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Isolation and Characterization of Endophytes from Different Plants: Effects on Growth of *Pennisetum typhoides*

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Studying symbiotic association can provide principles to improve crop yield under harsh conditions. In present study, influence of endophytes was studied on seedling emergence and growth of plumule and radicle of *Pennisetum typhoides.* The plants were sterilized on surface to prevent contaminations and then macerated in disinfected pestle and mortar to obtain pure colonies of in-habitat bacterial colonies on King's B media, Nutrient agar and Potato dextrose agar using conventional methods. From 08 plant species, 319 endophytic bacterial cultures were isolated. The isolated endophytes were applied on seeds of *Pennisetum typhoides* to check the influence on seedling emergence, and growth of radicle and plumule. Out of the 319 cultures, 224 (70.22%) cultures were inhibiting seed germination and growth, while 95 (29.8%) cultures were promoting growth of shoot and/or root of *Pennisetum typhoides*. Of these 95 growth promoting cultures, 49 (51.6 %) cultures were promoting growth of both shoot and root; 42 (44.2 %) were promoting shoot but inhibiting root growth and 4 (4.21%) were promoting root, but inhibiting shoot growth. Plant species *Catharanthus roseus* had maximum percentage of inhabitant cultures (58%) that promoted growth of shoot and root. Microbiological characterization of growth promotory cultures revealed that major number of bacteria belongs to Gram positive bacillus (21 cultures) group followed by Gram negative bacillus (16 cultures) group. The mechanism of growth promotion was observed as nitrogen fixation alone (38 cultures), phosphate solubilization along with nitrogen fixation (4 cultures) and other methods (7 cultures). The isolated endophytic strains have the potential to be used as seed inoculants or co-inoculants for improvement in growth, development and yields and thus help in sustainable development in the field of agriculture.

Key words: Endophytes, growth promotory, seed inoculant, Nitrogen fixation, phosphate solubilization.

The plants are constantly involved in interactions with a wide range of heterogeneous population of microorganisms. Plant-microorganisms association can be classified as Rhizospheric microorganisms (microorganism growing in rhizosphere), Epiphytic (microorganism growing in phyllosphere) and Endophytic (micro-organism growing inside plant tissue).

The endophytes are ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stem, roots and seeds of various plant species[1]. Endophyte can be colonize in the internal tissue of the plant but without showing external sign of infection or negative effect on their host[2,3]. Their route of primary entry in plant tissue is root zone; however, aerial portions of plants have also been route of secondary entry. Despite their discovery

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almost 50 years ago, there is very little knowledge about bacterial endophyte's diversity, population dynamics, and effects on host plant growth. Since their discovery, both Gram-positive and Gram-negative endophytes have been isolated from several tissue types in numerous plant species[1]. Broadly endophytes have been distributed among three main groups based on their effect on the host plant[4]; i. Plant growth promoting bacteria (PGPB), ii. Plant growth inhibiting bacteria (PGIB), and iii. Plant growth neutral bacteria (PGNB).

Bacterial endophytes colonize an ecological niche similar to that of phytopathogens, this makes them suitable as biocontrol agents[5]. In some cases, they can also accelerate seedling emergence, promote plant establishment under adverse conditions[6] and enhance plant growth[7]. Due to these reasons they have the potential to be used as seed inoculants or co-inoculants for improvement in growth, development, yields and pathogen resistance. The use of plant growth promoting endophytes as inoculum or co-inoculum with *Rhizobia* is becoming a practical method in development of sustainable agriculture. As reported by Rayan *et al.,*[8] exploitation of endophyte–plant interactions can result in the promotion of plant health and can play a significant role in low-input sustainable agriculture applications for both food and non-food crops.

However, endophytes are a rich and reliable source of genetic diversity, novel non-described species and novel bioactive compounds like new drugs for effective treatment of diseases in humans, plants and animals[9]. The natural ability of endophytes to degrade the xenobiotics has also been investigated with regard to improving phytoremediation[10-13].

