Evaluation of Endophytic Bacteria for Plant Growth Promotion and Pathogen Suppression Traits in *Saccharum officinarum*

Penumatsa Kishore Varma¹, Kotam Vijay Krishna Kumar¹, Vanapalli Chandra Sekhar¹, Dandu Adilakshmi², Madugula Suresh¹, Nambala Raja Kumar¹, Karnam Jayachandra² and Ravuri Anitha¹

1. Department of Plant Pathology, Regional Agricultural Research Station, Anakapalle 531001, Andhra Pradesh, India

2. Department of Genetics and Plant Breeding, Regional Agricultural Research Station, Anakapalle 531001, Andhra Pradesh, India

Abstract: Endophytes are beneficial microbes that are capable of promoting growth, besides protecting colonized plants against plant pathogens. These microbes are of either bacterial, fungal or actinomycetes in plants. In the study, the endophytic bacteria isolated from sugarcane with their characterization related to plant growth promotion and pathogen suppression have been reported. Roots, shoots and leaves of rooted tissue culture plantlets of sugarcane cultivars of 87A298 and 2009A107 were excised aseptically and isolated endophytic bacterial strains. The strains were identified using 16S rRNA gene sequence based homology. Molecular characterization of these strains was carried out for presence of antimicrobial genes. The results showed that the endophytes isolated from sugarcane tissue culture plantlets were of the genera *Bacillus* (*B. amyloliquefaciens*, *B. subtilis*, *B. cereus*, *B. safensis*, *B. siamensis*, *B. aryabhattai*, *B. flexus* and *B. velezensis*) and *Paenibacillus pabuli*. There were three antimicrobial peptides (AMPs) producing genes of bacilysin, bacillomycin and fengycin in *B. amyloliquefaciens* (SE1, SE7), *B. siamensis* (SE4, SE16), *B. subtilis* (SE2, SE3) and *B. velezensis* (SE15). The biochemical characterization assays showed that some of these strains could produce hydrogen cyanide (HCN), protease, cellulase and indole acetic acid (IAA). Few strains (SE1 and SE4) were phosphate solubilizers, whereas nine isolates were found to be diazotrophs. Most of the bacterial isolates were found antagonistic to *Fusarium sacchari*, the sugarcane wilt pathogen under *in vitro* conditions. Overall, the results suggested the scope and potentiality of sugarcane endophytic bacteria, isolated from tissue culture plantlets, in promoting plant growth and suppression of sugarcane pathogen.

Key words: *Bacillus*, *Fusarium sacchari*, *Paenibacillus*, antimicrobial peptides.

1. Introduction

Sugarcane is one of the main cash crops of India that provide sugar, besides biofuel, fibre, fertilizer and myriad of co-products with ecological sustainability. India is the second largest producer of sugar after Brazil, and accounts for 16% of world's production [1]. In Andhra Pradesh, sugarcane is grown in 1.39 lakh hectares with a production of 99.8 lakh tons. However, the sugarcane productivity (71 tons/ha) is very low compared to states, like Tamil Nadu, West Bengal, Kerala and Karnataka [2]. The productive potential of the crop is not completely harnessed due

to several biotic and abiotic factors. Among the biotic factors, diseases are the major constraints in sugarcane production, causing considerable yield losses. As majority of sugarcane diseases are sett or stalk borne and soil borne in nature, chemical management of diseases is not possible after certain crop growth stage owing to impermissible hard rind. Wilt incited by *Fusarium sacchari* is gaining importance in north coastal districts of Andhra Pradesh, wherever sugarcane is grown under irrigated conditions. The disease symptoms are evident in 6-8 months old plants as foliage drying followed by death of whole plant. Hence, protection needs to be offered from early stages of crop growth and should continue throughout the crop period. Inoculation of bacterial isolates with

Corresponding author: Penumatsa Kishore Varma, Ph.D., research field: plant pathology.

endophytic ability may offer continuous protection against the pathogen as they colonize sugarcane tissues effectively and suppress the pathogen colonization and thereby the disease development.

