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Bioprospecting Potential of Endophytic Bacteria from Leaves of *Gossypium hirsutum*

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A B S T R A C T

A total of 19 bacterial endophytes were isolated from leaves of cotton (*Gossypium hirsutum*) and characterized for plant growth promoting traits *viz*., siderophores production, phosphate solubilization, IAA and ammonia production; and preliminarily screened for chitinase, gelatinase, protease and lipase. Quantitative analysis of siderophores production and phosphate solubilization indicated that six endophyteseach *viz*., EB11, EB4, EB3, EB5, EB8 and EB9 were able to produce siderophores ranging between 80.26 to 92.58 % and EB9, EB11, EB14, EB17, EB4 and EB2 solubilized phosphorus ranging 10.24-13.58mg/ml within 48 hrs. After 72 hrs incubation, seven isolates EB3, EB13, EB8, EB11, EB15, EB2 and EB17 produced IAA in range of 15-28 µg/ml. Within 48 hrs, the ammonia production ranged from 0.8 to 1.6 (µg/ml). Primary screening of enzymes showed that chitinase was produced by EB5 (1.9 mm) and EB3 (1.1 mm); protease by EB15, EB12, EB11, EB1 and EB4 (11.80-16.60mm); gelatinase by EB1, EB9, EB1, EB12 and EB16 (10.00 -17.00mm); and lipase by EB10, EB13, EB17, EB14, EB12 and EB11 (10.00-15.00mm). Results suggested all endophytic bacterial strains studied are potential for the one or other parameter studied thus further study will help to make consortium as biofertilizer or bioprotectant to enhance plant productivity and protection.

Introduction

Plant growth promoting microbes is an attractive way to replace chemical or synthetic compounds in agriculture field because of its eco-friendly and economical feasibility (Bhattacharyya *et al.*, 2012; Kirti *et al.*, 2016). The 'biopropecting' word describes the collection and screening of biological material for commercial purpose. Every naturally propagated plant is colonized by divert communities of microbes referred to as '*endophytes'*. Endophytes defined as microorganisms that can be isolated from surface-disinfected plant tissues or extracted from within plants and that do not harm the host plants (Hallmann *et al.*, 1998). Recent studies have indicated that endophytes play many important beneficial roles in the metabolism and physiology of the host plant

via direct and indirect way. Direct way includes production of phytohormones, phosphorous solubilisation, nitrogen fixation, and siderophore production as an iron chelators etc., while indirect way includes suppression of plant diseases by elevating plant resistance mechanisms or by producing various enzymes or metabolites (Bakker and Schippers, 1987; DéFago *et al.,* 1990; Kachhap *et al.,* 2014). In last few decades a large array of endophytic bacterial species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthobacter, Burkholderia, Bacillus* and *Serratia* have been reported to enhance plant growth (Malfanova *et al.,* 2013).

