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Host-specific endophytic bacteria, *Sporosarcina pasteruii* enhances growth in *in vitro* shoot cultures of the bamboo, *Dendrocalamus longispathus,* an economically important bamboo

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Abstract: *In vitro* shoot cultures of *Dendrocalamus longispathus,* a tropical sympodial bamboo were found to be harboring the endophytic Gram positive bacteria, *Sporosarcina pasteruii,* which offered protection against contamination from other microbes and permitted shoot multiplication and increase in shoot biomass. Establishment of axenic cultures by treating with gentamicin (250µg/ml) resulted in significant reduction in shoot multiplication rates and growth. Indole 3-Acetic Acid (IAA) biosynthesis by the endophyte, correlated to the improved growth and multiplication rate of the shoots, was revealed by lift membrane assay. The production of IAA was eight times (98.76 µg/ml) in shoot cultures with the endophytes than that of the basal medium with the isolate alone (12.00 µg/ml). Growth promotion of *in vitro* shoot cultures by *S. pasteruii* was however limited to the host species and in shoot cultures of three non-host bamboo species viz. *Bambusa balcooa*, *Pseudoxytenanthera ritcheyi* and *Dendrocalamus strictus*. Production of siderophores by *S. pasteruii* was confirmed by CAS Blue agar test and an antagonistic effect against fungal and other bacterial contamination was demonstrated through dual culture. Detection and retention of potentially beneficial endophytes in tissue culture through the simple and rapid method employed can be a routine procedure to improve the efficiency of micropropagation in general.

Key words: Endophytes, bamboo, micropropagation, *Sporosarcina pasteurii, Bacillus pasteruii*, plant growth promotion.

INTRODUCTION

Plant micropropagation, especially of woody perennials, faces several constraints, many of them directly or indirectly linked to microbial contamination, that makes it difficult to establish sterile cultures. Even with numerous disinfecting agents in use for the preparation of aseptic explants, microbial contamination continues to be a serious threat to micropropagation, especially of woody perennials and long term maintenance of *in vitro* gene banks (Leifert and Woodward 1998; Leifert and Cassells

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2001; Herman, 2004). Contaminating organisms in plant tissue culture come under the categories of covert and endophytic bacteria (Thomas *et al.,* 2007), which include fastidious or viable but nonculturable (VBNC) bacteria, alcohol-tolerant and autoclaving-defying spore-formers. Endophytic bacteria that inhabit the internal plant tissues are known to form nonpathogenic and often mutualistic relationships with their hosts (Hardoim *et al.,* 2008; Compant *et al.,* 2010). Since latent organisms might hamper the establishment of successful micropropagation, several methods are adopted to eliminate or suppress bacterial growth like addition of antibiotics or other microbicides to the culture media. In contrast, Pirttila *et al.,* (2004) found that the elimination of bacterial endophytes from *Pinus sylvestris L* culture resulted in an altered morphology, which could be restored by conditioning the medium with *Methylobacterium extorquens* and *Rhodotorula minuta.* The growth promoting effect of *Rhodopseudomonas* species on tomato seedlings *in vitro* was attributed to the synthesis of IAA and 5-aminolevulinic acid (ALA) (Koh and Song 2007). Similarly, Quambusch *et al.,* (2014) reported that *Mycobacterium* sp, the endophytic populations of *Prunus avium* shoots is correlated with success in micropropagation. The beneficial role of endophytes in *in vitro* cultures is therefore beginning to be realized in several species and the conventional approach of aiming for axenic cultures for micropropagation should no longer be valid in all cases.

