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SEED GERMINATION ENHANCING ACTIVITY OF ENDOPHYTIC *STREPTOMYCES* ISOLATED FROM INDIGENOUS ETHNO-MEDICINAL PLANT *CENTELLA ASIATICA*

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ABSTRACT

Centella asiatica, a folk ethno-medicinal plant of Meghalaya was investigated for the isolation of endophytic actinomycetes and their performance in seed germination and seedling growth of *Phaseolus vulgaris* Linn was further tested. A total of thirty actinomycetes were isolated and tested for seed germination and seedling growth. Only two isolates CA10 and CA26 were showed, 100 percent germination and higher seedling growth comparison to control. It also revealed that germination percentage showed significant positive correlation with seedling length ($r=0.826$, $p<0.05$). The strains CA10 and CA26 were also evaluated for production of indole acetic acid which was quantified as 71µg/ml and 197µg/ml respectively. Based on morphological and biochemical criteria, the two isolates were tentatively identified as *Streptomyces.* From the present investigation, it can be suggested that the treatment of bean seeds with *Streptomyces* sp. might enhance seed germination and seedling growth.

KEYWORDS: ***Streptomyces*, Seed germination, Seedling growth, Indole-acetic acid, Medicinal plant**

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INTRODUCTION

Endophytic actinomycetes are widely distributed in natural plants, without exhibiting signs of pathogenicity [1]. Additionally, they are capable of colonizing internal tissues of plant; this made them as valuable tool in agriculture to improve crop production especially through seed germination and plant growth promotion [2]. They are well known high G+C containing gram positive bacteria and producer of bioactive metabolites with *Streptomyces* being the dominant [3]. *Phaseolus vulgaris* Linn. is commonly known as French bean is the most profitable crop in India which is cultivated extensively in north east India particularly in Meghalaya. Unfortunately, its production is less in comparison to the demand [4]. Meghalaya, is a state where seventy percent of total area is bounded by forest [5], has immense scope of ethano-botanical studies and *Centella asiatica* is a folk medicinal plant of the region. Keeping in view the importance of endophytic actinomycetes, the present investigation was carried out to obtain endophytic actinomycetes from *Centella asiatica* and to study its effect on seed germination and seedling growth of *Phaseolus vulgaris* Linn.

MATERIALS AND METHODS

Sample collection

Ten healthy *Centella asiatica* plants were collected from Permanent Campus, North-Eastern Hill University, Shillong, Meghalaya and were placed in sterile polyethylene bags, which were taken to the laboratory in an ice box and processed within 48 hrs.after collection.

Surface sterilization and isolation of endophytic actinomycetes

The root, stem and leaf of the plants were surface sterilized by sequential immersion in 5% $NaClO_3$ for 3 min, 2.5% $Na_2S_2O_3$ for 10 min, followed by 3 min wash in 75% ethanol, 10 min in 10% $NaHCO_3$ and a finally surface-sterilized samples were washed in sterile distilled water for three times to remove surface sterilization agents [6].The surface sterilized samples were aseptically sectioned and plated on Actinomycetes isolating agar (AIA) media which is prepared by dissolving Glycerol 5g, Sodium propionate 4g, Sodium caesinate 2g, Asparagine 0.1g, K_2HPO_4 0.5g, $MgSO_4.7H_2O$ 0.10g, $Fe_2SO_4.7H_2O$ 0.01g, Agar 15g in 1000ml of deionized water. Fifty µg ml^{-1} cyclohexamide and fifteen µg ml^{-1} nalidixic acid was added to inhibit the growth of fungi and Gram negative bacteria respectively. Plates were incubated at 28°C for 3-4 weeks and regularly observed for actinomycetes colony formation, the isolated colonies were further purified on the ISP-2 medium [7].

Screening for French bean seed germination and seedling growth

The ability of the isolates for seed germination was tested by surface-disinfesting healthy bean seeds in 70% ethyl alcohol for 5 min followed by 1.05% solution of sodium hypochlorite (20% household bleach) for 4 min followed by washing eight times for 1 min each with sterile distilled water, dried on sterile blotted paper and soaked them in actinomycetes suspension ($1x10^8$cfu ml^{-1}) incubated at 28°C for 6hrs., 150 rpm in rotary shaker and seeds treated with distilled water served as control [8]. Both treated and untreated seeds were placed on paper towel and incubated at 28°C in seed germinator for 7 days. After each day of incubation, the germinated seeds were counted and the germination percentage was calculated by using the following formula. On 7th day of germination test, the seedling length was also calculated

