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Isolation of putative endophytic plant growth promoting bacteria from xerophytic grass plants

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Abstract
Endophytes not only provide the basic nutrients for plants but also help them to adapt various environmental stresses. The present study is planned to isolate novel endophytic plant growth promoting bacteria from xerophytic grasses. A total number of 8 endophytic bacteria were isolated from xerophytic grasses and reinfection test was done to check its putative endophytic nature. Out of which, 6 turned out to be putative endophytes, which were further screened for plant growth promoting traits. Among all, isolate E2 produced higher amount of IAA (6.8 µg/ml) followed by E13 with 5.3 µg/ml. Thus results suggest that the endophytic isolates, E2 and E13 were found to be the potential tool plant growth promotion in agricultural crops.

Keywords: endophytes xerophytic grasses – IAA- PGP traits

Introduction
Agriculture is expected to provide food and income to nearly 36% of the world's population. Nowadays climate change has become a wreaking havoc on agriculture thereby putting food security at risk (Choudhary *et al*. 2017). Xerophytes are plants that are adapted to dry encironments. Endophytes are microorganisms which spends all or part of their life cycle within the plant, exhibiting no visible disease symptoms, in fact establishing a symbiotic relationship with the plant (Hallmann *et al*., 1997) [5]. Unfortunately, attempts to characterize endophytic bacteria of xerophytic grass plants for plant growth promotion are limited or in their infancy. In the present investigation an effort was made to isolate, characterize, identify, evaluate, and exploit plant growth promoting endophytic bacteria from xerophytic grass plants in order to harness their potential as microbial inoculant for agricultural crops.

Materials and Methods
Sampling and sampling site
Disease free nine different xerophytic grasses *viz.*, *Helictotrichon schmidii*, *Aristida setaceae*, *Brachiaria munae*, *Anthoxanthum odoratum*, *Eragrostis atrovirens*, *Agrostis peninsularis*, *Cenchrus setaceus*, *Cenchrus ciliaris* and *Bothriochloa pertusa* were collected from Yanaimalai hills (9.96º N, 78.19º E), Karuthapulliyampatty (10.02º N, 78.33º E), Surakkundu (10.01º N, 78.33º E), and Melur (10.02º N, 78.34º E) regions of Madurai district, Tamil Nadu, India.

Isolation of endophytic bacteria
Endophytic bacteria were isolated according to Araujo *et al.*, (2002) [1] method with slight modifications. The collected plant samples were first washed in running tap water and then surface sterilized with 70% ethanol for 3 min, 2% Sodium hypochlorite for 5 min, and 70% ethanol for 30 sec followed by 3 rinses with sterile water. Then the samples were macerated, serially diluted and plated on LB media. The plates were kept for incubation, distinct isolates were chosen for morphological and biochemical characterization.

Reinfection test for putative endophytes
Reinfection test is a confirmatory test to check whether the isolated cultures are endophytes or not. In this test the isolated bacterial cultures were re-infected to a model plant (i.e. Paddy) grown under *in vitro* condition and re-isolation was done (Stoltzfus *et al.*, 1997). Under aseptic condition the paddy seeds were surface sterilized and soaked in 2% ketoconazole solution for 12 hr, later placed in petriplate and kept for pregermination. The pregerminated seeds were

placed in Phyta jar containing Murashige and Skoog media and after 3 days 20µl of 24 hr old culture was inoculated below the hypocotyl region of the rice seedling and allowed to grow at room temperature. After 15 days, the rice plants were taken off and the isolation procedure was repeated. The re-isolated cultures were checked for similarity with the inoculated cultures. If both the cultures were the same it confirms that the isolated cultures were putative endophytes.

Screening of bacterial isolates for plant growth promoting traits

The isolates were screened for plant growth promoting traits *viz.*, IAA production (Glickmann and Dessaux, 1995) [4], ARA activity (Hardy *et al.*, 1968), phosphate solubilization (Murphy and Riley, 1962) [6], potassium solubilization (Stanford and English, 1949) and siderophore production (Schwyn and Neilands, 1987) [7]. Two isolates E5 and E11 which failed in putative test were taken as control isolates.

Results and Discussion

Isolation of endophytic bacteria and reinfection test

From the xerophytic grass samples, a total number of 30 distinct endophytic bacterial cultures were isolated and selected for further study. Morphological and biochemical characterization was carried out for all the cultures. Reinfection results showed that out of 8 isolates, 6 were found to be putative endophytes as they were able to reinfect the sterile rice seedlings. The cultures that were confirmed as putative endophytes were alone selected for further analyses.