Many reports strongly suggest that endophytes have an excellent potential to be used as plant growth promoters and to positively affect plant nutrient status, plant growth and the ability to tolerate abiotic and biotic stressors (Reinhold- Hurek and Hurek 2011). Growth promoting activity of endophytes are due to production of plant growth regulators (Lee *et al.,* 2004), siderophores (Costa and Loper 1994) or due to their phosphate solubilizing activity (Wakelin *et al.,* 2004) or nitrogen fixation (James *et al.,* 1994). In perennial ryegrass, *Lolium perenne*, increased seed set and improved germination (Clay 1987) as well as increased shoot growth and tillering (Vessey 2003) was attributed to the production of auxin-like compounds. In addition, the endophytes are known for activation of defense mechanisms enabled by production of iron-chelating siderophores, antibiotics, biocidal volatiles, lytic enzymes and detoxification enzymes (Bais *et al.,* 2004, Glick 1995, Sturz and Christie 2003).

Since in rye grass, it gave positive results, it was decided to test in a perennial grass species of the bamboo. *Dendrocalamus longispathus*, a large tufted bamboo, native to the north-eastern region of India is one of the 15 commercially important species that finds a variety of traditional and industrial uses (Williams and Rao 1994). The present study was initiated when apparently healthy shoot cultures of *D. longispathus* were found to harbor an endophytic bacterium and attempts to eliminate the endophyte with the aim of establishing axenic shoot cultures resulted in reduced growth of shoots. This paper reports the ability of the host- specific bacterial endophyte to enhance growth in *in vitro* cultures of bamboo and to act as an *in vitro* bio-control

agent against other microbial contaminants through IAA production and siderophore formation respectively.

MATERIALS AND METHODS

Plant material and culture conditions

Aseptic seedlings of the bamboo, *Dendrocalamus longispathus,* were initiated from seed collected from Tripura, India. The seeds were dehusked and given a thorough wash for 60 min. under running tap water followed by a treatment for 60 min. with a 1% (w/v) solution of a broad-spectrum commercial fungicide (Bavistin, BASF Chemical Company Ltd). Surface sterilization was then carried out with a treatment of 0.1percentage HgCl2 solution for 3 min. followed by repeated rinses with sterile distilled water to remove the traces of sterilants. The seeds were then inoculated under a laminar flow hood, on a hormone free basal medium containing the basal salts of Murashige and Skoog's (1962) media (MS) solidified with 0.8% (w/v) Agar-agar (Tissue Culture grade, Meron Hydrocolloids, Kochi) and supplemented with 2% (w/v) sucrose. The pH of the medium was adjusted to 5.8 using 1N NaOH or 1N HCl prior to autoclaving (at 121°C at 1.06 $kgcm^{-2}$ pressure) for 20 min. All the cultures were maintained at 25 ± 2°C under a16 h photoperiod and an average light intensity of 25 $\mu E\ m^{-2}\ s^{-1}$ provided by cool white fluorescent tubes and at a RH of 50 to 60%. The seedlings after 7 days of germination were transferred to liquid MS media supplemented with 2 % sucrose and 2.21µM BAP (SMM) for induction of multiple shoots following, which the shoots were maintained in vitro through 15-day subcultures in the above media.

Identification of the bacterial strain

The contaminating organism was isolated by streaking a loop full of media onto sterile Luria Bertani (LB) Agar Medium (Himedia Ltd, Mumbai, India) and Potato Dextrose Agar (PDA) (Himedia Ltd, Mumbai, India). The isolate outgrowths from LB agar were collected and sub-cultured onto LB agar and the culturesincubated at 28°C for 24 hours. Pure bacterial isolates were obtained by repeated subculturing using a serialdilution technique (Collins and Lyne 1984) and maintained on LB agar slants. The purified isolates were identified based on colony morphology, cell morphology, Gram reaction and other biochemical tests (IMViC tests) (Collins and Lyne 1984, Krieg and Holt 1984; Sneath *et al.,* 1986).