Keeping these aspects in mind the present study was carried out with the aim to isolate and purify endophytic bacteria from various plant sources and to evaluate their effects on the growth of *Pennisetum typhoides*. *Pennisetum typhoides* was used as the test plant because of its economic value as food as well as fodder crop and also being a C-4 plant, it grows faster, and therefore the effect of endophytes on its growth would be more prominent and clear. Moreover, it exudates more, so is apt for rhizobacterial interaction also. The further objectives included selection of the promising growth promontory cultures, their characterization and finally to pinpoint the possible mechanism(s) of growth promotion employed by them.

MATERIALS AND METHODS

Plant Materials

Plants and seeds (RGC-1017) of *Cyamopsis tetragonoloba* and plants of *Cassia siamea* were taken from Birla Institute of Scientific Research (BISR), Jaipur. Plants of *Pennisetum typhoides* (Pt) were obtained from a Farmer's field located in Jaipur and the seeds of *Pennisetum typhoides* (Hybrid, Pioneer-86M32) were purchased from Chowdhary Seeds of Chandpole Anaaj Mandi, Jaipur. All the materials were cross checked by Birla Institute of Scientific Research (BISR), Jaipur.

Endophytic cultures

The endophytic bacterial cultures used for the study were also provided by Birla Institute of Scientific Research (BISR), Jaipur, which were isolated from a broad range of agronomic and medicinal plants common to the Indian regions and included:

*Asparagus recemosus (*Ar*) ; Cassia angustifolia* (Ca)*; Cassia siamea* (Cs)*; Cyamopsis tetragonoloba* (Ct)*; Cyprus rotandus* (Cyr)*; Trianthema portulacastrum* (Tp) and *Zea mays* (Zm).

Isolation and purification of Endophytic Bacteria:

To isolate endophytes from *Cyamopsis tetragonoloba*; *Cassia siamea* and *Pennisetum typhoides*, young and healthy plants were gently uprooted, washed under tap water to remove soil and separated into stems, roots and leaves. Stems, roots and leaves were cut into sections 2-3 cm long. 1 g of respective plant parts (stem, root, leaves and seeds) were weighed and surface sterilized separately with 2% sodium hypochlorite (5 min for stem, root and leaves; 10 min for seeds), followed by 10 washings with sterile water, then further treated with 95% ethanol for 5 min and finally given 5 washings with sterile water. Sterilized seeds of *Cyamopsis tetragonoloba* were soaked overnight in sterile water before further processing. The sterilized plant part was macerated in disinfected pestle and mortar and then mixed properly with 100mL of sterile water to form a uniform suspension. Then 50 µL of the suspension

was spreaded (Spread-plate method) on three different culture media: King's B medium; Nutrient Agar (NA) and Potato dextrose agar (PDA). The unmacerated, small but surface sterilized plant part was inoculated on petridishes of the same three culture media, to act as control, to ensure proper sterilization. The inoculated plates along with the control plates were incubated at 28°C for 7 days. The plates were observed daily and different type of bacterial colonies were picked and streaked repeatedly (streak-plate method) on fresh media plates, till pure cultures were obtained.

Screening for growth promotory bacterial endophytes:

The seeds of *Pennisetum typhoides* were surface sterilized with 0.1% $HgCl_2$ (1 min) and after 5 washings with distilled water treated with 95% ethanol followed by 5 washings again. The surface sterilized seeds (10 seeds) were placed with proper spacing in a center row of the petridishes containing water agar (0.7%) medium. All the seeds (10 seeds) in a petridish were inoculated with a single endophytic culture, by pouring 20µL of the bacterial inoculum over each seed. The petridishes containing uninoculated seeds were kept as control. This experiment was performed in triplicate and all the plates along with controls were incubated at 28°C for 7 days. On completion of the incubation period the length of shoots and roots of each seedling in a petridish was measured and recorded. The percent increase or decrease in the shoot or root length of the seedlings treated with a particular endophytic bacterial culture in comparison to control was calculated according to following formulae:

Percent increase/decrease (% increase/decrease) = [(LTEST - LCONTROL)/ LCONTROL] X 100

where LTEST = Average length (10 seedlings) of treated shoot/root

and LCONTROL = Average length(10 seedlings) of control shoot/root

The data is presented as the mean of the triplicate's readings along with the standard deviation from the mean value.

