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Characterisation of Plant Growth Promoting Endophytic Bacteria Isolated from *Lens culinaris* Medik with Antagonistic Potential against *Fusarium Oxysporum*

Swarnita Dixit[1*], R. C. Dubey[1] and P. K. Seth[2]

[1]Department of Botany and Microbiology, Gurukul Kangri University, Haridwar-249404 Uttarakhand, India.
[2]Biotech Park, Sector G, Jankipuram, Kursi Road, Lucknow-226021, Uttar Pradesh, India.

**Corresponding author: Swarnita Dixit; swarnitadixit@gmail.com*

Abstract

A total of 152 endophytic bacterial isolates belonging to *Rhizobium*, *Pseudomonas* and *Bacillus* spp. were isolated from the root nodules of *Lens culinaris* from seven different provinces of Uttar Pradesh (India) growing in different soil condition of varying pH 6.8-7.8. These isolates were characterized morphologically, physiologically and biochemically, and screened *in vitro* for their plant growth promoting traits such as production of indole acetic acid (IAA), ammonia (NH_3), hydrogen cyanide (HCN), siderophore, phosphate solubilization and antifungal activity against *Fusarium oxysporum*. Only 27 endophytic showed positive results for all plant growth promoting attributes and antagonism against *Fusarium oxysporum.* All physio-biochemical characters including carbon utilization were analyzed by a Jaccard's coefficient dendrogram, that classified the isolates into the three major groups *viz.,* RT1, RT9-RT16, RT26 and RT27 isolates in one group, RT2-RT8, RT17-RT18, RT24 and RT25 isolates in second group, and RT19 - RT23 isolates in third group. On the basis of UPGMA cluster analysis and similarity with the standard strains these isolates were identified as *Rhizobium* strains RT1, RT9-RT16, RT26, RT27, *Pseudomonas* strains RT2-RT8, RT17-RT18, RT24 and RT25, and *Bacillus* strains RT19 - RT23. The strains RT1, RT2 and RT11 showed synergism among them and also found to possess plant growth promoting and antagonistic properties.

Keywords: Plant growth promoting rhizobacteria, Endophytic bacteria, Antagonism, *Bacillus*, *Rhizobium*, *Pseudomonas*, *Lens culinaris.*

Plants constitute vast and diverse niches for endophytic organisms. Bacterial endophytes show great diversity not only in term of plant hosts but also in bacterial phyla (Bacon and Hinton, 2006). They inhabit majority of healthy and symptomless plants. The bacterial taxa isolated from the endorhizosphere niches consist of

numerous genera of both Gram-negative and Gram-positive species. Genera such as *Bacillus*, *Enterobacter*, *Klebsiella*, *Rhizobium*, *Pseudomonas*, *Burkholderia*, *Pantoea*, *Agrobacterium*, *Methylobacterium* constitute the endophytes commonly isolated from various leguminous and non-leguminous plants (Bacon and Hinton, 2006). Endophytic bacteria produce unusual secondary metabolites of plant importance (Taechowisan *et al*., 2005) thus they have an excellent potential to be used as plant growth promoters for legumes and non-legumes (Antoun *et al*., 1998; Bai *et al*., 2002). The direct promotion by endophytic bacteria entails either synthesis of plant growth promoting substance or facilitating the uptake of certain plant nutrients from the environment. The indirect promotion of plant growth occurs when endophytic bacteria lessen or prevent the deleterious effect of one or more phytopathogenic micro-organisms. Although exact mechanisms by which plant growth promoting rhizobacteria (PGPR) promote plant growth are not fully understood, but the activities of PGPR includes: the ability to produce or change the concentration of plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene (Arshad and Frankenberger, 1993; Glick, 1995), symbiotic N_2 fixation (Boddey and Dobereiner, 1995), antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), antibiotics (Shanahan *et al*., 1992) and cyanide (Flaishman *et al.,* 1996), solubilization of mineral phosphates and other nutrients (Gaur, 1990; De Freitas *et al*., 1997).

