

Plant growth-promoting potential of endophytic bacteria isolated from roots of wild *Dodonaea viscosa* L.

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Abstract *Dodonaea viscosa*, a wild and perennial shrub that can tolerate harsh environmental conditions, was used for the isolation of its endophytic bacteria and their potential was explored for the promotion of Canola growth. The bacteria identified through 16S rRNA gene sequencing, belonged to ten different genera namely *Inquilinus*, *Xanthomonas*, *Pseudomonas*, *Rhizobium*, *Brevundimonas*, *Microbacterium*, *Bacillus*, *Streptomyces*, *Agrococcus* and *Stenotrophomonas*. All the strains produced small amount of IAA (indole acetic acid) in the absence of tryptophan and comparatively more in the presence of tryptophan. All the bacterial strains were positive for ammonia production, cellulase and pectinase activity, but few of them showed phosphate solubilization, siderophore and hydrogen cyanide production. Only three strains showed ACC (1-aminocyclopropane-1-carboxylate) deaminase activity when tested using *in-vitro* enzyme assay. Members of genera *Bacillus*, *Pseudomonas* and *Streptomyces* showed positive chitinase, protease and antifungal activity against two phytopathogenic fungi *Aspergillus niger* and *Fusarium oxysporum*, while members of *Xanthomonas*, *Pseudomonas* and *Bacillus* showed significant root elongation of Canola which could be related with their positive plant-growth-promoting (PGP) traits. Among the three plant

growth promoting *Bacillus* strains, *B. idriensis* is never reported before for its PGP activities. These results showed the potential of *Dodonaea viscosa* endophytic bacteria as PGPBs, which in future can be further explored for their host range/molecular mechanisms.

Keywords *Dodonaea viscosa* · Plant–microbe interaction · Phytohormones · Canola

Introduction

Modern agriculture is relying on the use of chemical fertilizers, pesticides and growth regulators to enhance yield due to increase in population growth and food demand. This reliance is linked with difficulties, such as environmental contamination, health threats, disruption of natural ecological nutrient cycling and damage to biological communities that otherwise maintain crop production (Enebak et al. 1998). As a result, there has been a revival of awareness in eco-friendly, viable and organic agronomic practices (Esitken et al. 2005). Environment-friendly agricultural practices such as use of bio-fertilizers and bio-pesticides has become an ideal approach for many countries in the world.

Endophytic bacteria has been reported as good candidates for plant growth promoting activity, as they can serve their host effectively and efficiently under a wide range of environmental conditions compared to those that bind exclusively to the plant's rhizosphere (Compant et al. 2010). Plant growth-promoting endophytic bacteria have the ability to inhabit the plant tissues with no apparent harmful effect to the host plant (Reiter and Sessitsch 2006) and known to provide growth benefits to their host. They may establish a biological alternative to chemical fertilizers and increase crop yield. They possess such distinct qualities that make

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them best candidates for the plant growth promotion. They can safely be used as fertilizers or against disease causing agents, as they do not exert ecological pressures and are environment friendly (Pavlov et al. 2011). They promote plant growth by producing phytohormones (such as IAA), siderophores, solubilizing phosphate or lowering of ethylene levels in plants etc. (Ahemad and Khan 2011).

A large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance plant growth (Ji et al. 2014). Endophytic bacteria with plant growth promoting characters have been reported from different plants (Fernandes et al. 2013; Zhao et al. 2015). These endophytic bacteria improve the growth by improving the biological fixation of nitrogen, production of growth regulators or by increasing various resistances. Endophytes with diverse properties isolated from unexplored and wild sources will have much effective role to manipulate and improve plant growth (Jasim et al. 2014).

In this study, we selected *Dodonaea viscosa* L. (Sanatha) as a target plant for the isolation of endophytic bacteria. It is an evergreen, woody, perennial and wild shrub, though it is a native plant of Australia, wide spread throughout the tropics known for its medicinal use (Rajamanickam et al. 2010). *Dodonaea* is drought tolerant, shade tolerant, wind hardy and has the ability to withstand wildfires (Duvauchelle 2009). The aim of the present study was to isolate endophytic bacteria of wild *Dodonaea viscosa* for the evaluation of their plant growth promoting activities. Canola (*Brassica napus*) was selected as the experimental crop for the inoculation of isolated endophytic bacteria with potential for the improvement of plant growth.