Antibiotic sensitivity of bacterial isolates

In a bid to remove the bacterial infection found in the shoot cultures, antibiotic susceptibility of the isolate was determined by using commercially available antimicrobial susceptibility test discs (Icosa G-I Minus, Himedia Ltd., Mumbai; 6 mm diameter) by Kirby- Bauer method (Bauer *et al.,* 1966). Twenty antibiotics, having

various antibacterial ranges of activity at various cellular sites in bacteria were selected for testing their inhibitory effects on the endophytic bacteria. Mueller-Hinton (MH) agar (Himedia Ltd, Mumbai, India) was inoculated with the bacterial isolate. Discs singly impregnated with various antibiotics at specific concentrations were placed onto the growth medium in plate after the bacterial inoculation. A treatment consisted of five discs of an antibiotic in a plate replicated three times. The inoculated plates were incubated at 28°C for 24 hours and the susceptibility of the bacterial isolates to the antibiotics was estimated based on the diameter of the inhibition zone measured using a ruler (Kneifel and Leonhardt 1992). Analysis of variance and student's t-Test were performed in order to determine the significance ($P<0.05$) of various antibiotics.

Effect of elimination of endophyte in shoot cultures with antibiotics

To eradicate the endophytic bacteria in shoot cultures, gentamicin was selected based on the sensitivity tests described above. This was added to the liquid SMM at concentrations ranging from 10 to 500µg/ml to determine the optimum bactericidal dose. To confirm the efficacy of the bactericidal treatment, shoot cultures were transferred to antibiotic free media after 15 days and observed for signs of bacterial growth. Shoots were maintained over three passages through subculture to evaluate the effect of eradication of endophyte.

Assessing the role of endophyte /culture filtrates on growth of shoot cultures of *D. longispathus*

Since antibiotic treatment of shoot cultures of *D. longispathus* resulted in the reduction of growth and multiplication rate of shoots, the role of the endophyte *in vitro* was tested by re-introduction of thebacteria into shoot cultures. The bamboo shoot cultures from antibiotic containing SMM was transferred into fresh SMM and inoculated with a loopful of the pure culture from agar slant and cultures maintained for three passages through subculture every 15 days.

The media from 15 day old shoot cultures of *D. longispathus* harboring the endophyte was centrifuged at 10000 rpm for 10 minutes to remove the bacterial cells and filter sterilized through a 0.22µm membrane filter to obtain the sterile conditioned filtrate. This filtrate was then mixed with equal quantities of fresh, sterilized stock of SMM of 2X concentration to formulate the Spent Media. Shoot cultures of *D.longispathus* with endophytes and those free of endophytes were additionally included in the experiment.

***In vitro* assay for IAA production by bacteria**

In order to check for the production of the auxin, IAA, pure culture of the bacteria was inoculated on to LB agar amended with 0.5mM L-Tryptophan (L-Trp). Petri plates (9 cm dia.) were inoculated with toothpicks into a grid pattern from agar cultures and the

membrane lift assay (Bric *et al.,* 1991).The plate was overlaid with Whatman No. 1 filter paper immediately after inoculation and incubated until colonies reached 0.5 to2 mm in diameter. Thereafter the paper was removed from the plate and treated with Salkowski's Reagent (Gordon and Webber 1951) and saturated in a Petri dish by overlaying on a reagent-saturated filter paper until adequate color developed at room temperature. Bacteria producing IAA were identified by the formation of a characteristic red halo within the membrane immediately surrounding the colony.

Estimation of IAA production by bacteria

The experiment was carried out in order to screen for the media for the optimal biosynthesis of auxin by the endophyte. Conical flasks (30 ml) containing 5 ml of LB broth, MS liquid media, with and without 5 mM of L-Trp, were inoculated with 1ml of the bacterial suspension and maintained at 28°C in the dark with constant agitation at 140 rpm. After growth for 48 h, the liquid cultures were centrifuged at 7,000 g at 4°Cfor 10 min and the supernatants were collected. The amount of IAA produced in culture was estimated by adding 1 ml of culture supernatant to 2 ml of Salkowski's Reagent. The mixture was kept for 120 min in dark until red color developed, with darker red indicating a higher amount of IAA. The color intensity was measured at an absorbance of 530 nm (Asghar *et al.,* 2002) in a spectrophotometer. IAA production in shoot cultures of *D. longispathus* harbouring the endophyte was assessed using the spent media after 3 days of culture.