The endophytic bacteria are known to show either synergism or antagonism among them. Interactions among endophytes influencing the plant productivity have been explained extensively. Some of the rhizobial isolates have been reported to display synergistic interaction with *Pseudomonas* (Dubey and Gupta, 2012).

Lentil (*Lens culinaris* Medik.) is one of the important *rabi* pulse crop of India, which is cultivated in the different states. It suffers from several microbial diseases including wilt diseases caused by *Fusarium oxysporum.* Exploitation of antagonistic endophytic bacteria for plant growth promotion and biocontrol of phytopathogens has earlier been attempted (Kumar *et al.,* 2011, 2012; Nejad and Johnson, 2000). The present work was aimed at the isolation, characterization and identification of plant growth promoting and antagonistic endophytic bacteria from *Lens culinaris* and their exploitation for enhanced production and protection in future.

Material and Methods

Sample collection

Sample of *Lens culinaris* (20-30 days) were collected from seven districts *viz*, Jhansi, Kanpur, Lucknow and Varanasi (Uttar Pradesh) India, during November – December, 2011. These fields were never been inoculated with any bioinoculants.

The fields have variation in soil properties as well as different cultivar variety of *Lens culinaris*. Plants were gently uprooted along with rhizospheric soil, kept in sterile sampling bags and brought immediately to the laboratory for further study.

Isolation of endophytic bacteria from root nodules of Lens culinaris

Endophytic bacteria were isolated from pink colored healthy root nodules of *Lens culinaris*. The roots were washed with tap water and healthy nodules were picked from the roots. Then the nodules were washed thoroughly with sterile distilled water, surface sterilized in 95% alcohol for 20–30 seconds and subsequently immerged in 3% sodium hypochlorite solution for 5 minutes. Subsequently nodules were washed 7–8 times with sterile distilled water to remove the traces of sodium hypochlorite. Standard microbiological technique was followed for isolation of root nodulating endophytic bacteria (Dubey and Maheshwari, 2012). The surface-sterilized nodules were transferred to a sterile culture tubes containing 100µl sterile distilled water. Nodules were crushed with the help of sterile glass rod. A milky bacterial suspension was obtained that was streaked on the YEMA, CrYEMA, Pseudomonas Agar medium, and Bacillus Agar medium (Himedia™) (Vincent, 1970). The plates were incubated at 28±2°C for 72 h and observed for the specific features of *Rhizobium* spp., *Pseudomonas* spp. and *Bacillus* spp. The cultures were identified by conducting the tests as described in Bergey's Mannual of Determinative Bacteriology (Holt *et al*., 1994) and compared with the standard culture of *Rhizobium leguminosarum* MTCC99, *Sinorhizobium meliloti* MTCC100, *Mesorhizobium loti* MTCC2378, *Pseudomonas fluorescens* MTCC103, *Pseudomonas* sp. MTCC129, *Pseudomonas aeruginosa* MTCC1934, *Bacillus* sp. MTCC297, *Bacillus subtilis* MTCC441, *Paenibacillus polymixa* MTCC122, procured from Microbial Type Culture Collection Center (MTCC), Institute of Microbiological Techniques (IMTECH), Chandigarh, India.

Characterization of bacterial isolates

Phenotypic Characterization

Differential staining: Gram's staining of the log phase culture was done to provide information on presumptive test of isolates following Dubey and Maheshwari (2012).

Colony morphology: Colony colour, form, elevation and margin of bacterial isolates streaked on YEMA, crYEMA, Pseudomonas Agar medium, and Bacillus Agar medium (Himedia™) were recorded after their growth (Dubey and Maheshwari, 2012).

Physiological and Biochemical Characterization

Motility test: Motility was examined following hanging drop method. One small drop of exponentially grown broth culture of strains was dropped in the center of a cover glass which was hanging in cavity slide. The cavity was sealed with paraffin wax and observed for bacterial motility under 100 X objective of compound light microscope.

