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Potential of Lactic Acid Bacteria in Plant Growth Promotion

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ABSTRACT

Background: Lactic acid bacteria are regarded the most important bacteria concerning food fermentation, pharmaceutical and special dietary applications. Strains have been isolated from environments rich in available carbohydrate substrates, such as food and feed, but also in human and animal cavities and in sewage and plant material. Besides lactic acid, other side products include acetate, ethanol, CO_2, formate and succinate. The most important advantage of Lactic acid bacteria making them suitable for the use in food biotechnology, is that they are generally recognized as safe (GRAS). Studies on Lactic acid bacteria isolated from aerial parts of plants are scarce. Bacteria that colonize plant roots and promote plant growth are referred to as plant growth-promoting rhizobacteria. Several substances produced by antagonistic rhizobacteria have been related to pathogen control and indirect promotion of growth in many plants. The present work explores the potential use of Lactic acid bacteria in promotion of plant growth.

Methods: Three isolates were obtained from aerial parts of pomegranate plant and confirmed by 16S rRNA sequencing to belong to *Leuconostoc sp*. The isolates were checked for plant growth promoting traits *viz.* antifungal activity, production of plant growth hormones, enzymes and 1-amino cyclopropane carboxylate deaminase activity.

Result: As LAB showed plant growth promoting traits, they can be suitably used for plant growth promotion.

Key words: Antifungal activity, 1-amino cyclopropane carboxylate deaminase activity, Lactic acid bacteria, Plant growth promotion.

INTRODUCTION

Lactic acid bacteria (LAB) are regarded the most important bacteria concerning food fermentation, pharmaceutical and special dietary applications. The most commonly used strains of different LAB species in food include the genera of *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Bifidobacterium* based on physiological and biochemical characteristics. They are found in environments rich in available carbohydrate substrates, such as food and feed, but also in human and animal cavities and in sewage and plant material. Indeed, strains have been isolated from all these environments. Besides lactic acid, other side products include acetate, ethanol, CO_2, formate and succinate. The general characteristics of LAB are that they are Gram-positive, catalase negative, microaerophilic, acid-tolerant and non-sporulating rods and cocci (Rattanachaikunsopon and Phumkhachorn, 2010; De Vuyst and Vandamme 1993; Axelsson 1998; Stiles, 1996). The most important advantage of LAB, making them suitable for the use in food biotechnology, is that they are generally recognized as safe (GRAS) (Stiles, 1996). Studies on LAB isolated from aerial parts of plants are scarce. Though LAB are abundantly distributed on plants this source remains underexplored. Their presence on plants definitely puts forth their benefits to plants one of which could be plant growth promotion.

Bacteria that colonize plant roots and promote plant growth are referred to as plant growth-promoting rhizobacteria (PGPR). Their effects can occur via local antagonism to soil-borne pathogens or by induction of systemic resistance against pathogens throughout the entire plant. Several substances produced by antagonistic rhizobacteria have been related to pathogen control and indirect promotion of growth in many plants, such as siderophores and antibiotics. Both types of induced resistance render uninfected plant parts more resistant to pathogens in several plant species (Beneduzi *et al.,* 2012; Lugtenberg and Kamilova, 2009).

Rhizobacteria belonging to the genera *Pseudomonas* and *Bacillus* are well known for their antagonistic effects and their ability to trigger induced systemic resistance (ISR). Resistance-inducing and antagonistic Rhizobacteria might be useful in formulating new inoculants with combinations of different mechanisms of action, leading to a more efficient use for biocontrol strategies to improve cropping systems (Kumar *et al.,* 2012).

Only few studies elucidated the role of lactic acid bacteria (LAB) in the rhizosphere and their plant growth promoting properties. (Chen, *et al.* 2005 and Fhoula, *et al.* 2013). The ability of LAB to act as plant growth promoting bacteria and biocontrol agent against *R. solanacearum in vivo* has been studied (Murthy *et al.,* 2012).

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MATERIALS AND METHODS

The work was carried out at Department of Microbiology, Haribhai V. Desai College and Department of Microbiology, SPPU, Pune during the period of 2015 to 2018.

Characterization and identification of the isolates

The isolates of lactic acid bacteria were obtained from Department of Microbiology. The said organisms were isolated from aerial parts of pomegranate plants like leaves, buds, flowers and fruits.

The morphological, physiological and biochemical tests were performed according to Bergy's Manual of Determinative Bacteriology.

Each isolate was grown in MRS broth under microaerophilic conditions for 48 hours.The suspensions was centrifuged for 25 minutes at 10,000 rpm.Supernatent was discarded and cell pellet was collected. This cell pellet was processed for DNA isolation according to the protocol (Ausubel *et al.*, 1987).

