05	18±0.11	18±0.15	0±0.0
13	MCC40	0±0.0	0±0.0	19±0.15	0±0.0	19±0.08	0±0.0	19±0.07	0±0.0
14	MBV41	19±0.07	18±0.03	25±0.05	22±0.03	26±0.02	0±0.0	22±0.08	21±0.04
15	MBV42	19±0.06	16±0.05	0±0.0	0±0.0	0±0.0	0±0.0	24±0.11	20±0.09
16	MCM3	20±0.15	18±0.06	0±0.0	0±0.0	25±0.11	0±0.0	19±0.05	15±0.07
17	MCM8	23±0.05	19±0.15	0±0.0	0±0.0	0±0.0	0±0.0	23±0.01	18±0.11
18	MCM11	0±0.0	0±0.0	23±0.02	0±0.0	0±0.0	0±0.0	22±0.03	20±0.05
19	MCM29	16±0.11	16±0.04	0±0.0	0±0.0	0±0.0	0±0.0	20±0.05	19±0.11
20	LDSC 2	17±0.06	16±0.11	22±0.08	20±0.05	0±0.0	0±0.0	19±0.06	18±0.08
21	LGPC 5	0±0.0	16±0.15	24±0.05	21±0.11	0±0.0	0±0.0	0±0.0	0±0.0
22	LMCB10	20±0.08	17±0.02	0±0.0	0±0.0	22±0.04	20±0.13	21±0.11	19±0.04
23	LMC13	21±0.02	19±0.06	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
24	LMCB14	16±0.03	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	20±0.05	0±0.0
25	LMC16	22±0.04	15±0.05	0±0.0	0±0.0	22±0.05	18±0.06	0±0.0	0±0.0

*= zone of inhibition including borer diameter (7mm); SE ±

Salt tolerance

Lactobacillus MBV 41, when grown in 5% NaCl concentration for 144 h was able to survive till 96 h with average growth of 9.3cfu/mL at 24 h which gradually decreased with time. No significant difference in the absorbance and growth was observed when compared with the control throughout the experiment (Fig. **4**).

Seed treatment and germination of tomato seeds

We observed significant increase in the germination percentage and seedling vigor index of seeds coated with the strain MBV41 as compared to control. The test strain demonstrated average germination percentage of about 51% with average seedling vigor index of about 558.4.

Simultaneously, the sterilized tomato seeds coated with Lactobacillus MBV 41 showed germination percentage 44.4% with seedling vigor index of about 460.4. Whereas the control of sterile seeds showed germination percentage and seedling vigor index of about 10 and 60.6 respectively (Fig. **5a and 5b).** Also the tomato plants grown from coated seeds showed an increase in root and shoot length as compared to the uncoated ones.

The data of Fig. 5a when calculated for X^2 using 2 x 2 contingency table (Table 3) (Bishop, 1968), gave X^2 = 11.11 ($p < 0.001$) against a tabulated value of 10.83 at df = 1 (Simpson *et al.*, 1960) showing significant difference in germination of tomato seeds under two sets of conditions. The coefficient of strength of association (V) appeared to be 0.2865 indicated a positive association between freshness of seeds and coating of *Lactobacillus* MBV 41 but not strong enough.

The coating of *Lactobacillus* MBV 41 on seeds had highly significant effects on seedling vigor (Fig. 5b; Table 4). The magnitude of X^2 (as given by 2 x 2 contingency table) was equal to 105.56 which was highly significant ($p < 0.0001$) against a tabulated value of 10.83 (df = 1). The magnitude of coefficient of association (V) was found to be 0.8701 indicating significantly strong positive association of particularly the freshness of seeds with application of coating of MBV 41 on to the seeds. The coating of seeds with MBV 41 was more effective on seedling vigor than on the seed germination.