Generation time: A loopful log phase culture of each isolate was inoculated separately in flasks containing respective media at 28±2°C and 150 rpm. Bacterial growth of each isolates was assessed by measuring the optical density at 600 nm after every 6 h in UV-VIS spectrophotometer (Labtronics-model LT 2800, India). Generation time was calculated by using the formula: generation time = $(T2-T1)/3.3\ \log_{10}(OD_2-OD)_{10}(T2-T1)/3.3\ \log_{10}(OD_2-OD)_{10}$, where $(T2-T1)(T2-T1)$= time interval taken at any two points in the log phase of growth and $\log_{10}(OD_2-OD_1)\log_{10}(OD_2-OD_1)$ = difference between the $\log_{10}\log_{10}$value of OD at time T_2 h to $\log_{10}\log_{10}$ value of OD at time T_1 h (Dubey and Maheshwari, 2012).

Temperature tolerance: All the isolates were streaked onto the respective medium and incubated at different temperature regime viz., 5, 15, 25, 35, 45 and 55°C°C for 72h to examine the temperature tolerance ability of the isolates (Jida and Assefa, 2011).

pH Tolerance: The ability of the bacterial isolates to grow in alkaline or acid media was assessed using respective agar medium at different pH viz., 3, 4, 5, 6, 7, 8, 9 and 10 (by using HCL or NaOH) and incubated at 28±2°C °C for the 72h (Jida and Assefa, 2011).

Salt tolerance: Bacterial isolates were tested for their salt tolerance on respective medium supplemented with 0.5, 1, 2, 3, 4, 5, 6 and 7 % (w/v) NaCl (Ben Romdhane *et al*., 2006).

Catalase activity: Drops of hydrogen peroxide (3%) were added over to 48 h old bacterial cultures. The plates were observed for the liberation of effervescence of oxygen around the bacterial colonies (Graham and Parker, 1964). Bacterial isolates exhibiting oxygen evolution confirmed the presence of catalase activity.

Gelatin Hydrolysis: Culture tubes containing sterilized agar medium amended with 0.4% (w/v) gelatin were separately stabbed by exponentially grown culture of bacterial isolates and incubated at 28±2°C for the 7 days. The tubes were observed for liquefaction of gelatin. Culture tubes showing liquefaction of gelatin confirmed for gelatin hydrolysis (Sadowsky *et al*., 1983).

Hydrolysis of urea: Log phase cultures of isolates were inoculated in the tubes containing broth amended with 2% (w/v) urea and 0.012% phenol red and

incubated at 30°C for 7 days. Appearance of red color in tubes indicated urea hydrolysis (Lindstrom and Lehtomaki, 1988).

Citrate utilization: Sodium citrate broth medium was modified by replacing the carbon source with sodium citrate and bromothymol blue (25 mg/l). The tubes containing modified media were stab-streaked with log phase culture of bacterial isolates separately and incubated at 28±2°C for 24 h (Koser, 1923).

Growth in presence of KNO_3: The ability of isolated strains to grow in presence of 8% KNO_3 was tested by inoculating the isolates separately on YEMA and NAM plates containing 8% KNO_3. The plates were incubated for 28±2°C for 24 h (EI Idrissi *et al*., 1996).

Polyhydroxy Butyrate (PHB) Accumulation: Bacterial isolates were inoculated with respective media separately and incubated at 28±2°C and 150 rpm for 72 h. After incubation, isolates were centrifuged at 10,000 rpm for 45 min to collect the supernatant. Exopolysccharide (EPS) was precipitated by adding double volume of chilled ethyl alcohol. The extracted EPS was dried at 45°C till constant weight was achieved (Navarini *et al.,* 1992).

Utilization of carbon sources: Utilization of various carbon sources by bacterial isolates was done following El Idrissi *et al.,* 1996. Himedia Carbohydrate™ Kit (Himedia Laboratories Pvt. Ltd., Mumbai, India) was used to determine the 35 carbon source utilization that consisted of monosaccharides pentoses, monosaccharides, hexoses, disaccharides, trisaccharides, polysaccharides, organic compounds and sugar alcohol. Results of carbon utilization were analyzed by using and MVSP (Multi Variant Software Package) version 3.21 software with UPGMA clustering method.