Materials and methods

Isolation of endophytic bacteria

Endophytic bacteria were isolated from stress resistant, perennial and wild shrub *Dodonaea viscosa* collected from Quaid-i-Azam University, Islamabad. Young plants were collected in the month of February 2014. Roots were cleaned thoroughly with tap water and cut into small pieces, which were sterilized with chlorox (Commercial bleach) for 5 min and five washes with autoclaved distilled water. Surface sterilized roots were macerated in mortar and pestle, followed by the serial dilution of the extract in 0.03 M MgSO_4 up to 10^{-3} and plated on Tryptic Soya Agar (TSA) medium at 30 °C. Pure and morphologically distinct colonies were selected for further studies and stored on TSA slants at 4 °C and glycerol stocks at –80 °C.

Plant growth promoting activities

Indole-3-acetic-acid (IAA) production

Ability of bacterial endophytes to produce IAA was assessed as described by Rashid et al. (2012). Bacterial cells were grown in tryptophan supplemented medium at 37 °C for 24 h cell harvesting (10,000 rpm for 10 min) was followed by addition of 2 ml of Salkowski's reagent. Appearance of pink color indicated IAA synthesis after 25 min. OD was monitored at 535 nm using a Spectrophotometer (UV 3000 spectrophotometer). IAA was used as standard.

Phosphate solubilization

Mineral phosphate solubilization activity was determined following Kuklinsky-Sobral et al. (2004). NBRIP medium was supplemented with 5 g l⁻¹ tricalcium phosphate. Bacterial culture was stabbed and incubated as described before. Appearance of halo zone around the colony was taken as positive result.

Siderophore, ammonia and hydrogen cyanide production

Siderophore production of isolates was checked on CAS agar medium according to the modified method of Schwyn and Neilands (1987). Bacterial isolates were streaked on Chrome azurol S agar medium and incubated at 30 °C for 48–72 h. Development of a yellow to orange halo around the bacterial growth showed a positive result for siderophore production.

Bacterial culture were grown in peptone water and incubated for 48 h at 30 °C for determining the ammonia production potential. After the addition of Nessler's reagent, development of yellow to brown color indicated positive activity (Marques et al. 2010).

Hydrogen cyanide (HCN) production by the bacterial isolates was analysed by amending the TSA medium with 4.4 g glycine. Isolates were streaked on this modified agar plates. Whatman no.1 filter paper soaked in a 2% sodium carbonate in 0.5% picric acid solution was placed on top of streaked plates and incubated at 30 °C for 4 days, after which development of orange to red color indicated HCN production by the isolates (Ahmad et al. 2008).

ACC deaminase enzyme production

ACC deaminase enzyme activity was assayed following the method of Penrose and Glick (2003). The absorbance of the bacterial extracts in the presence of assay reagent, with ACC (the substrate) and without ACC was measured by UV Spectrophotometer at 540 nm. The amount of

α -ketobutyrate produced was calculated by preparing its standard curve ranging between 0 and 10 μ mol.

Production of cell wall degrading enzymes

Cellulose Congo Red agar was prepared to analyse the cellulose activity of the isolates. Bacterial cultures were inoculated on the cellulose- Congo red agar and incubated for 7 days at 30 °C. Following incubation, the colonies exhibiting zones of clearing were taken to be cellulase positive (Hendricks et al. 1995).

For pectinase activity, isolates were spot-inoculated on a selective agar medium supplemented with polyglacturonic acid (5 g l⁻¹) as a source of substrate. The plates were observed for clear zones around the colonies after flooding with 1% iodine solution (Kumar and Sharma 2012).

For assessing protease activity of isolates, they were inoculated on LB supplemented with 2% skimmed milk agar medium (Adinarayana et al. 2003). Chitinase medium was prepared by using ½ strength TSA supplemented with 0.6% w/v colloidal chitin. Bacteria were spot inoculated on medium and incubated at 30 °C for 5 days. The chitinolytic activity was confirmed by the presence of clear zone around the inoculated bacteria (Bibi et al. 2012).