Characterization of the growth promoting bacterial endophytes and the mechanism of growth promotion

The bacterial cultures promoting growth of *Pennisetum typhoides* were characterized for various traits including color, form, elevation, margin, diameter, surface, opacity, and texture and Gram's reaction, according to Bensons Manual.

In order to determine the possible mechanism of growth promotion the cultures were tested for Nitrogen-fixing and Phosphate solubilizing capability. The Nitrogen-fixing capability was determined by inoculating the cultures onto petridishes containing Nitrogen-free Malate medium and uninoculated medium plates were kept as control. All the culture and control plates (in triplicate) were incubated at 28°C for 4 days. The change in the colour of medium from green to blue would be the indicative of nitrogen fixation. In order to test phosphate solubilizing capability the cultures were inoculated (in triplicate) on Pikovaskaya's medium plates and incubated along with uninoculated control plates at 28°C for 4 days. The development of a clear zone around the bacterial growth would be an indication of phosphate solubilization.

RESULTS

Isolation and purification of Endophytic Bacteria

With the completion of the purification step 319 bacterial cultures were obtained from all the 8 different plant sources (Table 1). Maximum numbers of endophytes were contributed by *Cymopsis tetragonaloba* (176), followed by *Trianthema portulacastrum* (32); *Cyprus rotandus* and *Pennisetum typhoides* (29 each); *Zea mays* (20), and least number of endophytes were obtained from *Cassia angustifoila* (09). The maximum numbers of the endophytes were obtained from roots (225) followed by stem (60) and the leaves (30) and the lowest number of endophytes were from the bulb/ tuber (4).

Screening for growth promotory and inhibitory endophytes

The study on the effects of the endophytic cultures on the germination and seedling emergence of the *Pennisetum typhoides* (Table-2) showed results ranging from stimulation of growth (Fig-1a and 1b) to inhibition (Fig-2a and 2b) in comparison to controls. However some cultures also showed neutral effects. Out of the 319 endophytic cultures tested as seed inoculants 95 (29.8 %) cultures were found to be promoting growth of shoot and/ or root of *Pennisetum typhoides*. All cultures

(100% i.e 13 cultures) from *Cassia siamea* were showing growth inhibitory effects. The single culture showing neutral effect on root growth was from *Cyprus rotandus* although it was inhibiting shoot growth, while two cultures from *Trianthema portulacastrum* were showing 100% inhibition of root growth (no root development) along with shoot growth inhibition. Surprisingly most of the cultures (90% i.e. 26 out of 29 cultures) from *Pennisetum typhoides* itself were having negative effect on the

Table 1. The plant-wise contribution of the endophytes

S. No.	Plant	Plant Part	Number of Bacterial Endophytes	Total Number of Bacterial Endophytes
1	*Asparagus recemosus*	Stem	1	11
		Roots	4	
		Leaves	4	
		Tuber	2	
2	*Cassia angustifolia*	Stem	4	9
		Roots	3	
		Leaves	2	
3	*Cassia siamea*	Root	13	13
4	*Cymopsis tetragonaloba*	Stem	19	176
		Roots	149	
		Leaves	8	
		Seeds	0	
5	*Cyprus rotandus*	Stem	7	29
		Roots	10	
		Leaves	10	
		Bulb	2	
6	*Pennisetum typhoides*	Stem	7	29
		Roots	22	
		Leaves	0	
7	*Trianthema portulacastrum*	Stem	11	32
		Roots	15	
		Leaves	6	
8	*Zea mays*	Stem	11	20
		Roots	9	
		Leaves	0	

Table 2. The plant-wise distribution of growth promotory and inhibitory endophytes