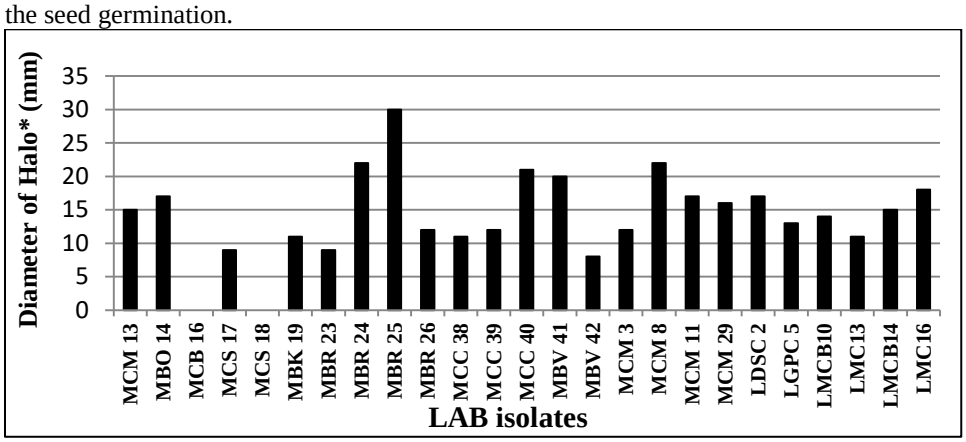

Fig. 2. Phosphate Solubilization of LAB isolates.
*= Diameter of halo excluding colony diameter (mm)

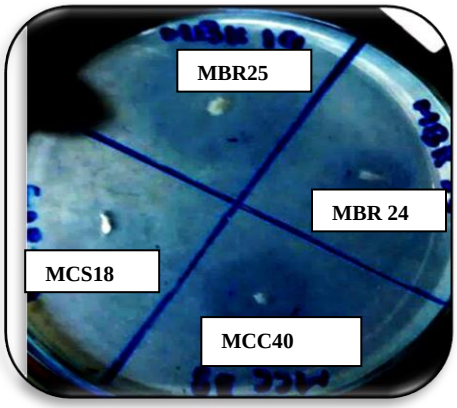

Fig. 3. LAB isolates demonstrating clear halo around the colony.

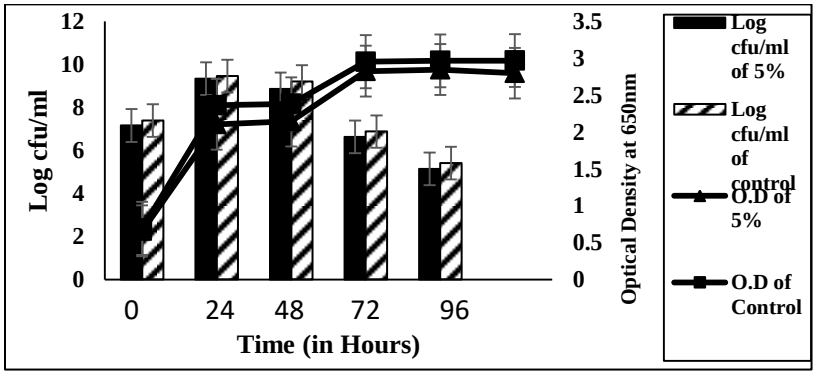

Fig. 4. Salt tolerance by LAB isolate MBV 41 at 5% NaCl.

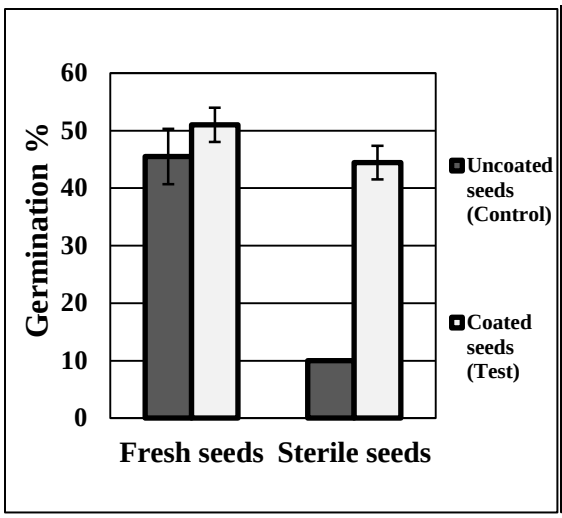

Fig. 5a. Germination % of Tomato seeds coated and uncoated with MBV41.