Antagonistic activities against pathogenic fungi

Bacterial endophytes were tested for their anti-fungal capabilities against *Aspergillus niger* (accession no: 1109) and *Fusarium oxysporum* (Accession no: 1114) obtained from Fungal culture bank, University of the Punjab, using a dual culturing technique on screening media containing half strength PDA and half strength TSA. Bacterial isolates were inoculated 2 cm away from fungal disc on media plate and uninoculated plate with fungal disc served as negative control (Kumar et al. 2012). Antifungal activity was evaluated after incubating inoculated plates at 30 °C up to 7 days.

In vitro assay for plant growth promotion

Gnotobiotic canola root elongation assay

Canola seeds were obtained from National Agricultural Research Centre (NARC), Islamabad. To evaluate the ability of bacterial isolates to promote root growth *in vitro*, method described by Penrose and Glick (2003) of gnotobiotic Canola root elongation was followed. Bacterial inoculated and un-inoculated seeds (control) on filter paper plates were placed in a plant growth chamber under controlled conditions (12 h light/dark cycles), constant temperature (25 °C) and relative humidity (60%). Root lengths of the resulting plantlets were measured on the fifth day.

Molecular identification of isolated endophytic bacteria

The selected isolates were identified through 16S rRNA Gene Sequencing. Total genomic DNA from each isolate was extracted using alkaline lysis method (Afzal et al. 2015). 16S rRNA gene was amplified by performing colony PCR using universal bacterial primers 27F and 1492R (Chen et al. 2010), which produced a product of size 1465 base pairs (Bibi et al. 2012). The PCR was run for 35 cycles (denaturation at 94 °C and 30 s, annealing at 56 °C and 30 s, extension at 72 °C and 90 s) with one initial denaturation step (94 °C, 5 min) and a final extension step (72 °C, 7 min). PureLink™ Quick PCR Purification Kit (Invitrogen) was used to purify PCR product and were commercially sequenced for the bacterial 16S rRNA gene using 27F through Sanger sequencing technology of ABI from Macrogen (South Korea). Depending on the quality of the sequencing result EzTaxon-e server version 2.1 and National Centre for Biotechnology Information (NCBI) database using Mega BLAST algorithm (<http://www.ncbi.nlm.nih.gov/BLAST>) were used to determine the closest match (Kim et al. 2012).

Statistical analysis

Data was analyzed statistically by ANOVA using Statistix 8.1 software. Significance was determined at $P \leq 0.05$.

Results and discussion

Isolation and identification of isolates by 16S rRNA gene sequencing

A total of 15 morphologically distinct bacterial isolates were selected and evaluated for their PGP and antagonistic traits. The isolates were identified through 16S rRNA gene analysis. The identified bacteria belonged to ten different genera namely *Inquilinus*, *Xanthomonas*, *Pseudomonas*, *Rhizobium*, *Brevundimonas*, *Microbacterium*, *Bacillus*, *Streptomyces*, *Agrococcus* and *Stenotrophomonas*. The nucleotide sequences obtained in this work were deposited in GenBank under accession numbers KJ591012-KJ591026. Details of 16S rRNA gene based identification of the bacteria isolated from *D. viscosa* are given in Table 1.

Bacillus as predominant genera in the present study is same as reported by Kim et al. (2011). *Bacillus* has also been reported as an endophyte of *Polygonum cuspidatum* (Figueiredo et al. 2009); Tomato (Feng et al. 2013); *Aquilaria* sp. etc. (Krishnan et al. 2012). *Bacillus* and *Pseudomonas* have been extensively reported and studied to colonize the roots (Kumar et al. 2011). Rashid et al. (2012) reported *Microbacterium* sp. as an endophyte of tomato. Recovery of these bacteria from surface sterilized canola

Table 1 Identification of isolated strains from wild *Dodonaea viscosa* by 16S rDNA sequence analysis

