

Screening of Endophytic Bacterial Isolates of Tea (*Camellia sinensis* L.) Roots for their Multiple Plant Growth Promoting Activities

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Abstract

A total of eighteen endophytic bacterial isolates from tea roots were screened for their multiple plant growth promoting activities including indole acetic acid production (IAA) production, phosphate solubilization, ammonia production and siderophore production. All the isolates subjected for characterization were IAA producers. Production of IAA was found to be remarkable in ER7 (10.45 µg/ml), ER14 (11.80 µg/ml), ER15 (11.40 µg/ml) and ER17 (16.22 µg/ml). Among the isolates, seven were siderophore producers, five were phosphate solubilizers and twelve were found to be ammonia producers. Proper implementation of these plant growth promoting endophytic bacteria will open a new area in organic tea cultivation.

Highlights

Endophytic isolates ER 15, ER17, and ER 18 may be used as potential biofertilizer agents to produce organic tea.

Keywords: Endophytic bacteria, siderophore, phosphate solubilization, IAA, plant growth promotion.

Microorganisms present within the intercellular spaces of any plant tissues without showing any visible symptoms of their presence are termed as endophytic microorganisms (Saikkonen *et al.*, 2004). Endophytic microorganisms are the inhabitants of different plant tissues including roots, stems, leaves and seeds (Johri, 2006). Though endophytes are ubiquitous in occurrence, their relationship with host plants is still not well known. Benefits of harboring the endophytic microorganisms for the plant itself are in various dimensions. From the support of a large number of experimental evidences it is revealed that endophytic microorganisms support the plant growth, development

and yielding by synthesizing different plant hormones as well as solubilization and mobilization of insoluble mineral salts (Bai *et al.*, 2003; Bottini *et al.*, 2004; Castro-Sowinski *et al.*, 2007). They promote plant growth and confer enhanced resistance to various pathogens (Arnold *et al.*, 2003) by producing antimicrobial agent (Ezra *et al.*, 2004). Besides these endophytic microbes produce some unusual secondary metabolites which are important to their hosts (Taechowisan *et al.*, 2005). It is also assumed that endophytes act as a biological trigger to activate the stress response system of plants more rapidly than that of non-mutualistic plants (Redman *et al.*, 2002).



Endophytes have an excellent potential to be used as plant growth promoters. They promote plant growth in various ways by secretion of growth hormones like indole acetic acid, solubilization of insoluble phosphate salts enhancing hyphal growth and micorrhizal colonization, production of siderophores and supplying biologically fixed nitrogen. In addition, endophytic microbes supply essential vitamins to plants. Endophytic rhizobial strains of rice significantly increase root and shoot growth of different crop plants. Inoculation with *Azotobacter diazotrophicus* wild type strain increases height of N-limited sugar cane plants compared with uninoculated plants. (Bai *et al.*, 2003; Lee *et al.*, 2004; Wakelin *et al.*, 2004).

Tea, one of the widely cultivated crop of Assam as well as India, depends on high agricultural inputs which are not only detrimental to non-targeting organisms but for human health also. Over application of chemical fertilizers is responsible for altering the soil quality and soil microbial populations (Bhatt *et al.* 2012; Bhaduri and Gautam. 2012). Agusta *et al.*, (2006) isolated and characterized six different fungal groups from *Camellia sinensis* collected from west Java and established their phylogenetic relationship to other groups. It is also reported that *Trichoderma* strains from healthy tea plants when inoculated to tea seedlings resulting resistant capacity to some common pathogens. As endophytic microbes are native, less detrimental to their hosts and promote plant growth, therefore, the use of endophytes may open a new area in crop production with minimal use of agrochemicals.

Materials and methods

Collection and preparation of samples

Three years old tea (*Camellia sinensis*) roots were collected from four different tea gardens of upper Assam. Healthy and disease free young roots were collected in ice box, packed immediately in poly bags and stored at 4 °C for further use.