S. No	Plant	Symbol	Total Endophytes	Growth promoting Endophytes		Growth inhibiting Endophytes	
				Number	Percent (%)	Number	Percent (%)
1	*Asparagus recemosus*	Ar	11	4	36%	7	64%
2	*Cassia angustifolia*	Ca	9	5	56%	4	44%
3	*Cassia siamea*	Cs	13	0	0%	13	100%
4	*Cymopsis tetragonaloba*	Ct	176	75	43%	101	57%
5	*Cyprus rotandus*	Cyr	29	4	14%	25	86%
6	*Pennisetum typhoides*	Pt	29	3	10%	26	90%
7	*Trianthema portulacastrum*	Tp	32	2	6%	30	94%
8	*Zea mays*	Zm	20	2	10%	18	90%

Table 3. The plant-wise distribution of the growth- promoting endophytes

S. No.	Plant		Growth Promoting Endophytes	Growth Promotion of the Plant Part			Growth Promotion of the Plant Part by more than 50% over Control		
				Shoot only	Root only	Shoot and Root	Shoot only	Root only	Shoot and Root
1	*Asparagus recemosus* (Ar)	NE	4	1	1	2	2	-	-
		PE	36%	9%	9%	18%	18%	-	-
2	*Cassia angustifolia*(Ca)	NE	5	2	-	3	-	-	1
		PE	55.5%	22%	-	33%	-	-	11%
3	*Cassia siamea*(Cs)	NE	-	-	-	-	-	-	-
		PE	-	-	-	-	-	-	-
4	*Cymopsiste tragonaloba*(Ct)	NE	75	35	3	37	5	9	6
		PE	43%	20%	2%	21%	3%	5%	3%
5	*Cyprus rotandus*(Cyr)	NE	4	-	-	4	1	1	-
		PE	14%	-	-	14%	3%	3%	-
6	*Pennisetum typhoides*(Pt)	NE	3	1	-	2	-	-	1
		PE	10%	3%	-	7%	-	-	3%
7	*Trianthema portulacastrum*(Tp)	NE	2	1	-	1	-	-	-
		PE	6%	3%	-	3%	-	-	-
8	*Zea mays* (Zm)	NE	2	2	-	-	-	-	-
		PE	10%	10%	-	-	-	-	-

If NE = Number of growth promotory endophytes then & PE = Percent of growth promotory endophytes PE is calculated as follows: PE = [NE/total endophytes studied] X 100

growth and seedling emergence.

Among the 95 growth promoting cultures: - 49 (51.6%) cultures were promoting growth of both shoot and root; 42 (44.2 %) were promoting shoot but inhibiting root growth and 4 (4.21 %) were promoting root, but inhibiting shoot growth (Table-3). It was observed that growth of root only was promoted mostly by cultures obtained from *Asparagus recemosus* and *Cymopsis tetragonaloba*. Maximum percentage of cultures promoting growth of shoot only were from *Cassia angustifolia* (22%) and those promoting growth of both shoot and root were from *Cassia angustifolia* (33%). The cultures increasing growth of shoot as well as root of *Pennisetum typhoides* were considered as growth promontory endophytes and studied further for characterization and mechanism of growth promotion. It was observed that majority of the growth promotory cultures (28 out of 49 cultures) were promoting root growth more efficiently than that of shoot (Table-4). Although in many cases the differences in promotion of shoot and root growth was not prominent. Interestingly all the growth promotory cultures from *Cassia angustifolia* (3 cultures) and *Trianthema portulacastrum* (1 cultures) and more than half of the cultures from *Cymopsis tetragonaloba* (19 out of 37 cultures) and *Cyprus rotandus* (3 out of 4 cultures) were more efficient in root growth promotion. Maximum shoot promotion was shown by Ct-329 (100.1 ± 0.1%) and root promotion shown by Cyr-60 (130.7 ± 0.1%). Only one culture Ct-330 (plant source *Cymopsis tetragonaloba*) was showing shoot as well root growth promotion by more than 90%, thus it was the most efficient culture. The other promising growth promotory cultures promoting growth of both shoot and root by more than 50% were: - Ca-157; by more than 60% were Ct-217, Ct-

Table 4. Percent increase in growth of shoot and root of *Pennisetum typhoides* due to application of the endophytes as seed inoculum