Assessment of growth promotion by endophyte and spent medium in shoot cultures of three non-host bamboo species

The potential for growth promotion by the endophytic bacteria isolated from *D. longispathus* on growth in other non-host bamboo species was evaluated. Shoot cultures of three species of bamboo viz. *Bambusa balcooa*, *Pseudoxytenanthera ritcheyi* and *Dendrocalamus strictus* derived from axillary buds of adult plants and maintained through 15 day subcultures on SMM, were used in the experiments.

A single colony of the bacteria from LB agar slant was inoculated into SMM and shoot cultures of the three species transferred to this media and maintained through subculture every 15 days. Cultures of the three species on SMM without the endophyte were maintained as control.

The filtrate was prepared by the procedure mentioned above. This filtrate was then mixed with equal quantities of fresh, sterilized stock of SMM of 2X concentration to formulate the Spent Media. Shoot cultures of the three bamboo species were inoculated into this media and maintained by subculture to the fresh reconstituted media every 15 days. Shoot cultures of the three species on SMM were maintained as control.

In order to examine whether exogenous supply of IAA could induce the same results

as that of the filtrate-conditioned media, 5.71 µM of the auxin was added to SMM into which shoot cultures of the four bamboo species were inoculated and maintained by subculture every 15 days.

Fresh weights of shoot cultures at beginning of experiment were calculated as the differences in the weight of the culture vessel with fresh media and that of the same vessel after inoculation, under sterile conditions, with the shoot culture. Enhancement in growth of shoots at the end of 3rd subculture were assessed by removing the shoot cultures onto sterile blotting paper in the laminar flow chamber for 60 seconds to remove the liquid media before weighing. The rate of shoot multiplication was assessed as percent increase in shoot number over 3 passages, using the formula:

$$\text{Shoot Multiplication Rate} = \frac{\text{No. of shoots after 3rd subculture} - \text{No.of shoots at 1st subculture}}{\text{Total no. of explants}} \times 100$$

Only shoots that had attained a length of 1 cm and above were counted. The data is reported as the mean often replications for each treatment. Means and the standard error of the means were calculated for the effect of different treatments. Student's t-Test was performed in order to determine the significance ($P<0.05$) of treatments in different species.

Siderophore production by the growth promoting bacterial isolate was determined through Blue agar CAS medium containing Chrome Azurol sulphonate (CAS) and hexadecyl trimethyl ammonium bromide (HDTMA) as indicators as described by Schwan and Neilands (1987). The Blue agar medium was aseptically poured on to sterile plates and allowed to solidify. After solidifying, a paper disc was placed on the center of the CAS blue agar and the bacterial suspension cultured in LB broth was dropped onto the paper disk and incubated at 30°C for 48 h.

A 5 mm mycelial mat from a pure culture maintained on PDA was taken to test the antagonistic effects of bacterial isolate on *Fusarium oxysporum*, placed on one side of a fresh PDA plate and endophytic bacteria isolate was streaked on the other side of the plate and cultured at 28ºC for 7 days. During the incubation period, antagonistic effects of the bacterial isolate against the fungal isolate was confirmed by the inhibition zone formed between the bacteria isolate and fungal isolate. The dual culture was performed in three replicates.