Bacterial IDs	Database NCBI	Similarity % age	Database EzTaxon	Similarity % age
MOSEL-RD 1	<i>Inquilinus limosus</i>	99	<i>Inquilinus limosus</i>	99.71
MOSEL-RD 2	<i>Xanthomonas sp</i>	99	<i>Xanthomonas sacchari</i>	99.60
MOSEL-RD 3	<i>Streptomyces sp</i>	99	<i>Streptomyces alboniger</i>	98.90
MOSEL-RD 7	<i>Bacillus idriensis</i>	99	<i>Bacillus idriensis</i>	99.30
MOSEL-RD 9	<i>Xanthomonas translucens</i>	99	<i>Xanthomonas translucens</i>	99.80
MOSEL-RD 12	<i>Rhizobium huautlense</i>	100	<i>Rhizobium huautlense</i>	100.00
MOSEL-RD 14	<i>Microbacterium sp</i>	99	<i>Microbacterium trichothecenolyticum</i>	98.48
MOSEL-RD 17	<i>Streptomyces caeruleatus</i>	100	<i>Streptomyces caeruleatus</i>	99.90
MOSEL-RD 19	<i>Bacillus simplex</i>	100	<i>Bacillus simplex</i>	100.00
MOSEL-RD 23	<i>Pseudomonas putida</i>	100	<i>Pseudomonas taiwanensis</i>	99.90
MOSEL-RD 25	<i>Brevundimonas sp</i>	99	<i>Brevundimonas subvibrioides</i>	98.90
MOSEL-RD 27	<i>Bacillus cereus</i>	100	<i>Bacillus cereus</i>	100.00
MOSEL-RD 28	<i>Bacillus subtilis</i>	100	<i>Bacillus subtilis</i>	99.90
MOSEL-RD 36	<i>Pseudomonas geniculata</i>	100	<i>Pseudomonas geniculata</i>	100.00
MOSEL-RD 40	<i>Agrococcus terreus</i>	99	<i>Agrococcus terreus</i>	98.60

plants, originating from the inoculated seeds, confirmed their endophytic nature (Rashid et al. 2012).

Characterization of endophytic bacteria as PGPB

Bacterial plant growth promotion is a deep-rooted and multifaceted phenomenon that is often proficient via the activities of more than one PGP trait showed by the associated bacteria (Lifshitz et al. 1987). In this study, we found that endophytic bacterial isolates of perennial and wild shrub *Dodonaea viscosa* possessed multiple PGP traits such as P-solubilization, IAA production, ACC deaminase, cell wall degrading enzymes, ammonia, siderophore, HCN production and antifungal activity against phytopathogens.

IAA production

All the isolates produced IAA in the absence of tryptophan and comparatively more in the presence of tryptophan. Amounts of IAA production by bacteria can produce positive effect on plant growth (Marques et al. 2010). IAA concentrations in 48 h grown liquid cultures supplemented with tryptophan ranged from 0.88 to 23.7 µg/ml and in the absence of tryptophan ranged from 0.36 to 9.02 µg/ml (Table 2). Six isolates produced more than 4 µg IAA ml⁻¹ culture. Few isolates *Pseudomonas taiwanensis*, *Pseudomonas geniculata*, *Bacillus idriensis*, *Bacillus cereus*, *Bacillus subtilis* and *Agrococcus terreus* showed significantly increased IAA production.

Phosphate solubilization

Endophytic bacteria are also known to increase the accessibility of nutrients like insoluble and fixed forms of

phosphorus for the host plant (Young et al. 2013). A number of isolates were also found to solubilize inorganic phosphate. *Bacillus*, *Pseudomonas*, *Serratia* and *Enterobacter* are reported to solubilize the insoluble phosphate compounds and assist in plant growth (Frey-Klett et al. 2005; Hameeda et al. 2008). These genera are well documented for their phosphate solubilizing abilities (Sharma et al. 2013). Among 15 isolates, 12 isolates exhibited the phosphate solubilizing activity by forming clear zones. Isolates having qualitative phosphate solubilizing activity belonged to genera *Bacillus*, *Pseudomonas*, *Xanthomonas*, *Rhizobium*, *Streptomyces*, *Brevundimonas* and *Inquilinus* (Table 2; Fig. 1a). It is a fact that improved phosphorous nutrition influences overall plant growth and root development (Jones and Darrah 1994). Isolates belonging to *Pseudomonas* and *Bacillus* genera were recently reported to induce the plant growth in green gram plants (Saravankumar et al. 2011).