Screening of plant growth promoting (PGP) endophytic bacterial isolates

The following PGP attributes of isolates were examined:

Phosphate Solubilization: Phosphate solubilization ability of isolates was examined using Pikovaskya agar medium (Pikovskya, 1948). Isolates were spot inoculated onto the Pikovaskya agar plates and incubated at 28±2°C for the 72h. A clear zone formation around the spot was recorded. Solubilization index (S.I.) was calculated as the ratio of halo zone diameter to colony diameter for positive isolates (Vazquez *et al*., 2000) following the formula:

$$SI = \frac{\text{colony diameter} + \text{halo zone}}{\text{colony diameter}}$$

$$\frac{\text{colony diameter} + \text{halo zone}}{\text{colony diameter}}$$

Indole -3 Acetic Acid (IAA) Production: To examine the IAA production by the bacterial isolates, their log phase cultures were incubated on the broth medium

amended with 5 mM/L tryptophan for 24 h (Bric *et al*., 1991). After centrifugation at 10,000 rpm for 15 min, supernatant was collected separately. 100 µl of 10 mM *o*-phosphoric acid and 4 ml of Salkowaski's reagent (1ml of 0.5 mM $FeCl_3$ in 35% of $HClO_4$) were added to 2ml of supernatant of each strain and incubated at room temprature for 25 min for developing the pink color. Absorbance was measured at 535 nm using UV-VIS spectrophotometer (Labtronics-model LT 2800, India). Un-inoculated broth supernatant with Salkowaski's reagent was taken as control.

HCN production: HCN (hydrogen cyanide) production was determined by modified method of Bakker and Shippers (1987). Log phase culture was streaked on to agar plate of respective media amended with 4.4g/l glycine with simultaneous addition of filter paper soaked in 0.5% picric acid in 1% Na_2CO_3 in the lid of plates. The control plates were un-inoculated. The plates were sealed with parafilm and incubated at 28±2°C for 72 h; development of brown color was examined for HCN production.

Siderophore production: Siderophore production was examined on Chrome-azurol S (CAS) medium following the method of Schwyn and Neiland (1987). Log phase cultures of isolated root nodule endophytic bacteria were spot inoculated onto CAS agar plates and incubated at 28±2°C for 72 h formation of orange to yellow halo around the colonies showed the production of siderophore.

Ammonia production: Ammonia production was examined on peptone broth following the method of Lannette *et al*., 1985. Log phase cultures of isolated endophytic bacteria were inoculated in peptone broth incubated at 28±2°C for 72 h. Yellow to brown precipitation were observed after adding of Nessler's reagent.

Antagonistic activity: Antagonistic properties of isolates were tested against *Fusarium oxysporum* on potato dextrose agar (250g peeled potato, 20g glucose, 15g agar, 1000ml distilled water, pH 6.5) plates using the dual culture technique (Skidmore and Dickinson, 1976). A loopfull log phase culture (24h old) of each bacterial strain was separately spot inoculated apart from 5mm agar disc containing mycelial growth of the fungal pathogen. Plates were incubated at 28±2°C for 3-7 days. The zone of inhibition (%) was recorded by using the formula: $100 \times (C\text{-}T)/C$, where, $C = C$ = radial growth in control; $T = T$ = radial growth in dual culture.

Results and Discussion

Plant samples collected from different field from various seven provinces had different soil pH varying from 6.8 to 7.8 (Table 1). Growth of lentil at various soil pH has also been reported by Jaiswal *et al*., 2011.

Table 1: Location of sampling sites and soil pH.