Several microbes with endophytic activity were found colonizing tissues of leaves, stalks [3] and roots of sugarcane without external sign of infection. These microbial endophytes include yeasts, fungi, bacteria and actinomycetes [4-7], with a potential for plant growth promotion and plant disease suppression. Plant growth promotion by microbial endophytes was attributed to their ability for nitrogen fixation, phosphate solubilisation, cellulase production, indole acetic acid (IAA) production, etc. [8-11]. Besides growth promotion, they were also found antagonistic to the sugarcane pathogens, viz., *F. sacchari* (wilt), *Collectotrichum falcatum* (red rot) and *F. moniliforme* (top rot) under laboratory conditions. Though, work has continued on sugarcane endophytes, little success has been attained on elucidating a suitable endophytic strain with both plant growth promoting ability and disease suppression. The present research aimed at identifying a potential plant growth promoting endophytic bacterial strain that promotes growth, besides managing wilt disease effectively.

2. Materials and Methods

2.1 Isolation, Purification and Characterization of Endophytic Bacteria

2.1.1 Plant Materials

Rooted tissue culture plantlets of sugarcane cultivars 87A298 and 2009A107 were obtained from tissue culture laboratory of Regional Agricultural Research Station (RARS), Anakapalle for isolation of endophytic bacteria.

2.1.2 Isolation and Purification of Bacterial Isolates

Leaves, stems and roots of tissue culture plantlets were separated aseptically and macerated in 1 mL of sterile distilled water using sterilized pestle and mortar. The homogenate was plated onto Luria Bertani agar and Bacillus agar (HiMedia) media and incubated at

28 ± 2 °C for 96 h. Distinct colony types were picked up and purified through three rounds of streaking and single colony selection on Luria Bertani agar.

2.1.3 Molecular Characterization of Bacterial Isolates

The identity of the bacteria was established through 16S rRNA gene sequence based homology analysis. The genomic DNA was isolated according to Sambrook and Russel [12]. Amplification of 16S rRNA genes of bacterial isolates was carried out by PCR using universal primers, FGPS6-63-GGAGAGTTAGATCTTGGCTCAG and FGPL 132-38-CCCGGTTTCCCCATTCGG [13]. The thermocyclic conditions included initial denaturation at 95 °C for 3 min followed by 35 amplification cycles of 95 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min followed by final extension at 72 °C for 3 min. The PCR products were analyzed in 1% agarose gel in 1× Tris-acetate EDTA, run for 90 min at 100 V, and the amplified products were excised and outsourced (BioServe Biotechnologies (India) Pvt. Ltd., Hyderabad) for partial sequencing. Similarity of 16S rRNA gene sequence was aligned using BLAST Programme of GenBank database (NCBI).

2.2 Pathogen Culture

Wilt infected sugarcane samples were collected from agricultural farm, RARS, Anakapalle and carried to the laboratory for isolation. Infected plant tissue was disinfected and plated onto potato dextrose agar (PDA). After 5 d of incubation at 28 ± 1 °C, the pathogen was identified as *F. sacchari*, based on the morphology of vegetative and reproductive structures according to keys of identification [14].

*2.3 In Vitro Antagonism against *F. sacchari**

The bacterial isolates were tested for antagonistic potential against *F. sacchari*, the causal agent of sugarcane wilt, by dual culture assay. An agar block (5 mm diameter) of 5-day-old culture of *F. sacchari* was placed in the centre of Petri plates (90 mm

diameter), containing PDA (HiMedia). A loopful of 24 h old culture of bacteria test antagonist was streak inoculated at either sides of *F. sacchari* disc at a distance of 2 cm apart. The pathogen culture was inoculated centrally on PDA plates, without bacterial streak served as control. Each treatment was replicated thrice in completely randomized design (CRD), and the inoculated plates were incubated at 28 ± 1 °C for 5 d and percent inhibition was calculated [15].

2.4 Evaluation of Endophytic Bacteria for Plant Growth Promoting Traits

2.4.1 Protease Activity

All bacterial isolates were screened for protease activity by inoculating on skim milk agar plates. Plates were incubated at 28 ± 1 °C for 72 h and observed for proteolysis, i.e., clear zone production around the inoculated bacterial disc.