Identification of bacterial isolates from aerial parts of the plants was performed by sequencing 16S rDNA of bacterial isolates. The genomic DNA was isolated as described previously. The PCR assay was performed using Applied Biosystems, model 2820 (Foster, California, USA) with 50 ng of DNA extract in a total volume of 25 µl. The PCR master mixture contained 2.5 µl of 10X PCR reaction buffer (with 1.5 M $MgCl_2$), 2.5 µl of 2 mM dNTPs, 1.25 µl of 10 pm/µl of each oligonucleotide primer 8F (5′- CCA GAGTTTGATCMTGGCTCAG-3′) and 1391R (5′- GACGG GCGGTGTGTRCA -3′) (Pidiyar, *et al.* 2002, Hauben, *et al.* 1997, Amann, *et al.* 1992 and Ben-Dov *et al.* 2006), 0.2 µl of 5U/ µlTaq DNA polymerase and 15.76 µl of glass-distilled PCR water. Initially denaturation accomplished at 94°C for 3 min. Thirty-two cycles of amplification consisted of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1.30 min. A final extension phase at 72°C for 10 min was performed.

The PCR product was purified by PEG-NaCl method (Hauben, *et al.* 1997). Briefly, all samples were mixed with 0.6 times volume of PEG-NaCl, 20% [PEG (MW 6000) and 2.5 M NaCl] and incubated for 20 min at 37°C. The precipitate was collected by centrifugation at 3,800 rpm for 20 min. The pellet was washed with 70% ethanol, air dried and dissolved in 15 µl sterile distilled water.

The thermocycling for the sequencing reactions began with an initial denaturation at 94°C for 2 min, followed by 25 cycles of PCR consisting of denaturation at 94°C for 10 s, annealing at 50°C for 10 s and extension at 60°C for 4 min using primers 704F (5′- GTAGCGGTGAAATGCGTAGA-3′) (Pidiyar, *et al.* 2002, Hauben, *et al* 1997 Amann, *et al* 1992 and Ben-Dov *et al.* 2006) and 907R (5′- CCGTCAATTCM TTTGAGTTT-3′) (Amann, *et al.* 1992 and Ben-Dov *et al.* 2006). The samples were purified using standard protocols described by manufacturer (Applied Biosystems Foster City, USA). To this, 10 µl of Hi-Di formamide was added and vortexed briefly. The DNA was denatured by incubating at 95°C for 3 min, kept on ice for 5-10 min and was sequenced in a 3730 DNA analyzer (Applied Biosystems, Foster City, USA) following the manufacturer's instructions.

The obtained sequences were analysed using Sequence Scanner (Applied Biosystems) software. The rDNA sequence contigs were analysed using online databases *viz.* NCBI-BLAST (Altschul *et al.,* 1997) to find the closest match of the contiguous sequence.

Antifungal activity of lactic acid bacteria against fungal phytopathogen

The fungal phytopathogen selected was *Fusarium* sp. The antifungal activity was studied by agar overlay method (Strom, 2005; Yang, 2000).

Production of enzymes by lactic acid bacteria

The production of enzymes *viz.*, amylase on nutrient agar (NA) [NA (g/l): peptone-10, yeast extract-3, NaCl-5, pH-7.2, agar-30, distilled water-1000 ml] with 1% starch (Collins and Lyne, 1984), cellulase on minimal agar medium [minimal agar (g/l): NH_4Cl-3, NaCl-3, K_2HPO_4-0.4, sodium citrate-3, glucose-10, pH-7.0, agar-30, distilled water-1000 ml] with 1% carboxy methyl cellulose (Teather and Wood, 1982), protease on skimmed milk agar [skimmed milk agar: Milk-100 ml, sterile NA- 200 ml. Sterilize the milk by autoclaving it and add to sterile NA. Add agar 20.0 g] (Allen *et al.,* 1999) and chitinase on chitin agar [chitin agar (g/l): K_2HPO_4-0.7, KH_2PO_4-0.3, $MgSO_47H_2O$-0.5, $FeSO_47H_2O$-0.01, $ZnSO_4$-0.001, $MnCl_2$-0.001, agar-30, distilled water-1000 ml] with 1% colloidal chitin (Collins and Lyne, 1984) were studied. The diameters of zone of clearance were measured to check for the production of enzymes.

Plant growth promoting traits

The plant growth promoting traits *viz.*, indole acetic acid (IAA) using Salkowasky reagent by spectrophotometric method (Vedpathak and Chincholkar, 2008); Gibberellins production (Holbrook *et al.*, 1961); Cytokinins production by the method of Tien *et al.*, (Tien 1979); Exopolysaccharides (EPS) production by solvent extraction method (Kumari *et al.*, 2009); 1-aminocyclopropane carboxylate deaminase activity (ACC) (Govindasamy *et al.*, 2009) were studied.