S.. No	Culture Number	*Percent Increase in Shoot Length (%)	*Percent Increase in Root Length (%)
		Asparagus recemosus (Ar)	
1	Ar-113	16.5 ± 0.6	32.5 ± 0.6
2	Ar-120	50.1 ± 0.1	1.8 ± 0.2
		Cassia angustifolia(Ca)	
3	Ca-155	36.4 ± 0.1	42.5 ± 0.1
4	Ca-157	52.3 ± 0.1	88.9 ± 1
5	Ca-158	22.4 ± 0.1	28.1 ± 0.1
		Cymopsiste tragonaloba(Ct)	
6	Ct-200	21.9 ± 0.1	99.1 ± 0.1
7	Ct-203	7.8 ± 0.2	61.1 ± 0.1
8	Ct-204	31.5 ± 0.1	127.7 ± 0.1
9	Ct-209	13.8 ± 0.1	15.6 ± 0.1
10	Ct-217	64.4 ± 0.2	79.4 ± 0.1
11	Ct-224	25.5 ± 0.1	33.7 ± 0.1
12	Ct-236	34.9 ± 0.1	53.1 ± 0.1
13	Ct-245	25.3 ± 0.1	21.2 ± 0.1
14	Ct-281	46.9 ± 0.1	82 ± 0.1
15	Ct-282	56.8 ± 0.1	42.2 ± 0.2
16	Ct-295	20 ± 0.1	22.7 ± 0.2
17	Ct-301	11.9 ± 0.1	7.5 ± 0.2
18	Ct-302	44.2 ± 0.2	48.9 ± 0.1
19	Ct-303	60.5 ± 0.1	69.8 ± 0.1
20	Ct-304	49.2 ± 0.1	54.1 ± 0.1
21	Ct-305	24.2 ± 0.2	13.4 ± 0.2
22	Ct-307	42.6 ± 0.1	34.1 ± 0.1
23	Ct-309	48.7 ± 0.1	10.9 ± 0.1
24	Ct-310	1.8 ± 0.2	20.2 ± 0.2
25	Ct-311	91.5 ± 0.1	5 ± 0.1
26	Ct-312	92.9 ± 0.1	72.2 ± 0.2
27	Ct-315	59.3 ± 0.1	38.6 ± 0.1
28	Ct-317	41.6 ± 0.1	50.7 ± 0.2
29	Ct-319	74.4 ± 0.2	43.1 ± 0.1
30	Ct-321	46.4 ± 0.1	21.6 ± 0.1
31	Ct-322	47.6 ± 0.1	41.1 ± 0.1
32	Ct-323	41.3 ± 0.2	58.2 ± 0.2
33	Ct-324	31.1 ± 0.1	73.1 ± 0.1
34	Ct-325	10.5 ± 0.1	31.3 ± 0.2
35	Ct-327	6 ± 0.1	13.3 ± 0.3
36	Ct-329	100.1 ± 0.1	72.5 ± 0.1
37	Ct-330	93.5 ± 0.1	92.6 ± 0.2
38	Ct-331	66.1 ± 0.1	60.8 ± 0.3
39	Ct-334	36.2 ± 0.2	5.3 ± 0.2
40	Ct-335	27.3 ± 0.3	12.1 ± 0.1
41	Ct-343	13 ± 0.3	0.6 ± 0.1
42	Ct-367	11.4 ± 0.4	13.2 ± 0.2
		Cyprus rotandus(Cyr)	
43	Cyr-55	18.9 ± 0.2	24.1 ± 0.1
44	Cyr-60	47.1 ± 0.1	130.7 ± 0.1
45	Cyr-63	25.9 ± 0.1	45.1 ± 0.1
46	Cyr-64	51.6 ± 0.1	1.9 ± 0.1
		Pennisetum typhoides (Pt)	
47	Pt-101	79.2 ± 0.2	124.4 ± 0.1
48	Pt-102	24.7 ± 0.1	10.8 ± 0.1
		Trianthema portulacastrum (Tp)	
49	Tp-51	30.9 ± 0.1	31.4 ± 0.2