Biochemically cotton plant is the source of valuable compounds such as terpenes, phenolics, fatty acids, lipids, carbohydrates and proteins. Especially, leaves of cotton plants contain camphene, limonene, myrcene, sabinene and other compounds (Egbuta *et al.,* 2017). Therefore, cotton leaf may harbor endophytes that are potential in plant protection, provide nutrients to the plants, and withstand abiotic stresses. Due to high diversity of soil bacteria and its high contribution to plant growth, traditional microbiological approaches are widely focused on root endophytic bacteria (Compant*et al.,* 2010). On the other hand, leaf endophytic bacteria have not received much attention so far. The present study aims to bridge this gap. Leaf endophytic bacteria are the collection of selective phyllosphere bacteria and they reside in the leaves and maintain endophytic symbiotic relationship with the host plant as well other microbe lives within the leaf. Every microbe within the leaf may have some beneficial function in terms of plant protection as well as to provide nutrients to the plants (Neilands, 1981; Lindow*et al.,* 2003). Studies indicated the predominance of endophytic bacteria in cotton viz., *Erwinia sp., Bacillus pumilus, B. brevis, Clavibacter species, Xanthomonas sp* (Misaghi and Donndelinger 1990), *Agrobacterium sp., Serratia sp., Burkholderia sp., Bacillus sp., Staphylococcus sp., Rhizobium, Variovorax sp., Pseudomonas sp., Acenitobacter sp., Artharobacter sp.* And *Enterobacter species* (McInroy and Kloepper 1995a and b; Mussone*t al.,* 1995). Some endophytes such as *Pseudomonas fluorescence* 89B-61 found as a controlling agent of *Fusarium* wilt of cotton caused by *Fusarium oxysporum f. sp. vasinfectum* (Chen *et al.,* 1995). McGee (2002) reported that due to presence of endophytes in leaves, plant is protected from fungal pathogens and pest. For example, methanol extract of endophytes isolated from the cotton leaf reduced the larval growth of insect pest. In another study significant reduction of damping off disease by *R. solani* was observed with the talc-based bioformulation with chitin that containe dendophytic bacteria such as *Bacillus EPCO102, Bacillus EPCO16* and *Pseudomonas fluorescence* Pf1 strains (Rajendran *et al.,* 2008). Therefore, the objective of this study is to isolate bacterial endophytes from cotton leaf and characterize their plant growth promoting traits *viz.*, siderophores production, phosphate solubilization, IAA and ammonia production; and preliminarily screening for chitinase, gelatinase, protease and lipase in order to bio-prospect the potential of these endophytic bacteria as biofertilizer or bioprotectant.

Materials and Methods

Collection of leaf sample and its surface sterilization for isolation of endophytes

Healthy leaf sample of cotton was collected from the farm at Main Cotton Research Station, Athwa farm, Surat, Gujarat. The collected leaf were taken to the laboratory and preserved at 4°C in sealed plastic bags and subjected to isolate endophytic bacteria.

Surface sterilization of leaf samples was performed according to the method of Musson *et al.,* (1995). Healthy cotton leaf sample was washed by running tap water for 1-2 min followed by 1 min wash with 75% alcohol to remove the soil particles completely.

Then the leaf sample was washed with sterile 1% (v/v) sodium hypochlorite embedded with 0.05% (v/v) Triton X-100 for3 min followed by four sequential wash with sterile phosphate buffer (pH 7.0). At this point, 0.1 ml of final phosphate buffer washed solution was spread onthe nutrient agar (Musson *et al.,* 1995).

Isolation and preliminary characterization of endophytic bacteria

After surface sterilization, leaf sample cut into 5 mm square pieces with sterile blade. Each of the leaf pieces embedded carefully on the surface of different media *viz.,* Nutrient Agar, R2A Agar and Soil Extract Agar (Musson *et al.,* 1995).

Four leaf pieces per plate were placed on each medium. Plating was done in triplicate and all plates were incubated at room temperature (27^{o}C) for three to four days.

After incubation, suspension of the bacteria which grown surrounding the leaf sample was prepared. Further, isolation and purification of endophytic bacteria was carried out using quadrant method (Sanders, 2012). Each of purified colonies than transferred to nutrient agar slants and were stored at 4^{o}C.

Bergey's manual of determinative bacteriology was used for recording preliminary cultural characteristics such as size, shape, margin, elevation, consistency, opacity, pigment and gram reaction of isolated bacterial endophytes (MacFaddin, 2000).

Evaluation of plant growth promoting traits of the endophytic isolates

Siderophores detection assay

Siderophores produced by the endophytic isolates were determined using qualitative and quantitative assay as described by Schwyn and Neilands (1985). For quantitative assay, test isolates were grown in iron free sterile succinic acid medium (Hi-media) for 48 hrs at room temperature at 120 rpm. Cell supernatant (0.5 ml) after centrifugation at 15,000 rpm for 5 min was mixed with 0.5ml of CAS solution (1:1) to observe the colour change from blue to orange or yellow. Optimal density was measured at 630 nm and the percentage of siderophore production was calculated as per formula [(Ar-As)/Ar] ×100=% siderophore units, where Ar = absorbance of reference (succinic acid media + CAS assay solution), As = absorbance of sample. As a reference sterile succinic acid medium free from inoculates was used. For qualitative analysis, 48 hrs old cultures of endophytic bacteria grown in succinic acid medium were spot inoculated on CAS agar plates and incubated at 27ºC for 48 hrs. Positive reaction was indicated by a color change of the CAS reagent from blue to orange surround the colony shows the siderophores production. Zones index was calculated by dividing yellow/orange zone on CAS agar by growth diameter of spot inoculants (Louden *et al.,* 2011).