Sites	Soil pH
Kanpur	6.8
Unnao	7.8
Lucknow	7.7
Hardoi	7.8
Jaunpur	7.6
Varanasi	7.5
Jhansi	7.3

In total, 152 endophytic bacteria were isolated from root nodules of *Lens culinaris*. Most of isolates differed in phenotypic and biochemical characteristics (Table 2). All the rhizobial isolates were Gram-negative, non-spore forming, and non-capsulated rod and motile. The isolates formed red, semi-translucent, rounded, smooth, mucoid colonies (2 to 4 mm diameter) on congo red yeast extract mannitol agar (CrYEMA) after 48 h of incubation. All *Pseudomonas* isolates were Gram-negative, non- spore forming, non-capsulated, motile and rod shaped structure. The colonies were smooth, large, 2-4 mm in diameter with regular edge on nutrient agar medium. Isolates (RT2, RT4, RT5, RT8, RT17 and RT18) produced greenish-blue fluorescent pigment on Pseudomonas agar medium. All the *Bacillus* isolates were Gram-positive, endospore forming, and motile rods. The isolates formed translucent, large, white colonies (2 to 4 mm diameter) on nutrient agar medium after 24 h of incubation. The Gram–negative isolates were more in number within the root nodules of lentil. Earlier workers have reported a predominance of Gram-negative bacteria in the tissues of various plants (Stoltzfus *et al.*, 1997; Elbeltagy *et al*., 2000; Stajković *et al*., 2009). Zinniel *et al*., 2002 reported an equal presence of Gram-negative and Gram-positive bacteria inside the root nodule.

Effect of temperature on all isolates was noticed and optimum growth was observed at 28˚C. The maximum and minimum temperature tolerated by the isolates was recorded as 4 and 50˚C respectively. Bacterial growth also influenced by pH and the optimum growth was observed at neutral pH. The pH value tolerated by the isolates was recorded as 4 and 9 respectively. It was observed that at high alkaline and acidic pH, growth was sharply affected. Effect of various salt concentrations on the growth of isolates was assessed and optimum growth was observed at 2-3% concentration of NaCl. Maximum and minimum concentration of salt tolerated by isolates was recorded as 0.5% and 6% respectively.

Table 2: Morphological and biochemical characterisation of endophytic bacteria isolated from root nodules of *Lens culinaris*

Isolates	1	2	3	4	5	6	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Generation time
RT1	-	Rod	+	+	-	+	+	-	-	+	-	+	+	-	+	N	N	+	+	+	2.7
RT2	-	Rod	+	-	-	+	+	+	+	+	-	+	+	-	+	+	+	+	-	+	1.2
RT3	-	Rod	+	+	-	-	+	+	+	+	-	+	+	-	+	-	-	+	-	+	1.4
RT4	-	Rod	+	-	-	+	+	+	+	+	-	-	-	-	-	+	-	+	+	+	1.2
RT5	-	Rod	+	-	-	-	+	+	+	+	-	+	+	-	+	+	+	+	+	+	1.3
RT6	-	Rod	+	-	-	-	+	+	+	+	-	+	-	-	+	-	-	+	-	+	1.3
RT7	-	Rod	+	+	-	+	+	+	+	+	-	-	+	-	+	-	+	+	+	+	1.2
RT8	-	Rod	+	-	-	-	+	+	+	+	-	+	+	-	+	+	+	+	+	+	1.4
RT9	-	Rod	+	+	-	+	+	-	-	+	+	+	+	-	+	N	N	+	+	+	2.3
RT10	-	Rod	+	+	-	+	+	-	-	+	-	-	-	-	-	N	N	+	-	+	2.8
RT11	-	Rod	+	-	-	+	+	-	-	+	-	+	+	-	+	N	N	+	+	+	2.4
RT12	-	Rod	+	+	-	-	+	-	+	-	+	+	+	-	+	N	N	+	+	-	2.8
RT13	-	Rod	+	+	-	+	+	-	-	+	-	+	+	-	+	N	N	+	+	+	2.4
RT14	-	Rod	+	+	-	-	+	-	-	-	-	+	+	-	+	N	N	+	+	-	2.7
RT15	-	Rod	+	+	-	-	+	-	+	-	+	+	+	-	+	N	N	+	+	-	2.3
RT16	-	Rod	+	-	-	-	+	-	-	-	-	+	+	-	+	N	N	+	+	-	2.5
RT17	-	Rod	+	-	-	-	+	+	+	+	-	+	-	-	+	+	-	+	+	+	1.2
RT18	-	Rod	+	-	-	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+	1.3
RT19	+	Rod	+	+	+	-	+	-	-	+	-	+	+	-	+	N	N	+	N	+	1.7
RT20	+	Rod	+	+	+	-	+	+	-	+	+	+	+	-	+	N	N	+	N	+	1.4

Contd.