2.4.2 Qualitative Determination of Phosphate Solubilisation

Phosphate solubilisation ability of bacterial isolates was detected by inoculating them on Pikovaskaya's agar plates [16]. The inoculated plates were incubated at 28 ± 1 °C for 3 d and observed for appearance of clearing zone around the colonies.

2.4.3 Qualitative Determination of Nitrogen Fixing Ability

Nitrogen fixing ability of bacterial isolates was determined by spot inoculation of log phase culture on nitrogen free medium (Burk's medium), comprising: 10 g dextrose, 0.41 g KH_2PO_4 , 0.52 g K_2HPO_4 , 0.05 g Na_2SO_4 , 0.2 g CaCl_2 , 0.1 g MgSO_4 , 0.005 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0025 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and 20 g agar [17]. The medium was adjusted to pH 7.0.

2.4.4 Hydrogen Cyanide (HCN) Production

HCN production was determined by modified method of Bakker and Schippers [18]. Exponentially grown cultures of bacterial isolates were streaked on to Luria-Bertani agar (LB agar) plates, supplemented with 4.4 g glycine/L. Simultaneously, a filter paper

soaked in 0.5% picric acid in 1% Na_2CO_3 was placed in the upper lids of each Petri plate along with uninoculated control. The plates were sealed with parafilm and incubated at 28 ± 1 °C for 4 d and observed for colour change from yellow to brown for putative HCN production.

2.4.5 Qualitative Screening of Bacterial Isolates for Cellulolytic Activity

Cellulolytic activity of bacterial isolates was determined qualitatively by inoculating individual strains on to Bushnell Haas medium (BHM) agar plates amended with carboxy methyl cellulose (CMC), containing CMC (10.0 g/L), K_2HPO_4 (1.0 g/L), KH_2PO_4 (1.0 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.2 g/L), NH_4NO_3 (1.0 g/L), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.05 g/L), CaCl_2 (0.02 g/L) and agar (20.0 g/L). For the secretion of cellulase enzyme, CMC agar plates were incubated at 30 ± 2 °C for 5 d. After incubation, culture plates were flooded with 0.1% Congo red solution for 20 min. The stain was poured off, and the plates were washed with 1 M NaCl for 15 min. A clear zone formation around the bacterial colonies indicates the hydrolysis of CMC [19].

2.4.6 Qualitative Test for IAA Production

A modified agar plate assay was used for qualitative estimation of IAA production by *Pseudomonas* isolates [20]. LB agar plate containing 100 µg/mL of tryptophan was prepared and poured in Petri plates. After solidification, cavities of 5 mm diameter and 0.2 cm depth were made by a sterile cork borer. Each cavity was filled with 50 µL of overnight grown culture and incubated at 30 °C for 24 h. After overnight growth, the cavities were filled with two drops of Salkowski reagent. The development of pink colour after addition of Salkowski reagent was considered positive for IAA production.

2.5 Distribution of Antimicrobial Peptide (AMP) Genes in Bacterial Strains

To study the distribution of three AMP genes (bacylisin, *bacA*; fengycin, *fenD* and bacillomycin,

bmyB) in bacterial isolates obtained from sugarcane tissue culture plantlets, PCR was carried out in a total volume of 25 µL, containing 1× PCR Buffer, 1.5 mM MgCl₂, 0.2 mM dNTP, 0.2 µM of each primer, 2.0 U of Taq DNA polymerase and 2.5 µL of genomic DNA. The cycling conditions for the amplification of all targets were as follows: 95 °C for 4 min, 40 cycles of 94 °C for 1 min, annealing temperature for 1 min and 70 °C for 1 min. The annealing temperatures were set to 58 °C for *bacA* and *fenD*, 55 °C for *bmyB* [21]. The oligonucleotide primers used to detect genetic markers in bacterial endophytes are given in Table 1. Amplifications were carried out in a thermal cycler and the amplified products were analyzed in a 1.5% agarose gel, and the gel images were captured with an imaging system.