Assessment of phosphate solubilization ability

Quantitative estimation of phosphate solubilization activity of isolates was performed using method described by King (1932). For that, bacterial endophytes inoculated into the sterile Pikovskaya's medium (Pikovskaya, 1948) kept for 48 hrs at room temperature at 120 rpm. Culture

supernatant after centrifugation used to estimate the concentration of soluble phosphate using stannous chloride method (King, 1932). For that, 1.0ml of the supernatant was mixed with 10 ml of 0.5M $NaHCO_3$, 10 ml of 1.5% ammonium molybdate, 1 ml of working stannous chloride and the final volume to 50ml using distilled water. Optimal density measured at 660nm after 15 min of incubation. The concentration of phosphate was estimated using standard curve of tricalcium phosphate in the range of 0.04-0.4 mg/ml.

Qualitative estimation of tricalcium phosphate solubilization was performed using the method of Pikovskaya (1948). Each endophytic cell suspensions were spot inoculated on Pikovskaya's agar plates and incubated at 27±2℃ for 48 hrs to observe the zone of solubilisation. Phosphate solubilisation index was calculated by dividing phosphate solubilisation zone on pikovskaya's agar by growth diameter of spot inoculants.

Indole Acetic Acid (IAA) production assay

The ability of bacterial endophytes to produce IAA was measured using colorimetric method described byLoper and Scroth (1986). For each of endophytic bacterial isolates was grown in sterile nutrient broth embedded with 0.2 ml of 1% L-tryptophan for 48 hrs. Further, 1.0 ml supernatant of each endophytic bacterial isolates mixed with 1.0ml of Salkowski's reagent to develop the pink colour for the positive result of IAA production and measured at 530 nm. Concentration of IAA produced was estimated against standard curve of IAA in the range of 10-100 µg/ml.

Production of ammonia

The ability of endophytic bacterial strains to produce ammonia was assessed as described by Cappuccino and Sherman (1992). In this method, each isolate inoculated into the 5.0ml of sterile peptone water broth and incubated at room temperature for 48 hrs at 120 rpm. After incubation, 1.0 ml of culture supernatant was mixed with 1.0 ml of Nesseler's reagent. Color change from brown to yellow was measured spectrophotometrically at 450nm. Concentration of ammonia produced was estimated against standard curve of ammonium nitrate in the range of 0.1–1.0 µg/ml.

Preliminary screening of endophytic bacterial strains for the chitinase, protease, lipase and gelatinaseproduction

Preliminary study of different enzyme production *viz*., chitinase, protease, lipase and gelatinase was performed using plate agar assay on the principle of zone of solubilization of the substrate. Solubilization index was calculated by dividing the respective substrate solubilisation zone on agar plate by growth diameter of spot inoculants.

Chitinase activity of bacterial isolates was determined by using chitin agar plates according to the method described by Kuddus and Ahmad (2013). Each bacterial isolate was spot inoculated and incubated for several days at 27 ± 2°C to study the zone of solubilisation.

Protease activity was performed using casein agar plate according to the method described by Olajuyigbe and Ajele (2005). Spot inoculation of each bacterial cell suspension was carried out on casein agar plate, kept for 48 hrs at room temperature to observe the zone of solubilization. Determination of lipase activity was performed using tributyrene agar plate according to method of Sirisha *et al.*, (2010). Endophytic cell suspension was spot inoculated on tributyrene agar plates and incubated for 48-72 hrs at

room temperature for the formation of zone of solubilization.

Presence of gelatinase was confirmed using preparation of gelatin agar plate according method described by Smith and Goodner (1958). Gelatin agar plates which were spot inoculated by each of the strains were flooded by Frazier's reagent to observe the zone of solubilization after 72 hrs.