RT21	+	Rod	+	+	+	+	-	+	-	-	-	-	N	N	+	N	+	1.6
RT22	+	Rod	+	-	+	+	-	-	+	-	+	+	-	N	N	+	N	+	1.7
RT23	+	Rod	+	+	-	+	+	-	+	+	-	+	+	N	N	+	N	-	1.5
RT24	-	Rod	+	-	-	+	+	+	-	+	+	-	+	-	-	+	-	+	1.3
RT25	-	Rod	+	-	-	+	+	+	-	+	+	-	+	+	+	+	-	+	1.2
RT26	-	Rod	+	+	-	+	+	-	-	+	+	-	+	-	N	N	+	+	2.4
RT27	-	Rod	+	+	-	+	+	-	-	-	+	+	-	+	-	N	N	+	2.8
MTCC99	-	Rod	+	+	-	+	+	-	-	+	+	+	-	+	-	N	N	+	2.7
MTCC100	-	Rod	+	+	-	-	+	-	-	-	+	+	-	-	-	N	N	+	2.4
MTCC2378	-	Rod	+	+	-	+	+	-	-	-	+	+	-	-	-	N	N	+	2.9
MTCC103	-	Rod	+	-	-	+	+	+	+	-	+	+	-	+	+	+	+	+	1.2
MTCC129	-	Rod	+	-	-	-	+	+	+	-	+	-	-	-	-	+	+	+	1.3
MTCC1934	-	Rod	+	-	-	+	+	+	+	-	+	+	-	+	-	+	+	-	1.4
MTCC297	+	Rod	+	-	+	+	+	-	-	-	+	+	-	+	N	+	N	-	1.7
MTCC441	+	Rod	+	-	+	-	+	+	-	+	-	-	-	-	N	N	+	N	1.5
MTCC122	+	Rod	+	-	+	+	+	-	+	-	+	+	-	+	N	N	+	N	-	1.6

Abbreviation: **1-** Gram reaction, **2-** shape, **3-** motility, **4-** capsule, **5-** endospore, **6-** KNO3 (8%) tolerance, **7-** gelatin hydrolysis, **8-** starch hydrolysis, **9-** urease, **10-** H_2S production, **11-** citrate utilization, **12-** Voges Proskaur test, **13-** methyl red, **14-** indole production,**15-**fluorescence, **16-** pigment, **17-** catalase, **18-** PHB accumulation, **19-** Hydrolysis of urea, **N-** not tested; + positive; **-** negative; *Rhizobium Leguminosarum* MTCC99, *Sinorhizobium meliloti* MTCC100, *Mesorhizobium loti* MTCC2378, *Pseudomonas fluorescens* MTCC103, *Pseudomonas* sp. MTCC129, *Pseudomonas aeruginosa* MTCC1934, *Bacillus* sp. MTCC297, *Bacillus subtilis* MTCC441, *Paenibacillus polymixa* MTCC122.

The bacterial isolates were identified on the basis of similarity with standard strains and their morphological and physio-chemical features along with carbon utilization characteristics as described in Bergey's Manual of Systematic Bacteriology (George *et al*., 2004). Results of physio-biochemical characters along with carbon utilization property were computed by MVSP version 3.21 software to get the Jaccard's coefficient dendrogram that classified the isolates into the three major groups *i.e*. RT1, RT9-RT16, RT26 and RT27 in one group, RT2-RT8, RT17-RT18, RT24-RT25 in second group and RT19-RT23 in third group.