***** The percent increase (Shoot/Root) is presented as the mean of the triplicate along with SD from mean

Table 5. Cell morphology, Gram's reaction, nitrogen fixation and phosphate solubilizing capabilities of the growth promotory bacterial endophytes

S. No.	Culture Number	Cell Morphology	Gram's Reaction	Nitrogen Fixation	Phosphate Solubilization
		Asparagus recemosus (Ar)			
1	Ar-113	streptococci	positive	positive	negative
2	Ar-120	bacillus	positive	positive	negative
		Cassia angustifolia (Ca)			
3	Ca-155	bacillus	positive	positive	negative
4	Ca-157	bacillus	positive	positive	negative
5	Ca-158	bacillus	negative	positive	negative
		Cymopsis tetragonaloba (Ct)			
6	Ct-200	coccoid	negative	positive	negative
7	Ct-203	bacillus	negative	positive	negative
8	Ct-204	bacillus	negative	positive	negative
9	Ct-209	bacillus	negative	positive	negative
10	Ct-217	bacillus	positive	positive	negative
11	Ct-224	bacillus	positive	positive	negative
12	Ct-236	bacillus	positive	positive	negative
13	Ct-245	bacillus	positive	positive	negative
14	Ct-281	bacillus	negative	positive	negative
15	Ct-282	bacillus	positive	positive	negative
16	Ct-295	staphylococci	positive	negative	negative
17	Ct-301	coccoid	negative	positive	negative
18	Ct-302	staphylococci	positive	negative	negative
19	Ct-303	bacillus	negative	negative	negative
20	Ct-304	bacillus	negative	negative	negative
21	Ct-305	bacillus	positive	positive	negative
22	Ct-307	bacillus	positive	positive	negative
23	Ct-309	bacillus	positive	positive	negative
24	Ct-310	bacillus	positive	positive	negative
25	Ct-311	bacillus	negative	positive	negative
26	Ct-312	bacillus	positive	positive	negative
27	Ct-315	bacillus	positive	positive	negative
28	Ct-317	diplococci	positive	positive	negative
29	Ct-319	bacillus	positive	positive	negative
30	Ct-321	bacillus	negative	positive	negative
31	Ct-322	bacillus	positive	positive	negative
32	Ct-323	bacillus	positive	positive	negative
33	Ct-324	bacillus	negative	positive	negative
34	Ct-325	diplobacilli	negative	positive	negative
35	Ct-327	bacillus	positive	positive	negative
36	Ct-329	bacillus	positive	positive	negative
37	Ct-330	bacillus	positive	positive	negative
38	Ct-331	bacillus	positive	negative	negative
39	Ct-334	coccoid	positive	positive	negative
40	Ct-335	diplococci	positive	positive	negative
41	Ct-343	bacillus	negative	negative	negative
42	Ct-367	staphylococci	positive	negative	negative
		Cyprus rotandus (Cyr)			
43	Cyr-55	bacillus	negative	positive	positive
44	Cyr-60	bacillus	negative	positive	negative
45	Cyr-63	bacillus	negative	positive	positive
46	Cyr-64	bacillus	negative	positive	positive
		Pennisetum typhoides (Pt)			
47	Pt-101	bacillus	negative	positive	negative
48	Pt-102	coccoid	negative	positive	negative
		Trianthema portulacastrum (Tp)			
49	Tp-51	coccus	positive	positive	positive

Fig. 1. Growth promotory effects of endophytic culture on shoot and root of *Pennisetum typhoides*, in comparison to control (A) single seedling, (B) petridish containing 10 seedlings

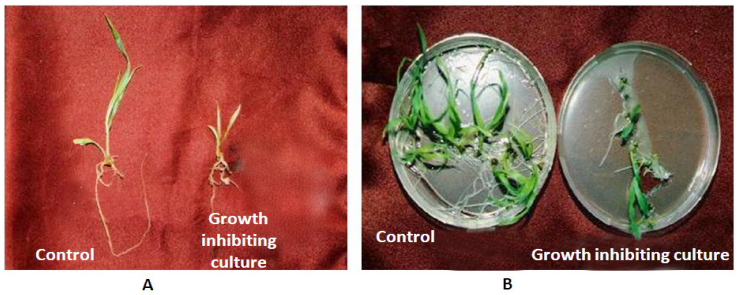

Fig. 2 Growth inhibitory effects of endophytic culture on shoot and root of *Pennisetum typhoides*, in comparison to control (A) single seedling, (B) petridish containing 10 seedlings