RESULTS AND DISCUSSION

Identification, characterization and elimination of the contaminating endophyte

In all the shoot cultures of *D. longispathus*, presence of bacteria was indicated by the turbidity in the media, as early as in the third passage (25 d). Since the contamination

from the phyllosphere microflora as a result of inadequate surface sterilization usually appears in the first passage itself and commonly consists of bacterial and fungal contaminants, the presence of latent microbes in the cultures was suspected. Moreover, the shoot cultures showed no signs of damage and growth continued unabated. Based on cultural characteristics, the IMViC test and the comparison with the Bergey's Manual of Determinative Bacteriology, the circular, creamy and smooth colonies were identified as Gram-positive *S. pasteurii (Bacillus pasteruii)* (Table 1). Molecular methods of identification of the strain were not deemed necessary for this study since the emphasis was for developing a routine simple and rapid method of identifying the potential for *in vitro* growth promoting activity.

Table 1. Identification of bacteria isolated from plant tissue cultures of *D. longispathus*

Criteria	Isolate
Gram reaction	+
Acid fastness	-
Morphology of bacterial cells	Rods
Endospores	+
Motility	+
Urease	+
Nitrate reduction	+
Carbon source	
Citrate	+
Starch	-
Indole	-
Methyl red	-
Voges paskuer	+
Methyl red	-

Antibiotic sensitivity

Out of 20 antibiotics tested, S. *pasteurii* was found to be sensitive only to tobramycin, kanamycin, gentamicin and streptomycin (Table 2) and the level of susceptibility was highest for kanamycin followed by gentamicin.

Table 2. Antibiotic sensitivity of the endophytic bacterial species of *D. longispathus*

Antimicrobial Agent	Disc content (µg)	Zone of inhibition (Diameter in cm)
Imipenem	10	0
Ciprofloraxin	5	0
Tobramycin	10	1.4066±0.075
Moxifloraxin	5	0
Ofloxacin	5	0
Sparfloxacin	5	0
Levofloxacin	5	0
Norfloxacin	10	0
Co-Trimoxazole	25	0
Colistin	10	0
Nalidixic acid	30	0
Augmentin	30	0
Kanamycin	30	2.590±0.05
Gatifloxacin	5	0
Gentamicin	10	2.563±0.076
Amikacin	30	0
Streptomycin	25	1.5533±0.1043
Ceftriaxone	30	0
Cefopodoxime	10	0
Ticarillin	75	0

The sensitivity assay revealed that at 250µg/ml, both kanamycin and gentamicin were bactericidal when added to shoot cultures of *D. longispathus* (Table 3). Both are broad-spectrum bactericides for Gram positive and Gram negative bacteria (Reed *et*

al., 1995). Although kanamycin treatment of shoot cultures for 15 days was found to be effective against bacterial contamination, it also caused severe damage to the expanded leaves and newly emerging shoots. Gentamicin at the safe bactericidal level of 250 µg/ml was therefore used in this study.

Table 3: Assessment of the influence of endophyte on shoot cultures of *D. longispathus*

Treatment	Fresh weight*	Multiplication rate*
Control	3.174 ± 0.087^{c}	5.40 ± 0.16^{a}
Antibiotic treated	0.089 ± 0.027^{a}	2.43 ± 0.39^{d}
Co-culture with isolate	2.716 ± 0.190^{b}	4.87 ± 0.21^{c}
Addition of Spent Medium	0.935 ± 0.001^{a}	2.69 ± 0.06^{a}

*Consolidated increase in shoot fresh weight in gm and percentage increase in shoot number rate after 3 passages. Values in a column followed by different letters are significantly different at $p>0.05$ level based on mean comparison range using t -Test.

Effect of endophyte on *in vitro* growth of *D. longispathus*

The beneficial role of the endophyte in maintaining steady growth and multiplication of shoot cultures was demonstrated when elimination of the endophyte with gentamicin resulted in a significant reduction in these parameters when compared to cultures with the endophyte (Table 3). Reintroduction of the endophyte through inoculation of the pure isolate and Spent Media retrieved the multiplication rate (an increase of 4.87% and 2.69% respectively compared to 2.43% in the antibiotic treated control), as well as the fresh weight enhancement (2.716 gm and 0.935 gm respectively compared with 0.089 gm in the antibiotic treated control) (Table 3). Pirtilla *et al.,* (2004) reported that the mitigation of browning in callus cultures of *Pinus sylvestris* through endophytic conditioning was due to the consumption of nutrients from the media by endophytes and production of metabolites. According to Thomas (2004a and b), these results indicate that the reproducibility of tissue culture protocols rely on the presence or absence of the endophytes which are involved in various pathways in the normal growth and development of the plants.

