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Isolation of bacterial endophytes for mitigating moisture stress in groundnut [*Arachis hypogaea* (L.)]

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ABSTRACT

Groundnut (*Arachis hypogaea*) in tropical and subtropical between 40°N and 40°S latitudes. It is valued for its high seed count and as such, it is the fourth most significant source of edible oil and significant source of vegetable protein of India and also an important agricultural export commodity. To increase drought tolerance in groundnut endophytic bacteria is isolated from weeds and inoculated in to groundnut crop in a pot culture experiment in which 12th isolate (CLR) *i.e., Enterobacter mori* showed higher yields and less water consumption. In consortium *Enterobacter mori* showed higher yield and less water consumption with *Brady rhizobium* and *Bacillus subtilis* which is suggested as best for drought tolerance.

Key words: *Bacterial endophytes, Drought, Groundnut and Yield*

Groundnut (*Arachis hypogaea* (L.)), a self-pollinated legume, is an important crop grown for edible oil extraction and food consumption. Groundnut, commonly known as peanut, is a significant legume crop used for oil, food, and feed cultivated in over 100 countries. Groundnut is widely cultivated in tropical, subtropical, and warm temperate countries, and it has cemented its place in global agriculture. Groundnut production, which covers an area of around 4.62 lakh hectares, had an outstanding yearly yield of 86.54 lakh tonnes tons in 2024 (per FAOSTAT). Notably, China is the biggest producer of groundnut, followed closely by India, demonstrating the crop's enormous economic and nutritional importance in both regions. 'Endophyte' is derived from the Greek word 'endon'(within) and 'phyte' (plant) (Carroll 1988; Clay 1988).Endopohytes are a type of micro-organisms which show endo-symbiotism with the plants i.e., they live inside the plant. Endophytes enter the plant through environment (horizontal transmission) or seed (vertical transmission). Endophytes doesn't cause harm to their host plant. The most common genera of endophytic bacteria include *Pseudomonas, Bacillus, Acinetobacter, Actinobacteria, Sphingomonas* and genera in the family *Enterobacteriaceae* (Pirttila and Frank, 2011). Endophytic bacteria are found in mostorgans of the plant including roots, stems, leaves, flowers (Compant *et al*. 2011), seeds (Trognitz *et al.* 2016), fruits (De Melo Pereira *et al.,* 2012), tubers, ovules, as well as inside nodules.

MATERIAL AND METHODS

All the weed plants used for isolation of endophytes are collected in the premises of Institute of Frontier Technology (13.6234422 lattitude and 79.3728773 longitude), Regional Agricultural Research Station (RARS), Tirupati, Andhra Pradesh, India and Tirupati city. A total of 8 weed plants were taken namely Crab grass (*Digitaria sanguinalis*), *Cymbopogon flexuosus*, Crow foot grass (*Dactyloctenium aegyptium*), Bermuda grass (*Cynodon dactylon*), Barnyard grass *(Echinochloa colonum*), *Chloris barbata, Prosopis juliflora,* Darbha grass (*Desmostachya bipinnata*)**.** From each plant, leaf segments, shoot segments and root segments were analysed.

The weed samples were collected and taken to the Department of Agricultural Microbiology for isolation of bacterial endophytes. All samples were washed with tap water to remove adherent soil particles. Surface sterilization of weed samples is very important step for isolation of bacterial endophytes which was carried inside the laminar air flow chamber.

They were surface sterilized with 70% ethyl alcohol for 3 minutes on shaking followed by washed with sterile distilled water to remove alcohol. After that, plant samples were surface sterilized with 1.2 % (w/v) of Sodium Hypochlorite solution (NaOCl)for 20 min on shaking at 110 rpm and followed by washed with sterile DW for 5-6 times. To check the sterility of samples, take 0.1 mL aliquot from final wash and was spread on nutrient agar plates. (Gyaneshwar *et al*. 2001). If any growth was detected in the sterility check, samples were discarded. The surface sterilized samples were then used for isolation of endophytic bacteria by culture dependent based technique.