Results and Discussion

Isolation of Endophytic bacterial strains from the leaf of *G. hirsutum* and their preliminary characteristics

Before isolation, surface sterilization of the plant tissue is a crucial step to ensure the isolation of endophytes. Thus, confirmation of proper surface sterilization of leaf carried out by inoculating last phosphate buffer wash into the nutrient broth and on nutrient agar plate. Absence of any growth after three days incubation indicated the proper surface sterilization of the leaf sample. The series of washes with tap water, 75% alcohol followed with sequential wash with sodium hypochlorite reduces 90% surface contaminants from the plant tissues and ensure proper sterilization (Musson *et al.*, 1995).

To get the diversity of endophytic bacteria, it is necessary to provide different nutrient conditions. Thus, the bacterial endophytes were isolated on three different media *viz*., Nutrient agar, R2A agar and soil extract agar. As nutrient medium is known for all types of microbes, R2A for oligotrophs and soil extract agar provides nutrients same as present within the soil. Figure 1 shows the growth of bacteria surrounding the leaf sample.

Initially a total of 23 colonies were isolated from the leaf of cotton plant. Of the 23 colonies, eight each were observed on Nutrient and R2A medium; and the other seven on soil extract agar. Further, all these colonies when grown on nutrient medium reduced to 19 colonies based on their similar colony characteristics. Basic cultural characteristics and gram reaction of all endophytic bacterial isolates are presented in Table 1. Out of 19 isolates, ten were gram positive and nine were gram negative rods. All the isolates were colourless except EB9 pigmented as fluorescent green; EB14 as fluorescent blue and EB 11 was brown pigmented. Further, all colonies were found to be round shaped with entire margin except EB2 and EB4 were punctiform and irregular in shape, respectively; while, EB4 showed repand margin. All the isolated bacterial endophytes were transparent except EB2 and EB4, they were opaque. The results are further in confirmation with the findings of Arunachalam and Gayathri (2010) who also reported the variation for cultural characterization of endophytic bacteria isolated from *Andrographis paniculata*.

Plant growth promoting traits of the endophytic bacterial isolates

Endophytes are known to provide the plant nutrients, plant health benefits along with protection against pest and disease. Therefore use of endophytes as an alternative eco-friendly method for the sustainable environment. Thus, an attempt has been made to study the endophytic bacteria from the leaf of *G. hirsutum* for their ability to produce plant growth promoting traits *viz.*, siderophores, indole acetic acid, ammonia and ability to solubilize phosphate. Besides these, preliminary study of enzyme production *viz*., chitinase, protease, gelatinase and lipase were carried out because of their role in plant protection. Table 2 summarizes the presence of plant growth promoting traits and enzyme activities of all 19 endophytic bacterial isolates.