***In vitro* screening of endophyte for IAA production**

The membrane lift assay confirmed the production of detectable levels of IAA by endophyte *S. pasteruii* when grown on media containing the precursor L-Trp. Color development was visible within minutes of overlaying and increase in intensity continued for a period of 30 min (Fig 1). The minimum detectable level of IAA in the test is approximately 50 pmol in a $2mm^2$ spot (Bric *et al.,* 1991). Many of the microorganisms associated with plants are involved in phytohormone biosynthesis and the amount of hormone produced may vary greatly between the strains within

a species, and some strains may not produce any phytohormone (Antoun *et al.,* 1998). This method is therefore suitable for rapid screening of endophytes associated with plants so as to take advantage of their growth promoting ability for improving the efficiency of *in vitro* cultures.

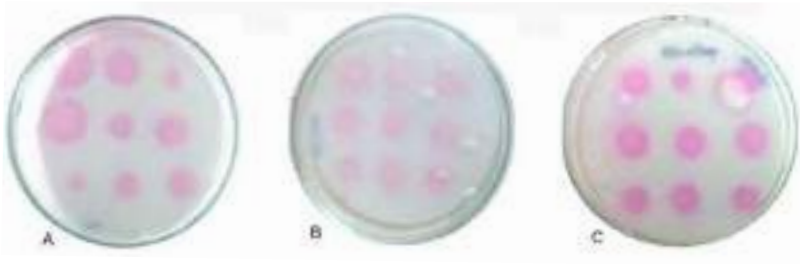

Figure 1. Color development due to the IAA production by *S. pasteruii* in different media. A: LB +Trp; B: MS+ Trp; C: Media from *D. longispathus* shoot culture

IAA was quantified from filtrates of bacterial cultures in LB broth, LB Broth + L-Trp, MS media, MS + L- Trp and from shoot cultures of *D. longispathus* after 48 hrs incubation with bacteria. The results (Fig 2) demonstrated that IAA production in shoot cultures harbouring the endophyte (98.76µg/ml) was more than 8 times that of the isolate grown in the basal tissue culture media (MS) (12.00 µg/ml) indicating the importance of the plant-microbe interaction for the growth promotion. Several reports suggest that IAA biosynthesis via the Indole-3- acetamide pathway (IAM) is widespread in the plant kingdom and that L-Trp can serve as the precursor for the auxin (Pollmann *et al.,* 2006; Mano *et al.,* 2010). Higher production of IAA in media containing L- Trp revealed that the bacteria relies on this Trp-dependent pathway for auxin production (Woodward and Bartel 2005; Pollmann *et al.,* 2006; Zhao 2010). Due to the simplicity of the colorimetric assay, this test can be recommended as a routine method of screening of auxin-producing endophytic bacteria in plant species for which micropropagation is attempted without the need for more sophisticated quantitative methods.

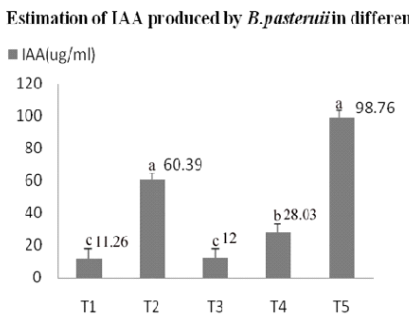

Figure 2. Spectrophotometric analysis of IAA production by endophytic *S. pasteruii* in different media T1: LB + Endophyte; T2: LB + L-Trp + Endophyte; T3: MS + Endophyte; T4: MS + L-Trp + Endophyte; T5: Filtrate from shoot culture with endophyte