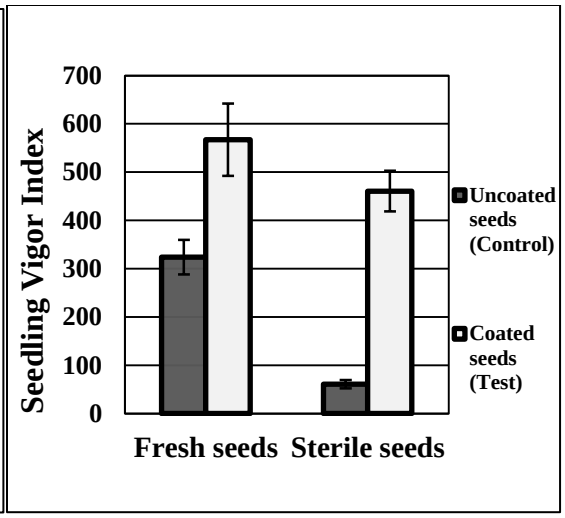

Fig. 5b. Vigor Index of Tomato seeds coated and uncoated with MBV41.

Table 3. 2 x 2 contingency table for mean germination data.

State		MBV 41		Total
		Uncoated	Coated	
Seed state	Fresh	a = 45.5	b = 51	96.5
	Sterilized	c = 10	d = 44.4	54.4
Total		55.5	95.4	
$X^2 = 11.11$ ($p < 0.001$); V = 0.2865				

Table 4. 2 x 2 contingency table for mean seedling vigor index data.

State		MBV 41		Total
		Uncoated	Coated	
Seed state	Fresh	a = 323.7	b = 567	890.7
	Sterilized	c = 60.6	d = 460.42	521.02
Total		384.3	1027.42	
$X^2 = 105.56$ ($p < 0.0001$); V = 0.8701				

$X^2 = n (ad – bc - n/2)^2 / (a + b) (c + d) (b + d) (a + c)$……here n = a + b + c + d. The item n/2 is correction factor.
Coefficient of association = $V = ad – bc / \sqrt{(a + b) (c + d) (b + d) (a + c)}$ …….here a, b, c and d are the usual entries of the 2 x 2 contingency table.

DISCUSSION

Lactic acid bacteria can be conventionally isolated from raw milk, dairy products and fermented foods (Widyastuti *et al*., 2014; Wouters *et al*., 2002 ; Kimoto *et al*., 2004; Tamang *et al*., 2005; Nomura *et al*. 2006; Kostinek *et al*., 2007; Tanasupawat *et al*., 2007) and from nontraditional source such as fecal material, soil and plant materials (Hartnett *et al*., 2002*;* Magnusson *et al.*, 2003*;* Cock and de Stouvenel, 2006; Siezen *et al*., 2008; Trias *et al*., 2008*)*. Lactic acid bacteria are commonly isolated from vegetables, aerial plant surfaces, pickled cabbage, grass silage, fermented cereals and also from soil (Gajbhiye and Kapadnis, 2016).

We have also isolated 16% of our LAB strains from meat resources that were observed to possess antibacterial and antifungal potential against *Streptococcus faecalis, Macrophomina phaseolina* and *Fusarium oxysporum*. In another study 61% LAB strains from fresh meat samples were isolated demonstrating broad spectrum of antibacterial activity against *S. aureus*, *Listeria monocytogenes* and *Listeria innocua* (Bromberg *et al*., 2004).

We were able to isolate 36% LAB from milk, out of which 77.8% were found to be Lactococci while the other 22.2% were Lactobacilli. Many researchers in earlier studies have demonstrated the isolation of LAB for the first time from milk (Carr *et al*., 2002; Metchnikoff, 1908; Sandine et *al*., 1972). Lactic acid bacteria species like

Lactobacillus plantarum and *Lactococcus lactis* have been commonly isolated from plant material (Kostinek *et al*., 2007; Escalante-Minakata *et al*., 2008; Trias *et al*., 2008). We have isolated 4% of the strains from soil samples demonstrating strong antifungal activity and increased phosphatase activity. Similarly, the isolation of LAB from rhizospheres of fruit trees exhibiting characteristic antibacterial potential against a number of Gram-positive bacteria was observed in another study (Chen *et al*., 2005).