ACC deaminase activity

The decrease of the level of the plant hormone ethylene by the activity of bacterial enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase is an important mechanism of plant growth promotion (Govindasamy et al. 2008). In this study, only three of 15 isolates gave the ACC deaminase activity. *Bacillus idriensis* (0.672775 µM), *Pseudomonas taiwanensis* (4.009774 µM), and *Pseudomonas geniculata* (0.727172 µM) showed ACC deaminase activity (Table 2). Some bacteria without ACC deaminase are also known to promote plant growth to similar extent compared to ACC deaminase producing bacteria (Sheng et al. 2008; Ma et al. 2011).

Table 2 Multiple plant growth promoting activities shown by isolates

Strain ID	Strain name	PGP properties					Cell wall degrading enzyme activity					
		Ammonia production	HCN production	Siderophore production	Phosphate solubilization	ACC deaminase activity (μM of α -ketobutyrate/mg of protein/h)	Indole acetic acid production ($\mu\text{g/ml}$)		Cellulase	Protease	Pectinase	Chitinase
							Without tryptophan	With tryptophan				
MOSEL-RD 1	<i>Inquilinus limosus</i>	+	-	-	+	-	0.44	1.28	+	+	++	-
MOSEL-RD 2	<i>Xanthomonas sacchari</i>	+	+	-	+	-	1.38	4.06	++	+++	+++	-
MOSEL-RD 3	<i>Streptomyces alboniger</i>	+	+	+	+	-	1.06	3.26	+++	+	+++	+
MOSEL-RD 7	<i>Bacillus idriensis</i>	+	+	+	+	0.672775	2.46	10.12	++	++	+++	+
MOSEL-RD 9	<i>Xanthomonas translucens</i>	+	+	-	+	-	0.84	2.54	+	+++	+++	-
MOSEL-RD 12	<i>Rhizobium huatluense</i>	+	-	-	+	-	0.36	0.88	++	-	++	-
MOSEL-RD 14	<i>Microbacterium trichothecemolyticum</i>	+	+	+	-	-	1.24	7.1	+++	+++	+++	-
MOSEL-RD 17	<i>Streptomyces caeruleatus</i>	+	+	+	+	-	0.8	2.86	+	++	+++	+
MOSEL-RD 19	<i>Bacillus simplex</i>	+	+	-	+	-	1.18	2.32	++	-	++	-
MOSEL-RD 23	<i>Pseudomonas taiwanensis</i>	+	+	+	+	4.009774	9.02	23.7	++	++	++	+
MOSEL-RD 25	<i>Brevundimonas subvibrioides</i>	+	+	-	-	-	0.9	3.44	+	++	++	-
MOSEL-RD 27	<i>Bacillus cereus</i>	+	+	+	+	-	5.32	10.8	+++	++	+++	+
MOSEL-RD 28	<i>Bacillus subtilis</i>	+	+	+	+	-	2.26	8.5	+++	+++	+++	+
MOSEL-RD 36	<i>Pseudomonas geniculata</i>	+	+	+	+	0.727172	3.72	15.32	++	++	++	+
MOSEL-RD 40	<i>Agrococcus terreus</i>	+	+	+	-	-	2.54	15.34	+	+++	++	-

In cell wall degrading enzymes activities, +shows small halos <10 mm, ++shows medium diameter of 10–20 mm whereas +++shows diameter >20 mm. In case of chitinase, ammonia, HCN, siderophore and phosphate activity +sign shows positive activity of bacterial isolates whereas -sign shows no activity