Assessment of growth promotion by endophyte and Spent Medium in shoot cultures of three non- host bamboo species

The pure culture of the endophyte when inoculated into shoot cultures of the three non-host bamboo species did not show the expected effect of growth enhancement as was seen in host species (Table 4 and 5). The bacteria instead caused a contaminated culture where the multiplication of the shoot culture was suppressed and damage to the shoots, typical of contaminated cultures, was seen within 3 days of inoculation. This confirmed the host specificity of the endophyte and ruled out its use as a beneficial organism for *in vitro* culture of other species.

Table 4. Assessment of growth enhancement in shoot cultures of three non-host bamboo species treated with Spent Medium and bacterial isolate

Treatment	*B. balcooa**	*D. strictus**	*P. ritcheyi**
Control	0.964 ± 0.049^{b}	0.353 ± 0.086^{b}	0.367 ± 0.237^{a}
Co-culture with isolate	0	0	0
Addition of Spent Medium	0.716 ± 0.273^{a}	0.456 ± 0.261^{b}	0.381 ± 0.331^{a}

*Increase in fresh weight of shoots in gm measured after 3 passages. Values in a column followed by different letters are significantly different at p>0.05 level based on mean comparison range using t-Test.

Table 5. Assessment of multiplication rate in shoot cultures of three non-host bamboo species treated with Spent Medium and bacterial isolate

Treatment	*B. balcooa**	*D. strictus**	*P. ritcheyi**
Control	5.23 ± 0.12^{a}	1.21 ± 0.41^{a}	2.38 ± 0.21^{b}
Co-culture with isolate	0	0	0
Addition of Spent Medium	5.54 ± 0.06^{b}	2.16 ± 0.11^{b}	2.53 ± 0.19^{a}

*Percentage increase in shoot number after 3 passages. Values in a column followed by different letters are significantly different at p>0.05 level based on mean comparison range using t-Test.

Addition of Spent Media as a supplement did not produce a significant increase in growth or multiplication rate in any of the four bamboo shoot cultures (Table 4 & 5). Although the presence of IAA in the filtrate has been established in this case, the benefit is limited since no further auxin production is possible in the absence of the endophyte and the auxin present is soon used up. The possibility of the filtrate containing other metabolites those are not conducive to growth and multiplication of bamboo cultures cannot be ruled out.

The effect of exogenous supply of IAA on growth of shoots and multiplication is clearly seen in all the four bamboo species (Table 6). In the host species, no significant

improvement in growth was seen even when the multiplication rate showed some improvement. Although the other non-host bamboo species do show significant improvement either in shoot growth or in multiplication rates, in the presence of added IAA, the levels of auxin required for optimum growth would need to be ascertained separately as is the case when media composition is being standardized.

Table 6. Assessment of growth in shoot cultures treated with exogenous IAA in four bamboo species

	Fresh weight		**Multiplication rate**	
	Control*	IAA(5.71 µM)*	Control*	IAA(5.71 µM)*
D. longispathus	0.8314 ± 0.0028^{a}	0.8569 ± 0.1239^{b}	2.61 ± 0.22^{c}	2.65 ± 0.18^{b}
B. balcooa	0.964 ± 0.049^{b}	1.1911 ± 0.09^{a}	5.23 ± 0.12^{a}	6.14 ± 0.01^{b}
D. strictus	0.353 ± 0.086^{b}	$0.6458\pm .0014^{b}$	1.21 ± 0.41^{a}	2.11 ± 0.08^{b}
P. ritcheyi	0.367 ± 0.237^{a}	0.6782 ± 0.0224^{b}	2.38 ± 0.21^{b}	3.09 ± 0.16^{a}

*Consolidated increase in shoot fresh weight in gm and percentage increase in shoot number after 3 passages. Values followed by different letters in a row for the same parameter are significantly different at $p>0.05$ level based on mean comparison range using t -Test.