The bacterial endophytes were isolated according to the procedure by Bacon *et al*. (2002). Root, shoot and leaf segments of 2 cm length were excised using flame sterilized scalpel by cutting little bit portion on either side of the leaf, stem and root section. All the samples individually were blotted dry with filter paper and then weighed to have final sample of 0.5 g. The surface sterilization of the shoot, leaf and root pieces was done with abovementioned sterilization steps. Efficiency of surface disinfestationdepend on selection of disinfectant, its strength, duration of immersion in disinfectant. The procedure for surface disinfestation and isolation conditions were standardised prior to experimentation. The cut ends of plant sample sections were removed with flame sterilized scalpel and were placed properly with the cut surface touching the agar media. The plates were incubated for three to five days at 28 ± 1^0C. Single colonies from the plates were picked up and purified by repeated quadrant streaking on NA medium and stored under refrigerated conditions for further studies.

RESULTS AND DISCUSSION

Plates 1 and 2 shows the endophytic bacteria and the weed plants which are used for extracting endophytes in the fields close to the Regional Agricultural Research Station in Tirupati. The plant samples that had been gathered were brought to the Department of Microbiology for additional examination. Plant tissues, such as the roots, stem, and leaves, were surface sterilized in order to isolate endophytic bacteria.

Naming of the Isolates

From the above studies, those bacterial colonies showing distinct colony morphology and distinct growth in broth were selected for further studies. Fifteen isolates from eight different weed grasses (Crabb grass, *Cymbopogan flexosa*, Crowfoot grass, *Cynodon doctylon*, *Echinocloa colonum*, *Chloris barbata*, *Prosopis juliflora* and Darbha grass) were obtained and purified by streak plate technique. They were named accordingly with different codes. Codes were given to the isolated bacterial endophytes in such a way that first and second letter indicating the weed followed by third letter representing the part of the plant from which the isolate has been isolated and are presented in Table 3.

25 distinct culturable endophytic bacterial morphotypes were identified, and the quadrant streak method was used to produce pure cultures of each. On the basis of colony shape, size, elevation, surface, margin, colour, pigmentation, motility, gram's reactivity, and cell shape, the isolates were characterized morphologically and physiologically (Table 1 & 2)

A) *Cymbopogan flexosa* B) *Chloris barbata*

Plate 1. Collection of different weeds for isolation of bacterial endophytes

Table 1. Cultural characteristics of the bacterial endophytes isolated from different weed plants

S. No	Isolate	GramReaction	Cell shape	Motility
1	CBR1	+	Cocci	-
2	CBR2	+	Rod	+
3	ECS	-	Rod	+
4	GGR	-	Rod	-
5	PJS	-	Cocci	+
6	CDR	-	Small rod	-
7	DBR	+	Small rod	+
8	CBS1	+	Rod	-
9	CBS2	+	Rod	-
10	DSR1	+	Rod	+
11	CBL	+	Cocci	-
12	CLR	-	Rod	+
13	CFR	-	Cocci	-
14	DSR2	-	Rod	-
15	CPR	-	Rod	-

All the isolates were stained for Gram reaction and the results are presented in Table 1. Among 15 obtained isolates 53.33 % (8 isolates) were found to be gram negative (–ve) and 46.66 % (7 isolates) of isolates were gram positive (+ve).About 60 % of isolates (9 isolates) were found to have motility function while 40 % (6 isolates) of bacterial isolates were found to be non-motile and motility is one of the attributes of being an endophyte.