Screening of bacterial isolates for plant growth promoting traits

Glick *et al.*, (2012) [3] reported that bacterial endophytes that produce IAA enhance the drought tolerance in plants by increasing the supply of endogenous IAA and also by altering the root architecture. The highest IAA production was observed in isolate E2 followed by the isolate E13 with the production 6.8 µg/ml and 5.3 µg/ml of IAA respectively. The results showed that all isolates produced siderophore, whereas the control isolates E5 and E11 were found to be negative for siderophore production. In this study the nitrogen fixing ability of the isolates were assessed through acetylene reduction assay and the results revealed that all the isolates exhibited higher ARA activity than the two check isolates. The isolate E2 recorded maximum ARA activity of 20.1 nmoles C_2H_4 mg protein^{-1}hr^{-1} whereas the minimum activity was recorded by the check isolate E11 (8.3 nmoles C_2H_4 mg protein^{-1}hr^{-1}). These results were in line with previous study conducted by Vendan *et al.*, (2019) in which 15 bacterial endophytes isolated from pulses were evaluated for N-fixing ability using ARA activity with results ranging from 29.4 to 15.6 nmoles C_2H_4 mg protein^{-1} hr^{-1}. The isolate E13 produced a maximum P solubilization of 161.1 µg/ ml wherein the control isolates E5 and E11 produced 42.7 and 37.5 µg/ ml of P respectively. Potassium-solubilizing bacteria play a vital role in plant nutrition by enhancing plant K uptake and thereby promoting plant growth and development. The potassium solubilizing ability of all six isolates was tested, and isolate E28 was found to be effective with 15.6 mg/l, while the check isolates E5 produced only 2.1 mg/l (Table 1).

Conclusion

From this study, it was evident that the endophytic bacterial isolates E2 and E13 were found to be promising and produced higher amount of plant growth promoting substances. Hence these novel endophytic plant growth promoting bacteria could be utilized as potential inoculants for plant growth and development in agricultural crops.

Table 1: Quantitative estimation of Nitrogen fixation, P solubilization, K release, IAA and siderophore production

Isolates	ARA (nmoles C_2H_4 mg protein^{-1}hr^{-1})	P solubilization (mg/l)	K release (mg/l)	IAA (µg/ml)	Siderophore
E2	20.1±0.24^{a}	90.3±0.74^{c}	12.2±0.10^{c}	6.8±0.13^{a}	+
E4	16.5±0.28^{c}	98.7±1.21^{b}	10.3±0.004^{d}	4.5±0.009^{c}	+
E10	13.5±0.13^{e}	62.1±0.26^{e}	6.8±0.06^{f}	3.6±0.004^{e}	+
E13	17.6±0.05^{b}	161.9±2.34^{a}	13.7±0.08^{b}	5.3±0.063^{b}	+
E26	15.3±0.16^{d}	70.8±1.05^{d}	8.1±0.03^{e}	4.1±0.069^{d}	+
E28	12.2±0.165^{f}	66.2±1.24^{de}	15.6±0.3^{a}	2.1±0.005^{f}	+
E5	9.6±0.16^{g}	42.7±0.63^{f}	2.1±0.027^{h}	1.2±0.002^{g}	-
E11	8.3±0.14^{h}	31.5±0.48^{g}	3.7±0.047^{g}	0.9±0.009^{h}	-
SEd	0.31	2.02	0.21	0.10	
CD	0.66	4.27	0.45	0.21	

+ +Positive; - negative; Values are mean of three replicates (± standard error) (n=3) and column values followed by different letters are significantly different from each other at 5% LSD

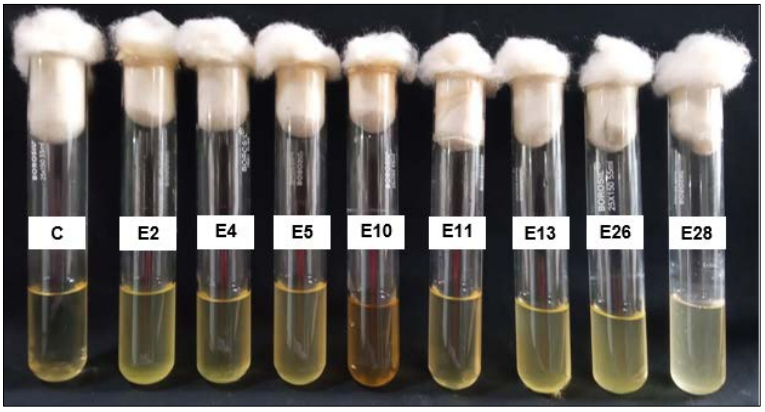

Fig 1: Growth of endophytic bacteria at -1.20 MPa PEG concentration

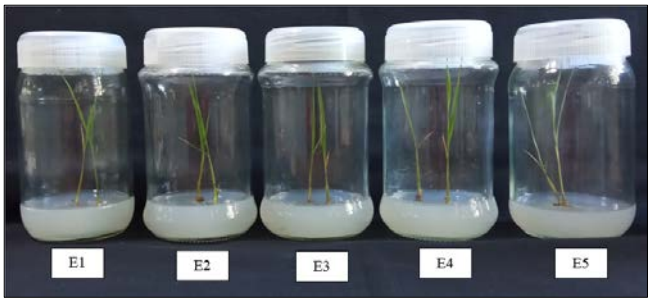

Fig 2: Reinfection test for putative endophytes

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