3. Results

3.1 Isolation and Identification of Endophytic Bacteria

All bacterial isolates isolated from the tissue culture plantlets of sugarcane cultivars, 87A298 and 2009A107, were found to be gram positive, rod shaped with opaque to white and irregular edged colonies on LB agar. The endophytic bacterial isolates were identified as bacteria of the genera *Bacillus* and *Paenibacillus* by amplification of 16S rDNA genes using universal primers, FGPS 6-63 and FGPL 132-38 [13]. Comparison of 16S rDNA amplified genes to sequences of GenBank has shown identity to *Bacillus amyloliquefaciens* (KY354250), *B. subtilis* (KY354251), *B. cereus*, *B. safensis* (KY354253), *B. siamensis* (KY354252 and KY354254), *B. aryabhattai*, *B. flexus*, *B. velezensis* and *Paenibacillus pabuli*.

3.2 Antagonistic Activity against *Fusarium sacchari*

The endophytic bacterial isolates were tested for their antagonistic activity against *F. sacchari* by dual culture method. Most of the endophytic bacteria tested have inhibited the mycelial growth of *F. sacchari* under *in vitro* conditions (Table 2). The highest inhibition (89.29%) of the test pathogen was recorded with *B. velezensis* followed by *B. subtilis* SE2 and *B. amyloliquefaciens* SE1 with an inhibition of 87.30% and 85.91%, respectively. These were followed by the endophytic bacteria *B. subtilis* SE3 (79.36%), *B. siamensis* SE16 (75.60%), *B. amyloliquefaciens* SE7 (73.41%) and *B. siamensis* SE4 (70.44%). The least inhibition of mycelia growth of *F. sacchari* was recorded with *B. flexus* (17.46%).

3.3 Qualitative Analysis of Endophytic Bacteria for Growth Promoting Traits

Screening of endophytic bacteria for growth promoting traits has revealed that some of the endophytic bacterial strains could produce protease, cellulase, HCN and IAA. Few strains were found to have the ability to solubilize phosphate, and nine isolates were found to be diazotrophs. The results pertaining to biochemical characteristics of endophytic bacteria were given in Table 3.

3.3.1 Protease Production

Protease production was observed in *B. amyloliquefaciens* SE1, *B. aryabhattai* SE6, *B. amyloliquefaciens* SE7, *B. cereus* SE8, *B. subtilis* SE9 and *B. safensis* SE10, as evidenced by the production of clear halo around the inoculated bacterial colonies on skim milk agar.

Table 1 Oligonucleotide primers to detect genetic markers in *Bacillus* species [21].

S. No.	Primer	Expression protein	Sequence (5'-3')
1	BACF BACR	Bacylisin	CAGCTCATGGGAATGCTTTT CTCGGTCCTGAAGGGACAAG
2	FENDF FENDR	Fengycin	GGCCCGTTCTCTAAATCCAT GTCATGCTGACGAGAGCAAA
3	BMYBF BMYBR	Bacillomycin	GAATCCCGTTGTTCTCCAAA GCGGGTATTGAATGCTTGTT

Table 2 *In vitro* antagonism of endophytic bacterial isolates against *F. sacchari*.

S. No.	Bacterial isolate	Percent of inhibition over control (%) [*]
1	<i>B. amyloliquefaciens</i> SE1	85.91 (67.93)
2	<i>B. subtilis</i> SE2	87.30 (69.11)
3	<i>B. subtilis</i> SE3	79.36 (62.97)
4	<i>B. siamensis</i> SE4	70.44 (57.04)
5	<i>P. pabuli</i> SE5	59.52 (50.47)
6	<i>B. aryabhattai</i> SE6	32.54 (34.76)
7	<i>B. amyloliquefaciens</i> SE7	73.41 (58.94)
8	<i>B. cereus</i> SE8	26.79 (31.15)
9	<i>B. subtilis</i> SE9	66.67 (54.72)
10	<i>B. safensis</i> SE10	35.53 (35.36)
11	<i>Paenibacillus</i> spp. SE11	32.34 (34.64)
12	<i>B. megaterium</i> SE12	29.17 (32.67)
13	<i>B. aryabhattai</i> SE13	30.75 (33.66)
14	<i>B. flexus</i> SE14	17.46 (24.66)
15	<i>B. velezensis</i> SE15	89.29 (70.87)
16	<i>B. siamensis</i> SE16	75.60 (60.38)
	Critical difference ($p = 0.05$)	1.67
	SE(M) $\pm$	0.57
	Coefficient of variation (CV)	2.04

^{*} Figures in parenthesis are the arc sine transformed values.