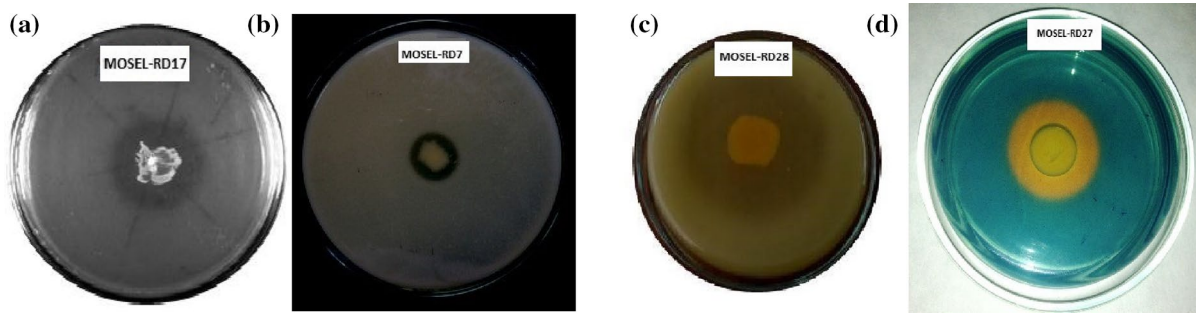


Fig. 1 Halos formation by selected strains for various activities. **a** Phospahte solubilization by MOSEL-RD17. **b** Pectinase by MOSEL-RD7. **c** Protease by MOSEL-RD28. **d** Siderophore by isolate MOSEL-RD27

Production of cell wall degrading enzymes

All the bacterial strains were screened in vitro for having different cell wall degrading enzymes activity such as cellulase, protease, pectinase and chitinase. Majority of the bacterial strains showed good activity for protease, cellulase and pectinase enzymes.

Plant cell-wall degrading enzymes

Enzymes such as cellulases and pectinases are important for the intracellular root colonization of plant growth promoting bacteria as these are hydrolytic enzymes with the ability to degrade cellulose/pectin material of the plant cell wall (Verma et al. 2001; Reinhold-Hurek and Hurek 2011) and to protect host plant (Hallmann and Kloepper 1996). All strains showed positive cellulase and pectinase activity. All the bacterial isolates showed positive pectinolytic activity and the zone of clearance formed ranged from 10 to 20 mm (Table 2; Fig. 1b).

Fungal cell-wall degrading enzyme

Fungal cell wall degrading enzymes such as chitinases and proteases are also produced by some of the isolated endophytic bacteria that can be employed for the control of plant fungal diseases. Largest zone size for protease activity was observed to be more than 20 mm (Fig. 1c). *Streptomyces (alboniger, caeruleatus)* *Bacillus (idriensis, cereus and subtilis)*, *Pseudomonas taiwanensis* and *Pseudomonas geniculata* showed chitinase activity. Gupta et al. (2006) related the degradation of fungal cell wall to chitinase. Proteases and chitinases are involved in combating the phytopathogenic fungi via biological control agents (Kim and Chung 2004). Plant growth promoting bacteria function by synthesizing hydrogen cyanide and enzymes such as chitinase for fungal cell wall degradation (Hayat et al. 2010).

Siderophore, ammonia and HCN production

Majority of the strains exhibited siderophore production (Fig. 1d) while all could potentially able to produce ammonia and HCN (except *Inquilinus limosus* and *Rhizobium huautlense*) (Table 2). Siderophore production by the isolate assumes significance for acquisition of nutrients such as iron availability to the plant (Glick 2003) and increasing competence for the nutrients with the pathogens in their ecological niche indirectly suppressing disease causing organisms (Pieterse et al. 2001; Wahyudi et al. 2011). Ahmad et al. (2008) reported that bacterial strains belonging to genera *Pseudomonas* and *Bacillus* may protect plants from phytopathogenic fungi due to HCN and ammonia production.

Antagonistic activities against pathogenic fungi

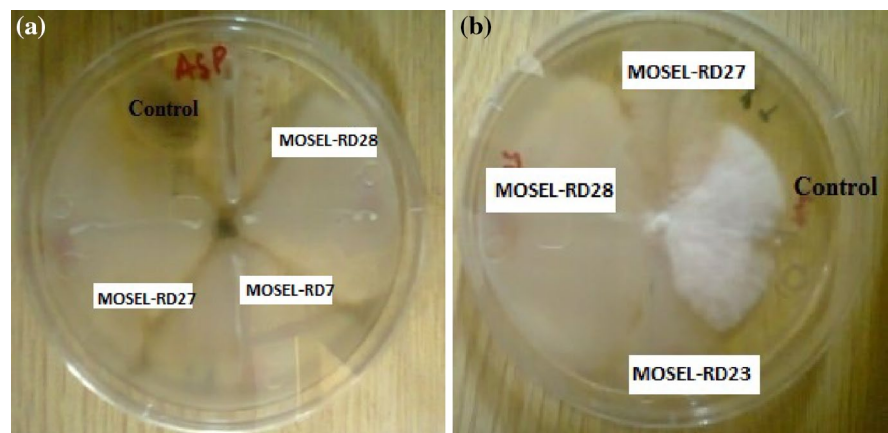
B. idriensis, *B. cereus*, *B. subtilis*, *P. taiwanensis*, *P. geniculata*, *S. alboniger* and *S. caeruleatus* showed antifungal activity against both pathogens *F. oxysporum* and *(A) niger* (Table 3; Fig. 2,b). *B. subtilis* and *B. cereus* are potentially useful as bio-control agents (Nagórska et al. 2007; Kumar et al. 2012). *Azotobacter* sp., *Pseudomonas* sp., *Streptomyces* sp. and *Bacillus* sp. are reported for antifungal activity (Ahmad et al. 2008).