Germination percentage = (No of seeds germinated x 100)/ No of seeds sown

Screening for IAA production

The production of indole acetic acid (IAA) by the isolates was determined by transferring them to 5 ml Yeast Malt extract broth containing 2 mg/ml L-trytophan. These cultures were incubated at 28°C in shaking incubator under

dark conditions at 125 rpm for 7 days and then harvested by centrifugation at 11,000 x g for 15 min. One milliliter of the supernatant was mixed with 2 ml of Salkowski reagent for the appearance of pink color indicating IAA production. Optical density (O.D.) was read at 530 nm [9] by using UV-Visible spectrophotometer (Perkin Elmer, USA). The level of IAA produced was estimated against the IAA standard and expressed in µg per ml of tryptophan.

Morphological and biochemical identification of endophytic actinomycetes

The strains showing the highest seed germination activity, seedling growth and IAA production was tentatively identified according to morphological criteria including Gram-staining, acid-fast staining, spore staining, cover slide culture which were recorded using Leica microscope with camera attachment. Biochemical characterization of the isolates was also performed following the standard protocol for the genus level confirmation [7, 10].

Statistical analysis

Descriptive and inferential statistics were used to evaluate the data. Statistical analysis has been carried out by calculating correlation coefficients between germination percentage and seedling length and t-test applied for checking significance. Probabilities less than 0.05 ($p<0.05$) were considered statistically significant. All statistical analyses were done by Microsoft Excel 97 and XLStat package from Addinsoft for windows.

RESULTS AND DISCUSSIONS

Most of the actinomycetes survived in environments such as the soil, rhizosphere and sediments as saprophytes. However, recently actinomycetes were proved to be closely associated with living plants and exert beneficial effects to the host plants [11]. Plants with an ethnobotanical history are expected to be more potent sources of endophytes than other plants [12]. In the present investigation, on screening the ten healthy *Centella asiatica* plants for endophytic actinomycetes, a total of thirty actinomycetes were isolated from root, stem and leaf of the plants. Out of these thirty isolates, the majority (n=12, 40%) were obtained from root, followed by stems (n=10, 33.3%) and leaves (n=8, 26.6%) respectively.

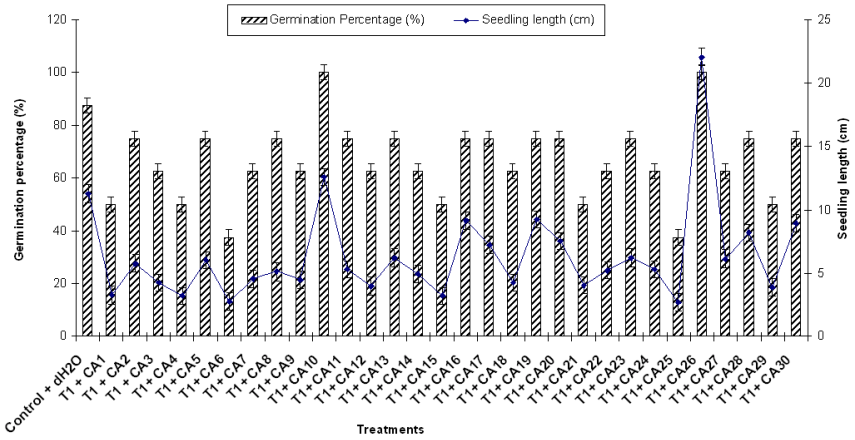

Figure 1
Graphical representation of relation between germination percentage and seedling length.

Several actinomycetes were previously reported to enhance the seed germination [13]. At this point, all the thirty isolates were selected to screen for the germination of French bean (*Phaseolus vulgaris* Linn.) seeds. Out of thirty, only two isolates CA10 and CA26 was found to enhance seed germination of French bean up to 100% which was higher than that of control sets (87.5%) after 7th day of incubation. Seedling growth was also measured in all the cases. It was observed that the growth of CA10 and CA26 treated seedlings were 12.56 cm and 22.06 cm whereas, that of control sets had a length of 9.86 cm (Fig.1 and 2). But the rest twenty eight isolates did not show any significant germination and seedling growth in comparison to control treatment. The correlation effects between the germination percentage and seedling length were also studied. It revealed that germination percentage showed a highly positive significant correlation with seedling length ($r=0.826$ with $p<0.05$).