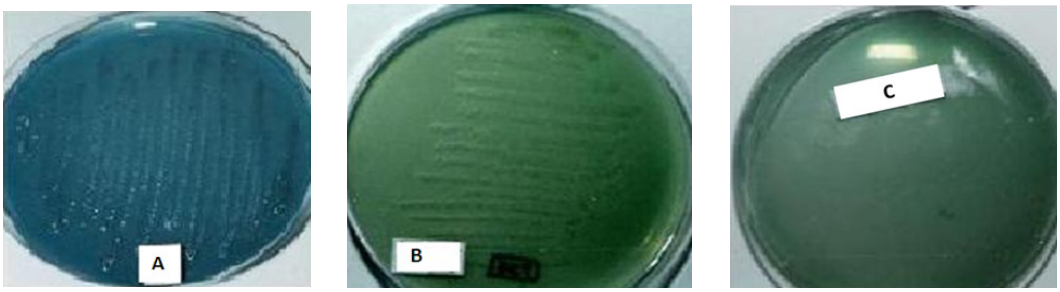

Fig. 3. Nitrogen fixing capabilities of endophytic isolates on N-free malate medium (A) culture fixing nitrogen, (B) culture unable to fix nitrogen and (C) control

Fig. 4. Nitrogen fixing capabilities of endophytic isolates on N-free malate medium (A) culture fixing nitrogen, (B) culture unable to fix nitrogen and (C) control

303 and Ct-331; and by more than 70% were: - Ct-312, Ct-329 and Pt-101 (Table-4). So these cultures have the potential to be used as seed inoculants for improvement in growth, development, yields of the economic crops.

Characterization of the growth promoting bacterial endophytes and the mechanism of growth promotion

The growth promotory cultures were mainly of two forms i.e. bacillus or coccus, with some variations like coccoid, diplobacilli, diplococci, streptococci and staphylococci. Majority of the growth promotory cultures were found to be Gram positive bacillus (21 cultures) followed by Gram negative bacillus (16 cultures). Other forms were very less in number i.e. Gram negative coccoid, Gram positive coccus and Gram positive staphylococci; Gram positive coccoid, Gram positive diplococci, Gram negative diplobacilli and Gram positive streptococci (1 culture each) (Table-5). The most efficient growth promotory culture (Ct-330) was Gram positive bacillus. In case of other promising growth promoters (promoting growth of both shoot and root by more than 50%) majority (5 out of 7 cultures) were Gram positive bacillus and remaining (2 cultures) were Gram negative bacillus. While finding the possible mechanism of growth promotion, it was observed that 38 cultures were employing the method of nitrogen fixation (Fig-3) alone, whereas only 4 cultures were promoting growth by Phosphate Solubilization (Fig-4) along with Nitrogen Fixation. Some cultures (7 cultures) were promoting growth but without employing any of the tested method (Table-5).

DISCUSSION

Our research goals were to isolate bacterial endophytes from various agronomic crops and medicinal plants and to study their effects on the experimentally inoculated test plant (*Pennisetum typhoides*) and finally to select the growth promotory cultures and to determine the possible mechanism employed. In this study, several hundred endophytic bacterial strains were obtained from the various plant sources. Similarly, other workers have also reported isolation of indigenous endophytic bacteria from various agronomic crops and prairie plants[14], soyabean[15], sweet potato[16], *Theobroma cacao*[17]. Thus these findings further prove the point that the most of the plants host one or the other endophyte. There appears to be significant variation in the types of indigenous bacteria isolated from diverse host plant species. Several factors may explain these differences, including host specificity, geographical distribution, plant genotype, plant age, tissue type, time of sampling and environment[1, 18].