RESULTS AND DISCUSSION

Morphological, physiological and biochemical characteristics of the isolates

All the three isolates were Gram positive cocci and non-motile. They were catalase negative and produced acid in case of galactose, sucrose, maltose and mannitol sugars (Table 1). None of the isolates showed gas production. All the three isolates were oxidative-fermentative.

No gas production was observed even after prolonged incubation. All the three isolates FL3(3), GYP3 and PFR88 showed acid production. However, none of the isolates showed gas production (Table 1).

16S rRNA sequencing of the isolates

The isolates *viz.*, FL3 (3), was identified as *Leuconostoc mesenteroides* where as GYP3 and PFR88 were identified

to be *Lactobacillus sp.* by the 16S rRNA sequencing (Table 2). NCBI database-For FL3 (3) bacterial gene sequences -(http://:www.ncbi.nim.nih.gov./nuccore/term).

Antifungal activity of lactic acid bacteria against *Fusarium sp*.

Values represent the mean of three replicate ± SD. All the three isolates showed antifungal activity against *Fusarium* sp. with highest activity shown by isolate FL3 (3) (Table 3).

Enzyme production by the isolates

All the three isolates showed protease production (Table 4).

Plant growth promoting traits of the isolates

The plant growth promoting hormones were studied for these LAB isolates. It was found that the isolate GYP3 showed IAA and Gibberellin production, both of which support root elongation and flowering respectively (Table 5). All the three isolates also showed EPS production.

All the three isolates showed ACC deaminase activity with highest activity shown by isolate GYP3 (Table 5). This enzyme is important to reduce the ethylene to non toxic levels to confer protection to the plants.

As seen from the results, the lactic acid bacteria were studied for various characteristics. The LAB isolates from aerial parts of the plants show various characteristics which can be related as support to plant growth. The characters *viz.*, production of enzymes, antifungal compounds, EPS, plant growth promoting hormones and ACC deaminase activity are especially important as PGPR traits. These have been observed in the present isolates with more or less intensity. LAB have not been very well explored for PGPR traits. This study supports the application of LAB to support plant growth. Though not from the rhizosphere, they are closely associated with the plant surfaces. This association certainly confers protection to the plant and also supports plant growth. Demonstration of antifungal activity and production of EPS confers protection to the plants. This property has been well studied in PGPR. This is the first report of LAB isolated from various parts of plants showing character parallel to PGPR.

Hence, it is observed that both the properties, *i.e.*, protection and growth promotion are well exhibited by LAB. Since these are plant isolates, they can well be used for the purpose. No such studies have been reported so far for LAB from parts of plants and hence, they need to be exploited for the same.

Table 1: Fermentation of sugars.

Isolates	Sugars			
	Galactose	Sucrose	Maltose	Mannitol
FL3 (3)	A	A	A	A
GYP3	A	A	A	A
PFR88	A	A	A	A

A: Acid.

Table 2: 16S rRNA sequencing of the isolates.

Isolates	Organism
FL3 (3)	*Leuconostoc mesenteroides*
GYP3	*Lactobacillus sp.*
PFR88	*Lactobacillus sp.*

Table 3: Antifungal activity of lactic acid bacteria against *Fusarium sp.*

Isolates	Zone of inhibition (mm)
FL3 (3)	13±0.00
GYP3	9±0.00
PFR88	10±0.00

Table 4: Production of the enzymes.

Isolates	Enzymes			
	Amylase	Cellulase	Protease	Chitinase
FL3 (3)	-	-	+	-
GYP3	-	-	+	-
PFR88	-	-	+	-

+: Positive, -: Negative.

Table 5: Plant growth promoting traits.

PGP traits	Isolates		
	FL3 (3)	GYP3	PFR88
IAA	-	5.0±0.00	-
Gibberellins	-	3.5±0.00	-
Cytokinins	-	-	-
EPS	0.005±0.00	0.005±0.00	0.003±0.00
ACC deaminase activity	370±0.00	450±0.00	370±0.00

PGP: Plant growth promoting; IAA: Indole Acetic Acid; EPS: Exopolysaccharide; ACC: 1- amino cyclopropane carboxylate deaminase. All the values except EPS are in µg/ml. Values represent the mean of three replicate ± SD.

CONCLUSION

The lactic acid bacteria inhabiting plant surfaces produce plant growth promoting hormones and hence can be used to promote the plant growth. Also they are found to exhibit antifungal activity which will reduce the use of chemical fungicides.

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