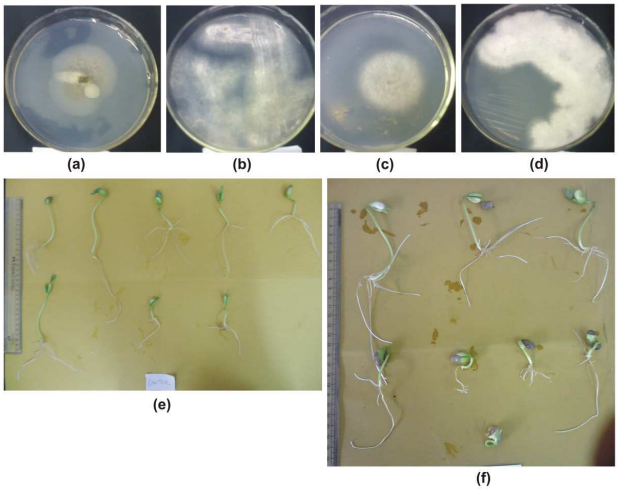

Figure 2
(a) CA10 actinomycetes isolates from Centella asiatica (b) Pure culture of CA10 (c) CA26 actinomycetes isolates from Centella asiatica (d) Pure culture of CA26 (e) Control seedling growth after 7 days of germination (f) CA26 inoculated seedling growth after 7 days of germination

IAA is a common natural auxin which is a product of L-tryptophan metabolism in microorganisms [14] which have the ability to promote seed germination [15]. The present investigation indicated that CA10 and CA26 can produce IAA from tryptophan. The concentrations of IAA produced by CA10 and CA26 isolates were found to be 71µg/ml and 197µg/ml respectively, these levels is well within the range of IAA production reported in *Streptomyces* isolates [16]. It can be presumed that the high levels of tryptophan may be present in root exudates of the medicinal plants which enhance IAA biosynthesis [16]. The enhancement of seed germination and seedling growth due to seed treatment with endophytic actinomycetes particularly of genus *Streptomyces* in the present study may be due to the *in situ* production of secondary metabolites including IAA. Previously it was reported that the culture filtrates of *Streptomyces olivaceoviridis* containing IAA stimulated growth and yield of wheat plants [17] and *Streptomyces* spp. from a tomato rhizosphere had the ability to produce IAA and improve tomato growth by increasing root dry weight [18].

Based on the seed germination and production of IAA, we selected only CA10 and CA26 for further analysis. Both the isolates were identified as *Streptomyces* sp. based on their morphological and biochemical criteria mentioned in Bergey's Manual of Determinative Bacteriology [10] and Shirling and Gottlieb (1966) (Table 1, Table 2 and Fig.2).

Table 2

Biochemical characteristics of the selected isolates.

Tests	CA10	CA26
Starch Degradation(D)	+	-
Tween-80 D	-	-
Tyrosine D	-	-
Xanthine D	-	-
Hypoxanthine D	-	-
Aesculin D	-	+
Casein D	-	-
Gelatin D	-	-
Lysozyme sensitive	+	+
Nitrate test	+	+
Phosphatase test	+	+
Catalase test	+	+
Urease test	+	+
Methyl Red	+	-
Voges-Proskauer	-	-
1% NaCl	+	+
2% NaCl	+	+
3% NaCl	+	+
4% NaCl	+	+
Kanamycin Resistance	-	-
Penillin G Resistance	+	+
Ampicillin Resistance	-	-
Lactose	-	-
Maltose	-	-
Fructose	+	+
Dextrose	+	+
Sucrose	+	+

Table 1

Morphological characteristics of the selected isolates

Tests	CA10	CA26
Substrate mycelium	+	+
Aerial mycelium	+	+
Hyphal organization	Unfragmented hyphae	Unfragmented hyphae
Colour on ISP-1	Cream	Cream
Colour on ISP-2	Cream	White
Colour on ISP-3	Cream	Yellow
Colour on ISP-7	Cream	Yellow
Grams stain	+	+
Motility	+	+

CONCLUSION

From the present investigation, it can be concluded that endophytic *Streptomyces* strains of *Centella asiatica* has the ability to produce secondary metabolites. Out of thirty actinomycetes isolates, only two isolate was found to induce seed germination and seedling growth by the production of indole acetic acid. This study will lead and support further to exploit *Streptomyces* for improving germination of seeds and seedling growth of *Phaseolus vulgaris* Linn.

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