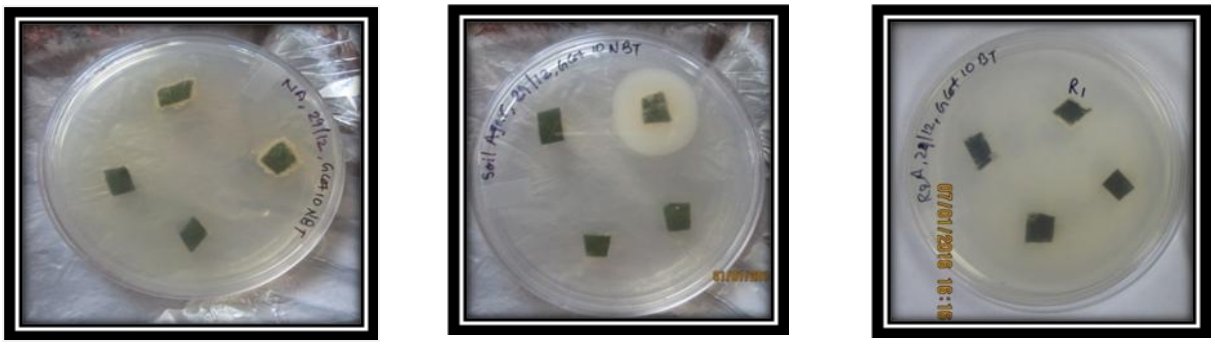
The siderophores play important role in the growth of plants with their ability to supply iron and suppress of plant pathogens. All isolates were studied qualitatively as well as quantitatively for their siderophores production activity. Among 19 isolates, six were confirmed to produce siderophores by color change from blue to orange. Qualitatively six isolates EB11 (92.58%), EB4 (88.94%), EB3 (86.02%), EB5 (85.41%), EB8 (84.44%) and EB9 (80.26%) produced higher siderophores as compared to control (55.04%) within 48hrs (Fig. 2a and 2b). These isolates showed orange color zone in range of 1.0- 2.2 cm of diameter on CAS plate agar assay. Sharma *et al.*,(2014) reported siderophore production by *Pseudomonas* sp in the of range 2.0-2.1 cm of diameter of orange color zone by two isolates *i.e.* one isolate from apple Ar-3-kul (2.0 mm) and one from pear i.e. Pn-1-Kul (2.1mm) site. According to Bashan *et al.,* (2005), siderophore produced by the endophytic bacteria have higher affinity for iron than the siderophore produced by the pathogens of plant; thus the former endophytic bacteria scarvenge most of the available iron, and thereby prevent proliferation of pathogens within the plant especially plant pathogens. Also, some plants can bind and release iron from bacterial iron-siderophore complexes and thus use iron for growth (Bashan *et al.*, 2005).

Table.1 Cultural characteristics and gram reaction of the endophytic bacterial strains

Sr. No.	Culture No.	Size	Shape	Margin	Elevation	Consistency	Opacity	Pigment	Gram reaction
1	EB1	Small	Round	Entire	Flat	Moist	Transparent	Colourless	Gram(-)
2	EB2	Pinpoint	Punctiform	Entire	Flat	Viscous	Opaque	Colourless	Gram(+)
3	EB3	Small	Round	Entire	Convex	Viscous	Transparent	Colourless	Gram(+)
4	EB4	Large	Irregular	Repand	Flat	Dry	Opaque	Colourless	Gram(+)
5	EB5	Large	Round	Entire	Convex	Moist	Translucent	Colourless	Gram(-)
6	EB6	Large	Round	Entire	Convex	Moist	Transparent	Colourless	Gram(+)
7	EB7	Small	Round	Entire	Flat	Viscous	Transparent	Colourless	Gram(-)
8	EB8	Pinpoint	Round	Entire	Convex	Dewdrop	Transparent	Colourless	Gram(+)
9	EB9	Small	Round	Entire	Convex	Moist	Transparent	Fluorescent blue	Gram(-)
10	EB10	Pinpoint	Round	Entire	Raised	Moist	Transparent	Colourless	Gram(+)
11	EB11	Small	Round	Entire	Convex	Viscous	Transparent	Brown	Gram(-)
12	EB12	Small	Round	Entire	Raised	Moist	Translucent	Colourless	Gram(-)
13	EB13	Small	Round	Entire	Raised	Moist	Translucent	Colourless	Gram(-)
14	EB14	Small	Round	Entire	Convex	Moist	Translucent	Fluorescent green	Gram(-)
15	EB15	Pinpoint	Punctiform	Entire	Raised	Dry	Transparent	Colourless	Gram(-)
16	EB16	Small	Round	Wavy	Flat	Dry	Transparent	Colourless	Gram(-)
17	EB17	Small	Round	Entire	Convex	Moist	Transparent	Colourless	Gram(+)
18	EB18	Larger	Round	Entire	Flat	Dry	Opaque	Colourless	Gram(+)
19	EB19	Pinpoint	Round	Entire	Convex	Moist	Transparent	Colourless	Gram(+)