Table 3 Plant growth promoting traits of endophytic bacteria isolated from tissue culture plantlets of sugarcane.

S. No.	Bacterial isolate	Protease	Phosphate solubilizing ability	Growth on N-free medium	Cellulase	HCN production	IAA production
1	<i>B. amyloliquefaciens</i> SE1	++	+	+	+++	+	-
2	<i>B. subtilis</i> SE2	-	-	+	-	+	+
3	<i>B. subtilis</i> SE3	-	-	+	-	+	+
4	<i>B. siamensis</i> SE4	-	+	+	-	+	-
5	<i>P. pabuli</i> SE5	-	-	-	-	-	-
6	<i>B. aryabhattai</i> SE6	+	-	-	-	-	-
7	<i>B. amyloliquefaciens</i> SE7	+	-	+	+++	+	+
8	<i>B. cereus</i> SE8	+	-	-	-	-	-
9	<i>B. subtilis</i> SE9	+	-	+	-	+	+
10	<i>B. safensis</i> SE10	++	-	+	++	+	+
11	<i>P. pabuli</i> SE11	-	-	-	-	-	+
12	<i>B. megaterium</i> SE12	-	-	-	+	-	-
13	<i>B. aryabhattai</i> SE13	-	-	-	++	+	-
14	<i>B. flexus</i> SE14	-	-	-	-	-	-
15	<i>B. velezensis</i> SE15	-	-	+	-	+	+
16	<i>B. siamensis</i> SE16	-	-	+	++	+	-

+: Positive reaction; -: negative reaction.

In case of protease, phosphate solubilising and cellulose activities, the size of the halo has been denoted as +, ++ and +++ based on the halo diameter (+: diameter of the halo up to 30 mm, ++: diameter of the halo from 31 mm to 50 mm, +++: diameter of the halo above 50 mm).

3.3.2 Phosphate Solubilization

Only few isolates could solubilize phosphate when inoculated on Pikovaskaya's agar. Clear halos were

produced by *B. amyloliquefaciens* SE1 and *B. siamensis* SE4; 48 h post inoculation onto Pikovaskaya's agar showed their ability to solubilize

tri-calcium phosphate.

3.3.3 Growth on N-Free Medium

Nine endophytic bacterial isolates have shown their ability to grow on Burk's medium and were found to be free living diazotrophs. Most of the *Bacillus* species, viz., *B. amyloliquefaciens*, *B. subtilis*, *B. siamensis*, *B. safensis* and *B. velezensis* were found to be diazotrophs.

3.3.4 Cellulase Production

To indicate the cellulase activity of the endophytic bacterial isolates, diameter of clear zone around the colony on Congo red agar media was measured. A total of six bacterial isolates was found to be positive on screening media producing clear zone. *B. amyloliquefaciens* SE1 and SE7 exhibited the maximum zone of clearance around the colony with diameter of over 50 mm. The isolates, *B. safensis* SE10, *B. aryabhattai* SE13 and *B. siamensis* SE16 were found to be moderate producers of cellulase with a zone of clearance between 31 mm and 50 mm. However, the zone of clearance on Congo red agar was less than 30 mm in case of *B. megaterium* SE12.

3.3.5 HCN Production

Most of the isolates have produced HCN as evidenced by the change in the colour of filter paper

from yellow to brown when grown on LB agar supplemented with glycine. HCN production was not observed in *P. pabuli* SE5, *B. aryabhattai* SE6, *B. cereus* SE8, *Paenibacillus* spp. SE11, *B. megaterium* SE12 and *B. flexus* SE14.

3.3.6 IAA Production

Only few isolates have produced IAA on LB agar amended with tryptophan. In agar plate assay, pink zone was observed around the cavities inoculated with the endophytic bacterial strains, *B. subtilis* SE2, *B. subtilis* SE3, *B. amyloliquefaciens* SE7, *B. subtilis* SE9, *B. safensis* SE10, *Paenibacillus* spp. SE11 and *B. velezensis* SE15, on addition of Salkowski reagent, indicating the ability to produce IAA in the presence of tryptophan.