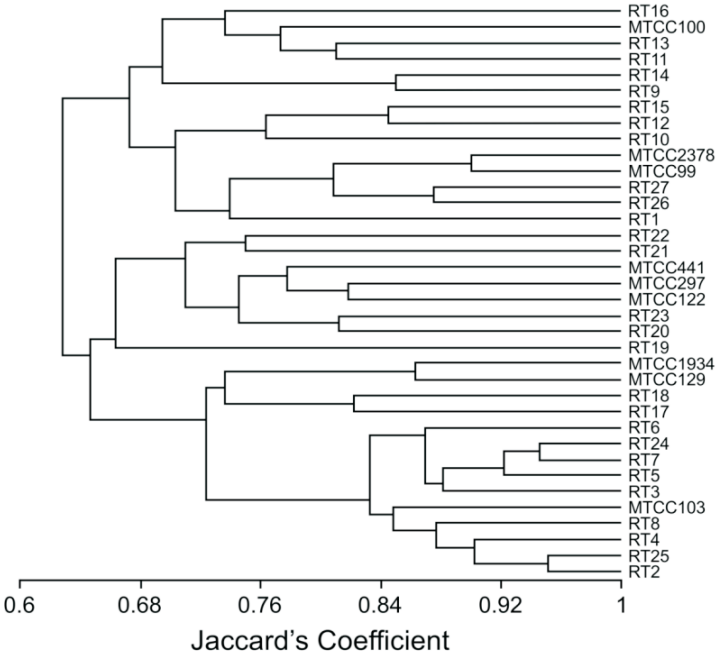

Fig. 1: Dendrogram was obtained from endophytic bacterial isolates RT1–RT27 and standard cultures *Rhizobium Leguminosarum* MTCC99, *Sinorhizobium meliloti* MTCC100, *Mesorhizobium loti* MTCC2378, *Pseudomonas fluorescens* MTCC103, *Pseudomonas* sp. MTCC129, *Pseudomonas aeruginosa* MTCC1934, *Bacillus* sp. MTCC297, *Bacillus subtilis* MTCC441, *Paenibacillus polymixa* MTCC122. UPGMA (unweighted pair-group method with arithmetic mean) clustering method based on Jaccards coefficien by using physico-chemical characteristics and carbon utilization.

On the basis of similarity with the standard strains these isolates were named as *Rhizobium* spp. for isolates RT1, RT9-RT16, RT26, RT27, *Pseudomonas* spp. for isolates RT2-RT8, RT17-RT18 and RT24-RT25, and *Bacillus* spp. for isolates RT19 - RT23 (Figure 1). Use of physiological and biochemical properties for establishment of similarity among bacterial isolates by using MVSP software has also been done by Dubey and Gupta, 2012.

Table 3: Plan growth promoting and antifungal properties of endophytic bacteria isolated from root nodules of *L. culinaris*.

Isolates	IAA production	Phosphate solubilisation	Ammonia production	Siderophore production	HCN production	Fusarium oxysporum growth inhibition
RT1	+++	+++	+++	++	+++	+++
RT2	+++	+++	+++	++	+++	+++
RT3	++	++	++	+	+	+
RT4	+	+++	++	+	++	++
RT5	+	+	+	+	+	+
RT6	+	+++	+	+	+	+
RT7	+++	++	++	+	+	++
RT8	++	++	+	+	++	++
RT9	+++	++	++	+	+	+
RT10	++	+++	+++	+	+++	+++
RT11	+	++	++	+	++	++
RT12	+	++	+++	+	+++	+++
RT13	++	++	++	+	++	++
RT14	+	++	+	+	++	++
RT15	++	++	+	+	+	+
RT16	+	++	+	-	+	+
RT17	++	++	+	+	+	+
RT18	+	+	+	-	+	+
RT19	+++	+++	++	+	+	++
RT20	+	+	+	+	+	++
RT21	+	++	+	++	+	+
RT22	+++	++	+	+	++	++
RT23	+	++	++	+	+	+
RT24	++	+++	+	+	+	+
RT25	+	++	++	+	+	+
RT26	++	+++	+	+	+	+
RT27	+	++	++	+	+	+
MTCC99	+	+	+	-	-	-
MTCC100	-	+	-	-	-	-

Contd.