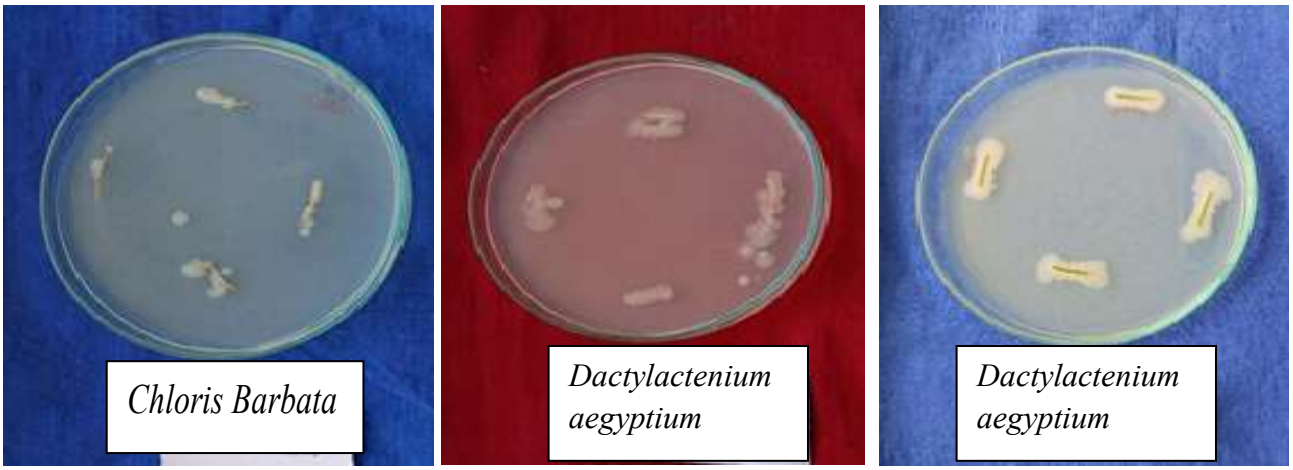

Plate 2. Isolation of different bacterial endophytes from different plant parts of weeds collected

Table 2. Morphological and physiological characters of endophytic bacteria isolated from weeds

S. No.	Isolate	Culture growth in broth			Colony characteristics in agar
		Surface Growth	Clouding	Sediment	
1	CBR1	Ring	Adequate	Scanty	Medium, Circular, Radium yellow colour, Opaque, Glistening
2	CBR2	None	Moderate	Flaky	Small, Circular, Pale white colour, Opaque
3	ECS	Ring	Adequate	Scanty	Medium, Circular, white colour colony surrounded by orange yellow border,

4	GGR	Ring	Adequate	Scanty	Medium, Circular, Yellow orange colour, Glistening, Transparent
5	PJS	None	Slight	Flaky	Large, Round, White colour, Dry colonies with rough ending, Opaque
6	CDR	Ring	Slight	Scanty	Small, Circular, Orange red colour, Glistening, Smooth margin, Opaque
7	DBR	None	Moderate	Flaky	Large, Circular, Cream colour, Glistening, Smooth margin, Opaque
8	CBS1	None	Moderate	Scanty	Small, Circular, White colour, Glistening, Smooth margin, Opaque
9	CBS2	Ring	Adequate	Flaky	Medium, Irregular shape, White colour, Dry colony, Rough margin
10	DSR1	None	Adequate	Scanty	Small, Circular, Milky white colony produces orange colour after 2 days of growth, Glistening, Rough margin
11	CBL	Ring	Moderate	Scanty	Small, Circular, Creamy white colour, Glistening, Smooth margin, Opaque
12	CLR	Ring	Adequate	Scanty	Medium, Circular, White colour, Glistening, Smooth margin, Opaque
13	CFR	None	Adequate	Scanty	Small, Circular, Pale yellow colour, Glistening, Smooth colony, Nearly opaque
14	DSR2	None	Adequate	Scanty	Small, Circular, White colour, Rough margin, Transparent
15	CPR	None	Moderate	Flaky	Small, Circular, Radium green colour, Dry colony, Rough margin, Opaque

Table 3. Details and naming of the bacterial endophytes isolated from different weed grass

S. No	Weed	Parts	Isolate
1	*Cymbopogan flexosa*	Root	CBR1
2	*Cymbopogan flexosa*	Root	CBR2
3	*Echinochloa colonum*	Stem	ECS
4	Guinea grass	Root	GGR
5	*Prosopis juliflora*	Stem	PJS
6	*Cynodon doctylon*	Root	CDR
7	Darbha	Root	DBR
8	*Cymbopogan flexosa*	Stem	CBS1
9	*Cymbopogan flexosa*	Stem	CBS2
10	*Digitaria sanguinalis*	Root	DSR1
11	*Cymbopoganflexosa*	Leaf	CBL
12	*Chloris barbata*	Root	CLR
13	Crow foot grass	Root	CFR
14	*Digitaria sanguinalis*	Root	DSR2
15	*Cyperus rotundus*	Root	CPR

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