Isolation of Endophytic bacteria

Samples were first washed properly with tap water for seven times followed by washing with distilled water for five times to remove debris. After washing, samples were dipped in 70% ethanol for 5 min and 0.1% HgCl₂ for 1 minute and then washed with sterilized distilled water for 10 times to remove the surface sterilizing agents (Bandara *et al.*, 2006). Five grams of the samples were homogenized in 50 ml of distilled water to prepare stock solution of

tissue homogenate. From this stock, appropriate serial dilution was made and inoculated in Tryptic soya agar (TSA) plates and incubated at 30⁰ C for 48 hrs. From these plates pure colonies were isolated by the process of streaking (Tejera *et al.*, 2006).

Estimation of IAA production by test isolates

Quantitative estimation of IAA was performed according to Brick *et al.*, (1991). For this purpose 25 ml of nutrient broth were poured into 100 ml Erlenmeyer flasks, autoclaved and allowed to cool at room temperature. The media was amended with filter sterilized L-tryptophan (1000 µg/ml). Flasks were inoculated with 1 ml of 0.6 OD fresh bacterial cultures and incubated at 30⁰ C for 72 hrs. After incubation the contents of flasks were centrifuged at 3,000 rpm for 10 minutes. For measuring the amount of IAA produced, 1ml of culture supernatant was pipetted into test tubes and mixed with 2 ml of FeCl₃-perchloric acid reagent (50 ml 35% perchloric acid + 1 ml 0.5 M FeCl₃ solution) (Gordon and Weber; 1951) and 2 drops of *ortho*-phosphoric acid. After 25 mins development of pink colour was measured at 530 nm wave length by UV-VIS spectrophotometer (CECIL CE 7250).

Screening of isolates for phosphate solubilizing activity

Solubilization of insoluble mineral phosphates was screened by growing the isolates on Pikovskaya's agar medium. Inoculated plates were incubated at 30⁰ C, clear zones around the colonies were observed after three days (Gour 1990).

Screening of isolates for ammonia production

Bacterial isolates were grown in 10 ml peptone water medium for 72 hrs at 30⁰C. After 0.6 OD growths 0.5 ml of Nessler's reagent was added. Development of brown to yellow coloration indicates ammonia production (Cappuccino and Sherman; 1992).

Screening of isolates for siderophore production

Siderophore producing activity of bacterial isolates was observed by using CAS (chrome azurol sulfonate) liquid medium. Bacterial cultures were grown in iron starved medium for 48 hrs. and then centrifuged at 3000 rpm for 5 mins. One ml of culture supernatant was added to 0.5 ml of CAS solution and left for 25 minutes to change the colour of the solution. Development of yellow to pink colour indicated siderophore production (Schwyn and Neilands. 1987).

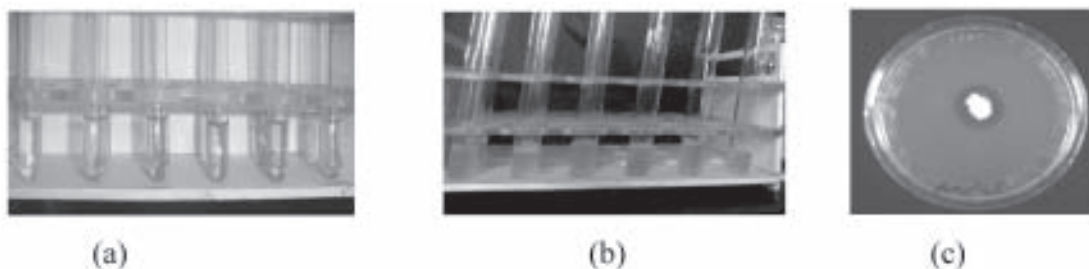


Figure 1: (a) IAA, (b) ammonia production and (c) phosphate solubilization activities of some bacterial isolates.

Results

The present study deals with the isolation and characterization of endophytic bacteria from tea (*Camellia sinensis*) roots and evaluates their plant growth promoting activities.

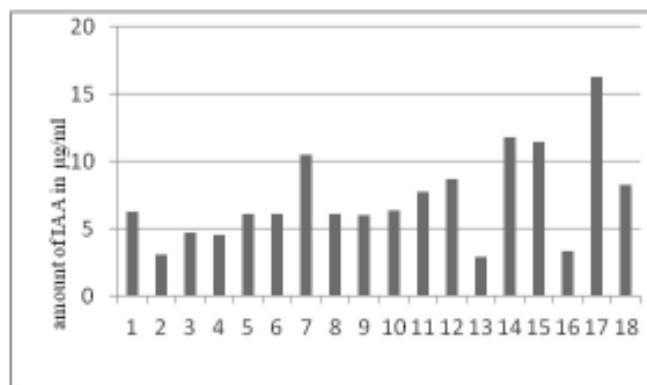


Figure 2: Bar diagram showing IAA production by endophytic bacterial isolates (1-18 refers ER1-ER18).