During the screening for antimicrobial activity of LAB we found that 40 % of our isolates were assumed to be bacteriocin producers inhibiting variety of the bacterial and fungal pathogens. Such broad spectrum of antibacterial potential of LAB was also demonstrated against *Staphylococcus aureus* and *Listeria innocua* (Bromberg *et al*., 2004). Efficient antibacterial activity was observed against *Streptococcus faecalis* and *Pseudomonas aeruginosa*, while reduced inhibitory action was observed against *Bacillus subtilis* and *Staphylococcus aureus.* Another research demonstrated widespread inhibition of Gram positive and Gram negative bacterial pathogens with varying potential by different LAB isolates (Kazemipoor *et al*., 2012).

Role of LAB as a biocontrolling agent against fungal spoilage was also demonstrated in earlier studies (Sathe *et al*., 2007).The cell free supernatants of 92% of our LAB strains showed antifungal activity against various fungal pathogens including *Aspergillus niger, Fusarium oxysporum, Macrophomina phaseolina and* specie belonging to genus *Penicillium*. The antifungal inhibition by cell free supernatants of LAB isolates was also observed in a study against different fungal pathogens like *Aspergillus niger, Aspergillus tubingensis* and *Penicillium crustosum* (Ndagano *et al*., 2011). All our LAB isolates were able to inhibit fungal pathogens after 24-30 h of incubation. Whereas, in one such study no inhibitory action of one of the LAB specie *Enterococcus durans* was observed against *Aspergillus fumigatus* even after 6 days (Kıvanc *et al.*, 2014). We isolated 12% of LAB strains from vegetables resources that were found to possess strong antifungal activity. Similarly, *Weisella paramesenteroides* and *Lactobacillus paracollinoides* were isolated from fresh vegetables possessing fungal antagonistic activity. This study also suggested that the antifungal activity of *Lactobacillus plantarum* was growth dependent which gradually increases with the extension of logarithmic phase and then ultimately declines during the stationary phase (Sathe *et al*., 2007).

Phosphatase enzyme production was demonstrated by 92% strains, thus solubilizing phosphate and making phosphorus available for plant absorption. Large halo was demonstrated by Lactobacilli MBR25 and MBV41 indicating maximum phosphate solubilization when inoculated on NBRIP medium. Strains of Lactobacilli demonstrating higher phosphatase activity was also demonstrated by other researcher (Palacios., *et al.*, 2005; Haros *et al*., 2008).

As it is an essential requirement for LAB to survive under harsh environments of soil such as high salt concentrations in order to promote plant growth and development, *Lactobacillus* MBV 41 was able to grow well for 96 h in 5% NaCl. One such study reported the survival of eight out of 17 LAB isolates at 4% NaCl while only the suspected *Lactobacillus casei* specie was found to grow at 6.5% NaCl (Ekundayo, 2014). Another research demonstrated the production of lactic acid by LAB isolate at 0%, 4%, 8%, 12% and 16% NaCl respectively. They found that the production of lactic acid gradually decreases with the increase in the concentration of NaCl (Huang *et al*., 2014).

The application of LAB for plant protection against bacterial and fungal pathogens and for plant growth promotion have been widely reported (Trias *et al*., 2008; Giassi *et al*., 2016). Our strain MBV 41 demonstrating significant results in antifungal activity, phosphatase enzyme production and salt tolerance also significantly improved the root length, shoot length and germination percentage when coating onto fresh tomato seeds. Another similar study observed the development of roots and shoots of tomato seeds soaked in different suspensions of *Lactobacillus plantarum.* They observed that eight of the ten tested strains promoted the root length while only three strains displayed increase in the shoot length (Limanska *et al*., *2013)*. The variable increase in the development of roots and shoots after treating the seeds with Lactobacilli was also demonstrated when the seeds were soaked for 1 hour with cell suspensions (Hamed *et al*. 2011).

Conclusion

The results obtained from our studies enable us to conclude that LAB have the potential to produce diversified antimicrobial compounds against both bacterial pathogens as well as phytopathogenic fungi. Our present study also demonstrated the phosphate solubilizing and salt tolerating potential of LAB that makes these organisms beneficial for plant growth and development and also aids in the survival of LAB in soil environment. Hence, LAB may be used as biofertilizers and plant growth promoting agents.

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