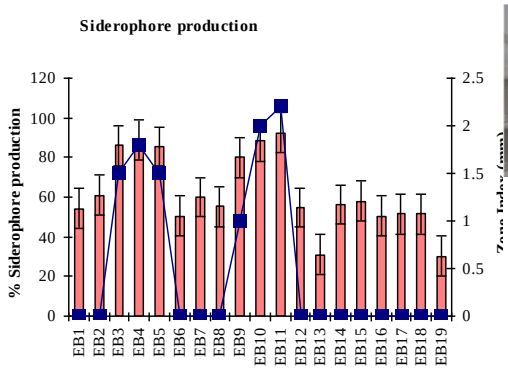
Table.2 Data on plant growth promoting traits and the enzyme production of all isolated endophytic bacteria from leaf of *G. hirsutam*

Isolates	Siderophore production	Phosphate Solubilization	IAA Production	Ammonia Production	Protease Production	Lipase Production	Gelatinase Production	Chitinase Production
EB1	-	-	+	+	+	+	+	-
EB2	-	-	+	+	-	-	-	-
EB3	+	-	+	+	-	+	-	+
EB4	+	+	+	+	+	+	-	-
EB5	+	-	+	+	-	+	+	+
EB6	-	-	+	+	+	+	-	-
EB7	-	-	+	+	-	+	+	-
EB8	-	-	+	+	-	-	-	-
EB9	+	+	+	+	+	+	+	-
EB10	+	-	+	+	-	+	-	-
EB11	+	+	+	+	+	+	+	-
EB12	-	-	+	+	+	+	+	-
EB13	-	+	+	+	+	+	-	-
EB14	-	+	+	+	+	+	+	-
EB15	-	-	+	+	+	+	+	-
EB16	-	-	+	+	+	+	+	-
EB17	-	+	+	+	-	+	-	-
EB18	-	-	+	+	+	+	+	-
EB19	-	-	+	+	+	+	-	-
Total	06	06	19	19	12	17	10	02



(a) **(b)** **(c)**

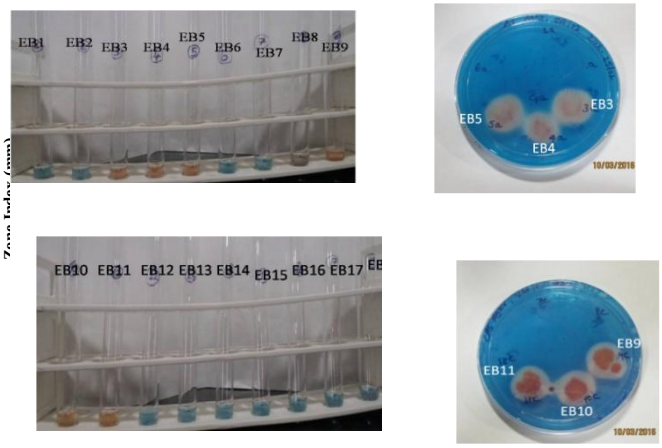
Fig.1 Growth of endophytic bacteria from leaf sample of*G. hirsutum* on (a) nutrient agar, (b) Soilextract agar and (c) R2A agar medium



Endophytic bacteria

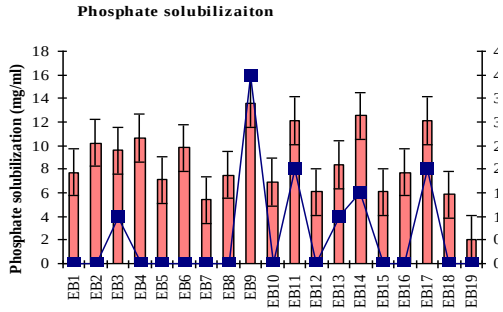
2(a)

Fig.2(a) Graphical data represents the qualitative and quantitative production of siderophores by endophytic bacteria



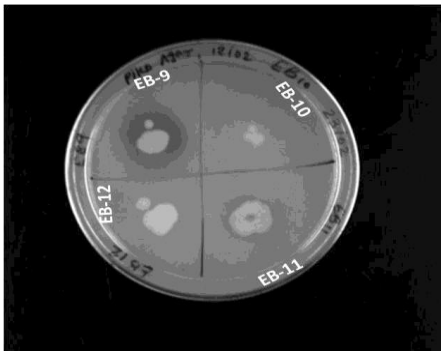
2(b)

Fig.2(b) Confirmation of siderophores production by color change from blue to orange quantitatively andqualitatively by CAS assay