In the present study bacterial endophytes have been obtained from all the plant parts taken but maximum numbers were contributed by roots followed by stem and then leaves. This might be because generally the endophytes enter plant tissue primarily through roots where their population is higher and thereafter decreases acropetally. The lowest number of endophytes from the bulb/tuber can be due to consideration of these tissues in only 2 out of the selected 8 plants. These observations are well supported by the study of Posada and Vega[19] which states that bacteria generally colonize the intercellular spaces, and they have been isolated from all plant compartments including seeds.

After the completion of seedling inoculation test to study the effect of the endophytes on the growth of *Pennisetum typhoides* appreciable number i.e. 49 (15.4 %) cultures out of 319 were promoting growth of both shoot and root, whereas the remaining cultures were showing variable effects ranging from complete inhibition to negative effect either on shoot or root only. It has been reported in the studies of Chanway[6] and Bent and Chanway[9] that bacterial endophytes can accelerate seedling emergence, promote plant establishment under adverse conditions and enhance plant growth. Interestingly it was found that most of the cultures (90% i.e. 26 out of 29 cultures) from *Pennisetum typhoides* itself were having negative effect on the growth and seedling emergence. This might be because of the difference in the varieties of the two plants (plant used for isolation of endophytes and the test plant) due to which the endophytic cultures could not recognize the test plant as the host plant and might become pathogenic. It has also been concluded in the study of Long et al.[20] that natural endophytic bacteria with Plant Growth Promotory traits do not have general and predictable effects on the growth and fitness of all host plants, although the underlying mechanisms are conserved. According to them the

different responses of host and non-host species to the natural endophytic bacteria may result from a combination of several factors including the different environmental conditions under which the host and non-host grow. Therefore in the present study, it can be pointed out that the Growth Promotory Cultures are not species specific unlike the inhibitory cultures.

On the basis of characterization of the growth promotory cultures, it could be concluded that majority of the cultures were Gram positive bacillus (21 cultures) followed by Gram negative bacillus (16 cultures). Similar findings have been reported by Sgroy *et al.,*[21] which states that of 29 endophytes obtained from *Prosopsis trombulifera*, 68.9% were positive bacillus and 31.1% negative bacilli.

During the study to find possible mechanism of growth promotion, it was observed that majority of the isolates were able to grow in nitrogen-free culture medium, and this capability could be attributed to the acquisition of atmospheric nitrogen by biological fixation, whereas only some isolates were found to solubilized phosphate along with Nitrogen Fixation. This however does not rule out the possibility of use of other growth promotory mechanisms besides the above two methods. Endophytic bacteria are believed to elicit plant growth promotion in one of two ways: either **(1)** indirectly by helping plants acquire nutrients, e.g. via nitrogen fixation[22-26], phosphate solubilization [20, 27-28] or iron chelation[29], by preventing pathogen infections via antifungal or antibacterial agents, by outcompeting pathogens for nutrients by siderophore production, or by establishing the plant's systemic resistance [30]; or **(2)** directly by producing phytohormones such as auxin or cytokinin[31], or by producing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which lowers plant ethylene levels[32]. A particular bacterium may affect plant growth and development using one or more of these mechanisms, and may use different ones at various times during the life cycle of the plant. Also there were some cultures that were promoting growth but without employing any of the tested method, this points towards some other mechanisms of growth promotion used.

These results are well supported by study of Sgroy *et al.,*[21], who reported endophytic growth promoting bacteria with nitrogen fixing capabilities but none with phosphate solubilizing capability. Similarly Khan and Doty[16] reported isolation of nitrogen fixing endophyte from sweet potato. Reiter *et al.*[33] also identified potential nitrogen fixing endophytes in seven sweet potato varieties collected in Uganda and Kenya. Other reports have indicated that bacterial endophytes provide nutrient and mineral input to the plants[4, 34-35].

CONCLUSION

This study demonstrated the occurrence and diversity of cultivable endophyte, in various unexplored plant species. Out of which the efficient growth promontory endophytes could find enormous use as seed inoculants or co-inoculants for improvement of growth, development, yields and pathogen resistance in the economically important crop plants.

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