Effect of endophytic bacteria as PGPB on growth of *Brassica napus* (Canola)

Canola seeds inoculated with endophytic bacteria showed that *X. sacchari*, *S. alboniger*, *B. idriensis*, *X. translucens*, *M. trichothecenolyticum*, *P. taiwanensis*, *B. cereus*, *B. subtilis*, *P. geniculata* and *A. terreus* significantly enhanced root length of Canola seedlings as compared to uninoculated control (Fig. 3). Differences in percent increases possibly lies in the plant-bacterial relationship which varies due to differences in the genetic makeup. Previous reports showed the role of *Bacillus* sp. and *Pseudomonas* sp. in the growth promotion of grape wine, tomato, maize, rice and sugar beet

Table 3 Antifungal activity of bacterial isolates

Strain ID	Strain name	<i>Fusarium oxysporum</i>	<i>Aspergillus niger</i>
MOSEL-RD 1	<i>Inquiliinus limosus</i>	–	–
MOSEL-RD 2	<i>Xanthomonas sacchari</i>	–	–
MOSEL-RD 3	<i>Streptomyces alboniger</i>	+	+
MOSEL-RD 7	<i>Bacillus idriensis</i>	+	+
MOSEL-RD 9	<i>Xanthomonas translucens</i>	–	–
MOSEL-RD 12	<i>Rhizobium huautlense</i>	–	–
MOSEL-RD 14	<i>Microbacterium trichothecenolyticum</i>	–	–
MOSEL-RD 17	<i>Streptomyces caeruleatus</i>	+	+
MOSEL-RD 19	<i>Bacillus simplex</i>	–	–
MOSEL-RD 23	<i>Pseudomonas taiwanensis</i>	+	+
MOSEL-RD 25	<i>Brevundimonas subvibrioides</i>	–	–
MOSEL-RD 27	<i>Bacillus cereus</i>	+	+
MOSEL-RD 28	<i>Bacillus subtilis</i>	+	+
MOSEL-RD 36	<i>Pseudomonas geniculatea</i>	+	+
MOSEL-RD 40	<i>Agrococcus terreus</i>	–	–

Fig. 2 Antifungal activity by selected strains against. **a***Aspergillus niger*. **b***Fusarium oxysporum*

through various mechanisms (Chauhan et al. 2013). Patel et al. (2012) explored that members of *Pseudomonas* enhanced root and shoot improvement by the members of *Bacillus* and *Pseudomonas* are reported by many researchers (Vivas et al. 2003; Patel et al. 2012). In the present study, *Bacillus idriensis* (MOSEL-RD 7) has been first ever reported as endophytic bacteria and shown to be positive for all plant growth promoting activities.

Bacteria producing IAA in the range of 2–10 µg/ml enhanced root elongation of the host plant under gnotobiotic conditions. IAA promotes root growth by directly stimulating plant cell elongation or cell division (Burd et al. 1998). Ammonia and hydrogen cyanide production by the isolates is positively associated to accumulation of nitrogen and elongation of the roots so, it seemed to influence the plant characters and biomass production of plant (Marques et al. 2010).