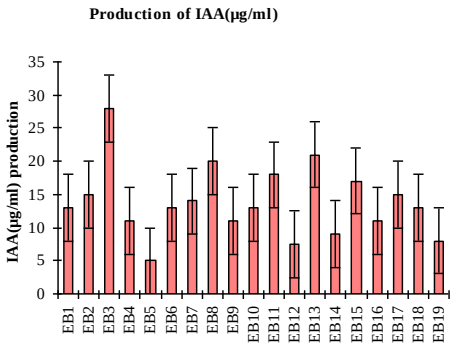
Endophytic bacteria 3(a)

Fig.3(a) Graphical data represents the qualitative and quantitative production of phosphate solubilization by endophytic bacteria



3(b)

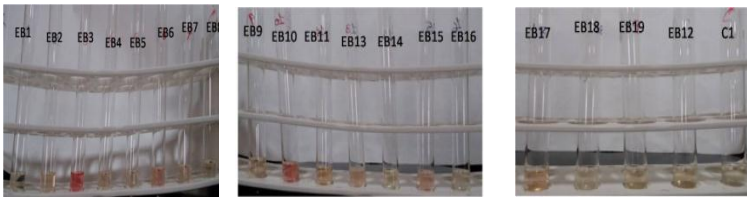
Fig.3(b) Solubilization of phosphate on Pikovkya's agar plate by EB9



Endophytic bacteria

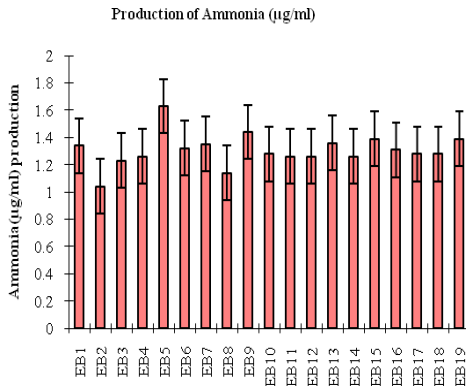
4(a)

Fig.4(a) Quantitative estimation of IAA(µg/ml)



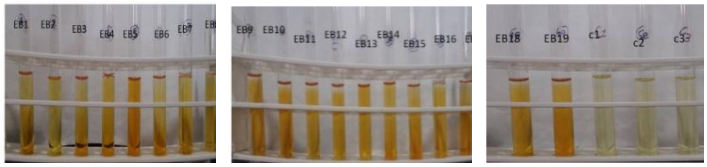
4(b)

Fig.4(b) Detection of IAA production by development of pink color



Endophytic bacteria 5(a)

Fig.5(a) Quantitative determination of ammonia production (µg/ml)



5(b)

Fig.5(b) Color change of yellow to brown due to production of ammonia after addition of Nessler's reagent

Endophytes move into plants from soil through cracks due to emerging lateral root; from roots bacteria spread in leaves, flowers and fruits *via* vascular plant system (Hardoim *et al.,* 2008; Compant *et al.,* 2011). Thus, endophytes from leaf may have possible mechanism of phosphate solubilization. Assessment of phosphate solubilization in the present work indicated that only one strain EB9 had shown significant phosphate solubilization both qualitatively (4.0 mm) and quantitatively (13.58mg/ml). Zones of solublization data were not exactly matched with the activity of solubilization with liquid assay within the duration of 48 hrs. The strains EB11 (12.12mg/ml), EB14 (12.54mg/ml), EB17 (12.12mg/ml), EB4 (10.65mg/ml) and EB2 (10.24mg/ml), which showed significant solubilization of tricalcium phosphate by stannous chloride method did shows less significant or no zone with agar plate assay. Data are presented in Figures 3a and 3b and that represents the solubilization of phosphate by EB9 strain on Pikovskaya's medium. According to Johnston (1952), it might be due to the different strategies used by bacteria to solubilize phosphate, the time duration, type and amount of organic acid produced by diversified bacterial strain etc. Detailed study will be required to know the factors behind the differences of results using agar and liquid assay.