Plant growth promoting activities of the isolates

All the isolates subjected for their characterization were IAA producers in presence of *L. tryptophan* (1000µg/ml). Among the bacterial isolates, production of IAA was found to be remarkable in in ER7 (10.45 µg/ml), ER14 (11.80 µg/ml), ER15 (11.40 µg/ml) and ER17 (16.22µg/ml). All other isolates were also found to be more or less good IAA producers.

Out of eighteen isolates, ER3, ER4, ER5, ER6, ER9, ER11, ER12, ER13, ER14, ER15, ER16, and ER18 were found to be positive for ammonia production, ER1, ER7, ER8, ER9, ER10, ER13, and ER17 were found to be siderophore producers and ER9, ER10, ER13, ER17an, ER18 were positive for tri calcium phosphate solubilisation.

Discussion

Among all the bacterial isolates screened for plant growth promoting activities, only a few were found to be efficient IAA producers. Production of IAA was found to be remarkable in ER7 (10.45 µg/ml), ER14 (11.80 µg/ml), ER15 (11.40 µg/ml) and ER17 (16.22µg/ml). Among these isolates, 12 were ammonia producers, seven were Siderophore producers, and five were phosphate solubilizers. Several earlier reports have revealed that endophytes have an excellent potential to be used as plant growth promoters (Bai *et al.*, 2003). They promote plant growth in various ways, by secretion of growth hormone such as indole acetic acid (Lee *et al.*, 2004), via phosphate solubilizing activity (Wakelin *et al.*, 2004), by enhancing hyphal growth and micorrhizal colonization, production of siderophores and supplying biologically fixed nitrogen. In addition endophytic microbes supply essential vitamins to plants. Endophytic rhizobial strains significantly increase root and shoot growth of different crop plants. Inoculation with *Azotobacter diazotrophicus* wild type strain increases height of N- limited sugar cane plants compared with uninoculated plants (Sevilla *et al* 2001). Several bacterial endophytes have been shown to support plant growth and increase nutrient uptake by providing phytohormones, low molecular weight compounds, and siderophores. The plant associated habitat is a dynamic environment in which many factors affect the structure and species composition of the microbial communities that colonize roots, stems, branches and leaves. It is previously been shown that endophytic communities vary spatially in plants or may be dependent on interaction with other endophytes or pathogenic microorganisms (Bottini *et al.*, 2004). In the present study it is clear that all the endophytic isolates taken into consideration were able to produce a remarkable amount of IAA. Other plant growth promoting substances like

**Table1:** plant growth promoting activities of the isolates.

Isolates. .	Siderophore production.	Ammonia production.	Phosphate solubilization.	IAA production in (µgm/ml)
ER 1	+	-	-	6.22 ± 0.87
ER 2	-	-	-	3.04 ± 0.85
ER 3	-	+	-	4.68 ± 0.97
ER 4	-	+	-	4.48 ± 0.77
ER 5	-	+	-	6.10 ± 0.89
ER 6	-	+	-	6.10 ± 0.77
ER 7	+	-	-	10.45 ± 0.96
ER 8	+	-	-	6.10 ± 0.92
ER 9	+	++	+	6.00 ± 0.67
ER 10	+	-	+	6.30 ± 0.62
ER 11	-	++	-	7.70 ± 0.43
ER 12		++	-	8.70 ± 0.88
ER 13	+	+	+	2.84 ± 0.57
ER 14		+	-	11.80 ± 0.99
ER 15		+	-	11.40 ± 0.86
ER 16		++	-	3.30 ± 0.65
ER 17	+	-	++	16.22 ± 0.98
ER 18	+	+++	+	8.22 ± 0.67

Values are average ±SD of five replicas.

siderophore production, ammonia production and phosphate solubilization activities confirm that endophytic bacterial isolates are good plant growth promoters and predicts their possibilities to be used as biofertilizers.

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