The plant growth promoting phenomenon can be endorsed to the ability of the isolates to give positive results

for different PGP attributes like IAA, siderophore production, phosphate solubilization, ACC deaminase and cell degrading enzymes activity. IAA positively influences root growth and development, thereby enhancing nutrient uptake (Khalid et al. 2004). However, among all PGP traits of the bacteria, the frequency of IAA-producers was found much higher than other PGP traits. IAA increases root size and spreading, resulting in greater nutrient absorption from the soil (Li et al. 2008). Many studies have been embarked on to understand the potential of these unique microbes, which harbor PGP traits.

Conclusion

Dodonaea viscosa can be the potential source of unique endophytic bacteria and for the development of an integrated strategy to be used as bio-inoculants for agronomic

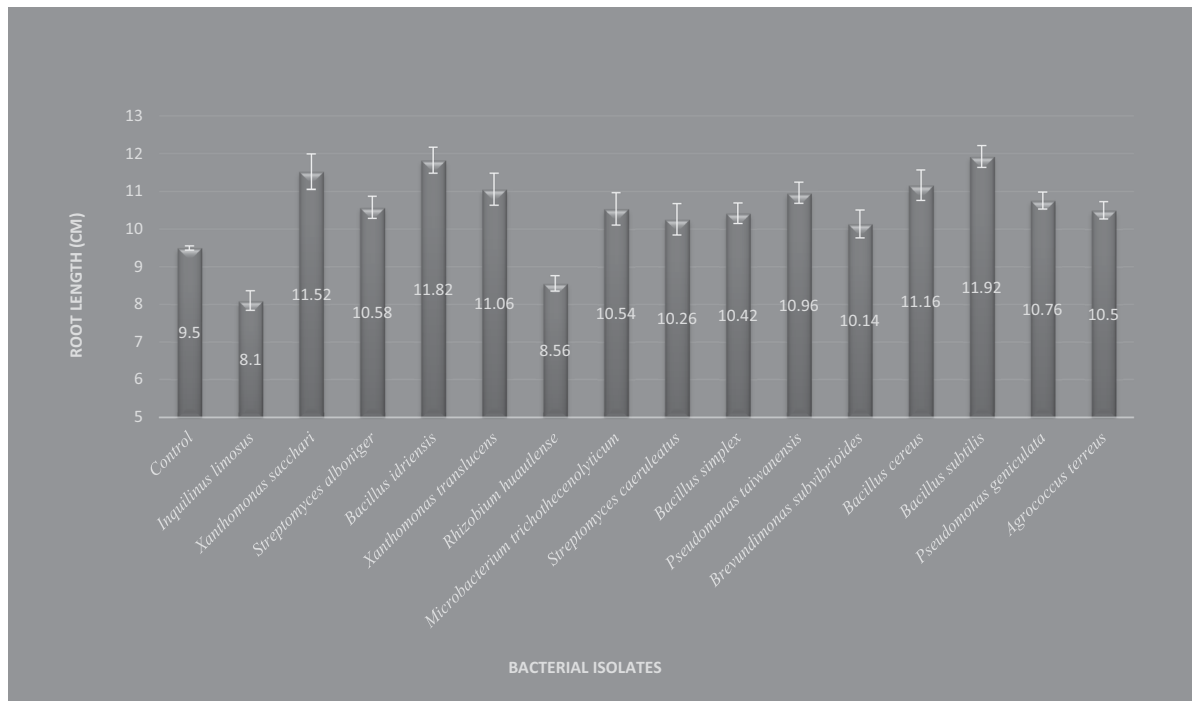


Fig. 3 Root elongation (cm) of *Brassica napus* (Canola). Results are expressed as mean of replicates. One way ANOVA was performed for each bacterial isolate. Means were compared using the least significant difference (LSD) method; $P \leq 0.05$ was considered significant.

The F-values of ANOVA for root length is 19.4 ($P < 0.05$). Dark bars belong to isolates significantly increasing root length compared to control

crops like canola to enhance their growth. Results revealed that plant growth promoting attributes like IAA production, ammonia, hydrogen cyanide, ACC deaminase and siderophore production may be positively associated with the elongation of the Canola roots in present study. *Bacillus idriensis* reported in the present study has the potential to promote seedling root growth and is first ever reported as plant growth promoting bacteria. Thus these isolates have potential to be used as plant biofertilizer for plant growth improvement. Experiments related to exploration of more bacterial traits related to growth promotion and field studies are required to further strengthen the findings.

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