Indole acetic acid production by bacteria is a part of mechanism between plant-microbial interactions. IAA is an auxin that regulates the plant growth and its cell division. To determine the IAA production each isolate was grown in nutrient medium embedded with tryptophan because tryptophan is believed to be the primary precursor for the formation of IAA in microbes (Monteiro *et al.*, 1988). After 72 hrs incubation, addition of Salkowski's reagent showed significant amount of IAA production with the bacterial endophytes EB3 (28 µg/ml), EB13 (21 µg/ml), EB8 (20 µg/ml), EB11 (18 µg/ml), EB15 (17 µg/ml), EB2 and EB17 (15 µg/ml). Other strains also produced IAA in small or negligible amount (Fig. 4a and 4b). Sharma *et al.,* (2015) reported IAA production on luriabertani broth by *Bacillus* spp. ranging from 12 to 25 µg/ ml.

Ammonia production is an important plant growth promoting trait, where microbes can able to breakdown complex nitrogenous materials and convert it into ammonia, which is taken up by plant as a nitrogen source.

Also, formation of ammonia leads to alkaline condition, which suppresses the growth of certain pathogenic fungi (Jha *et al.,* 2012). In our study, peptone was used as a nitrogenous compound which breakdown leads to formation of ammonia; it was detected by color change using Nessler's reagent. In the present study, it was observed that all endophytic isolates showed ammonia production in the range of 1.6-0.8 (µg/ml) within 48 hrs (Fig. 5a and 5b). Significant differences were not recorded for ammonia production among all 19 isolates.

Preliminary assessment of enzymes production of endophytic bacteria

On leaves of plant, limited sites are available where the pathogen can attack the plant. Bacteria capable of multiplying within the leaf can compete with pathogens by producing enzymes or such chemical compounds as a metabolic product. Diverse group of microbes as an endophytes lives within the plant tissues. From that, such microbes are able to synthesize and secret hydrolytic enzymes. Many microbes produce and excrete lytic enzymes such as chitinase, protease, gelatinase and lipase, which can hydrolysepolymeric compounds *viz*., chitin, proteins and lipids. Secretion of these enzymes by different microbes results in the suppression of plant pathogenic activity directly (Pal *et al.,* 2006). For example, fungal cell wall made up of chitin, which might be breakdown by endophytic microbial chitinase (Bashan *et al.,* 2005). Plate agar assay was used for the preliminary study of enzyme production by the isolates. Among 19 isolates, two bacterial strains EB5 (1.9mm) and EB3 (1.1mm) showed zone of solubilization with chitin agar plate after 7 days. Protease production was shown by six isolates, EB15 (16.60mm), EB12 (14.00mm), EB11 (13.60mm), EB1 (12.60mm) and EB4 (11.80mm). Gelatinase activity was recorded in five strains EB1 (17.00mm), EB9 (12.50mm), EB11 (15.50mm), EB12 (10.00) and EB16 (15.70mm) on gelatin agar plate. Significant lipase activity on tributyrene agar plate was recorded with EB10 (15.00mm), EB13 (13.70mm), EB17 (12.80mm) EB14 (12.20mm), EB12 (11mm) and EB11 (10mm). Rajendran (2006) reported that application of endophytes such as *Bacillus* species and *Pseudomonas* species to cotton plant induced the expression of chitinase and other enzymes which reduced the disease severity caused by *Xanthomonas axonopodis* pv. *malvacearum*. Similarly, Khianngam *et al.,* (2013) isolated and screened twenty endophytic bacteria from mangrove plants in Thailand for the presence of hydrolytic enzymes *viz*., proteases, lipases, amylases or cellulases.

From our study, it can be concluded that all the 19 endophytic bacterial strains studied are potential strains for the one or other plant growth promoting traits and hydrolytic enzymes involved in protection against pest and disease. These potential isolates could be used to make consortium as biofertilizer or bioprotectant to enhance plant productivity and protection. Among 19 isolates, EB9 and EB11 showed all activities except chitinase production, thus these two are the candidates endophytic bacterial strains to be studied at molecular level along with their effects on plant growth under pot and field conditions that would help us to understand the plant microbe interaction in detail.

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