MTCC2378	+	+	-	-	-	-
MTCC103	+	+	+	-	-	-
MTCC129	+	+	-	++	+	+
MTCC1934	-	-	-	+	-	+
MTCC297	+	+	+	+	+	+
MTCC 441	+	+	+	-	-	-
MTCC 122	+	+	-	-	-	-

Abbreviation: +++ very good production; ++ good production; + positive; **-** negative; *Rhizobium Leguminosarum* MTCC99, *Sinorhizobium meliloti* MTCC100, *Mesorhizobium loti* MTCC2378; *Pseudomonas fluorescens* MTCC103, *Pseudomonas* sp. MTCC129, *Pseudomonas aeruginosa* MTCC1934, *Bacillus* sp. MTCC297, *Bacillus subtilis* MTCC441, *Paenibacillus polymixa* MTCC122.

Among 152 isolates, only 27 isolates were found to have plan growth promoting traits *viz.*, IAA, HCN, ammonia, siderophore production, phosphate solubilisation and antagonism against *Fusarium oxysporum* (Table 3). Some endophytic bacteria have the potential to synthesize IAA by using tryptophan as the precursor. All the isolates were found to be positive for IAA production as observed by the development of pink color in the cell free supernatants and its production was induced in the presence of tryptophan. Six isolates (RT1, RT2, RT7, RT9, RT19 and RT22) produced the maximum amount of IAA. The ability of the production of IAA by different bacteria isolates has also been reported by many researchers (Lata *et al.,* 2006; Kumar *et al.,* 2012).The bacterial isolates formed clear halos zone around the inoculated spot which indicate phosphate solubilisation on the Pikovaskya's agar medium. Among all, nine isolates *viz.,* RT1, RT2, RT4, RT6, RT10, RT18, RT23, RT24 and RT26 showed to solubilize the maximum amount of inorganic phosphate. All the isolates produced cyanogen and siderophore onto their respective media. Only two isolates (RT1 and RT2) were found good in siderophore production, while the other six isolates (RT1, RT2, RT10, RT12, RT18 and RT21) were good in HCN production.

All selected strains were found ammonia producer but among them only four isolates (RT1, RT2, RT9 and RT11) were good ammonia producer. Similar results have also been reported for phosphate solubilization (Kumar *et al*., 2011), siderophore production (Kumar *et al*., 2011), HCN production (Ahmad *et al*., 2008), IAA production (Lata *et al.,* 2006), and ammonia production (Selvakumar *et al*., 2008). Isolates RT1, RT2, RT10, RT12 and RT18 showed strong growth inhibition *in vitro* against *Fusarium oxysporum*. Antagonistic properties by the isolates may be explained to be due to the production of antibiotics, HCN, and siderophore production, resulting in fungal growth inhibition. Similar works have been reported in other studies (Dubey and Gupta, 2012; Kumar *et al*., 2012). However, role of other inhibiting molecules such as toxins and enzymes in the growth inhibition process of the fungal pathogens cannot be ruled out. Growth inhibition of *F. oxysporum* (Kumar *et al.,* 2011) and *M. phaseolina* (Dubey and Gupta, 2012) has also been reported. Besides, several other factors such as staling growth products, pH of growth media, composition for nutrients and space and nutrient imbalance caused due to nutrient impoverishment may also be involved in microbial antagonism.

Conclusion

It may be concluded that *Rhizobium* sp. RT1, *Pseudomonas* sp. RT2 and *Bacillus* sp. RT11 has synergism among them and possess plant growth promoting and antagonistic properties. Hence these strains may be recommended to be used as co-inoculants for plan growth promotion and biocontrol of soil-born phytopathogen *Fusarium oxysporum* only after field trails.

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