It is thus established that the beneficial effect of the endophyte in shoot cultures of *D. longispathus* is not surpassed by the supplements of Spent Medium or exogenous auxin (Table 3), indicating the uniqueness and importance of host–endophyte interaction in producing the effect. The effectiveness of endophyte as growth promoting agents, besides host specificity, depends on factors like population dynamics and pattern of host colonization, the ability to move within host tissues, and the ability to induce systemic resistance to biotic stresses (Kandel *et al.,* 2017). It is to be assumed that the endophyte in *D. longispathus* meets one or more of these requirements.

CAS blue agar assay (Fig. 3) confirmed that the endophytic bacteria produce siderophores. Schwyn and Neilands (1987) reported that siderophores can remove the iron molecules to develop a typical colored zone of yellow to orange in the media. This biochemical property is important as a plant growth promoting trait as well as for microbial antagonism shown by the isolate. In addition to inhibiting the growth of pathogenic microorganisms, siderophores indirectly stimulate the growth of plant (Glick, 2012). Gururani *et al.,* (2012) reported that siderophore producing bacteria significantly influence the uptake of various metals, including Fe, Zn, and Cu by plants.

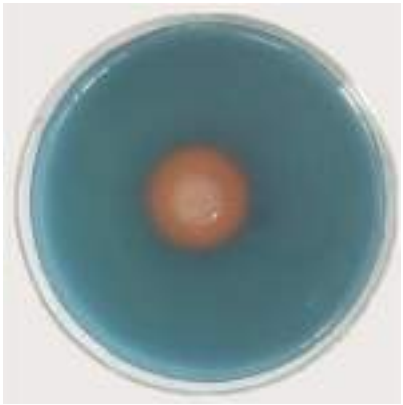

Figure 3. Siderophore production on CAS blue agar by the endophyte, *S. pasteruii Antagonistic effect of endophytic bacteria in vitro*

The formation of inhibition zones after 7 days incubation (Fig. 4) confirmed the biocontrol potential of *S. pasteruii*. The dual culture *in vitro* test conducted with *F. oxysporum* (an ascomycete with a chitin–glucan containing cell wall and a common fungal contaminant of plant tissue cultures), demonstrated that the siderophore producing endophyte from *D. longispathus* can control the emergence of other contaminating organisms. Kim *et al.,* (2008), through dual culture tests have shown that many *Bacillus* isolates have antagonistic effects against the isolates of soil-borne fungi, *F. oxysporum, P. capsici, R. solani AG-4,* and *S. sclerotiorum* and the degree of inhibition varied depending on strains and siderophore producing trait. Endophytes also stimulate the biosynthesis of various antimicrobial compounds in host plants which suppress the growth of pathogenic organisms viz., *F. oxysporum* and *R. solani* and function as stress factors in inducing host resistance (Haas and Défago 2005; Joseph *et al.,* 2007;Wahyudi *et al.,* 2011). The isolate from *D. longispathus* showing high siderophore producing activity can thus be further studied for its ability to confer disease resistance in host plants.

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Figure 4. Antagonistic activity of the endophyte, *S. pasteruii* against *F. oxysporum*

CONCLUSION

The study demonstrates decisively that the host-specific endophytic bacterium, *S. pasteurii* enhances the growth and multiplication of *in vitro* shoot cultures of *D. longispathus*. The presence of the endophyte given the additional benefit of prevention of the contamination from opportunistic fungi and bacteria. These growth enhancing and antimicrobial effects are shown to be due to the production of IAA and siderophores by the endophyte. The establishment of axenic cultures by removal of endophytes may therefore not be desirable in all instances. A routine screening of plants for growth promoting microbes using the simple methods adopted in this study may be a strategy that can serve to improve the efficiency of micropropagation in general.

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