3.4 Distribution of AMP Genes

The presence of AMP biosynthetic genes *bacA*, *fenD* and *bmyB* was examined in 16 sugarcane endophytic bacterial isolates. The distribution of AMP genes varied among the endophytic bacteria (Table 4). The genes, *bacA* and *bmyB* were the most frequent genes, and the gene *fenD* was less frequent. The isolates containing all the three genes accounted for 43.75%.

Table 4 Distribution of AMP genes in bacterial endophytes of sugarcane.

S. No.	Bacterial isolate	AMP genes		
		<i>bacA</i>	<i>fenD</i>	<i>bmyB</i>
1	<i>B. amyloliquefaciens</i> SE1	-	+	+
2	<i>B. subtilis</i> SE2	+	+	+
3	<i>B. subtilis</i> SE3	+	+	+
4	<i>B. siamensis</i> SE4	+	+	+
5	<i>P. pabuli</i> SE5	-	-	+
6	<i>B. aryabhattai</i> SE6	+	-	+
7	<i>B. amyloliquefaciens</i> SE7	+	+	+
8	<i>B. cereus</i> SE8	+	-	+
9	<i>B. subtilis</i> SE9	+	+	+
10	<i>B. safensis</i> SE10	+	-	+
11	<i>P. pabuli</i> SE11	+	-	+
12	<i>B. megaterium</i> SE12	+	+	-
13	<i>B. aryabhattai</i> SE13	+	-	-
14	<i>B. flexus</i> SE14	+	-	-
15	<i>B. velezensis</i> SE15	+	+	+
16	<i>B. siamensis</i> SE16	+	+	+

4. Discussion

In the present study, bacteria present in the tissue culture plantlets of two sugarcane clones was isolated, characterized and tested for their antagonism and plant growth promoting traits. The procedure for production of tissue culture plantlets includes extensive sterilization procedures, which are generally expected to limit the viability of microorganisms [22]. However, several bacteria were found to colonize roots, shoot and leaves of *in vitro* tissue culture plantlets without any deleterious effect on their growth. Several studies have proved the association of endophytic bacteria with tissue culture plantlets or embryonic suspension cultures of banana [22, 23]. The endophytic nature of the isolated bacteria was confirmed by plating the tissue culture medium (in which the tissue culture plantlets were grown) onto nutrient agar. No bacteria could be recovered from the inoculated plates.

The results showed that most of the bacteria inhabiting tissue culture plantlets of the sugarcane cultivars, 87A298 and 2009A107, are of *Bacillus* and *Paenibacillus* species. Endophytic bacteria reside within host plants without any pathogenic effects, and various endophytes have been found to functionally benefit plants by suppressing plant diseases [24]. Several *Bacillus* species were documented as endophytes of cotton, maize [25], banana [26] and rice [27].

In the present study, *B. velezensis*, *B. subtilis* and *B. amyloliquefaciens* were highly antagonistic to *F. sacchari*. The antagonistic potential of endophytic *Bacillus* species against *Fusarium* species was reported earlier. Three endophytic *Bacillus* species, viz., *B. amyloliquefaciens*, *B. subtilis* subsp. *subtilis* and *B. thuringiensis*, were found highly antagonistic to *F. oxysporum* f.sp. *cubense*, the incitant of Panama disease of banana [26]. Similarly, endophytic *Bacillus* species (*B. cereus* str. S42, *B. tequilensis* str. SV39, *B. subtilis* str. SV41, *B. methylotrophicus* str. SV44, *B. amyloliquefaciens* subsp. *plantarum* str. SV65 and *B.*

mojavensis str. SV104) isolated from wild Solanaceae species (*Datura metel*, *Solanum nigrum*, *S. elaeagnifolium* and *Nicotiana glauca*) were found to inhibit the mycelial growth of *F. oxysporum* f.sp. *lycopercisi* by 36%-46% [28]. The antagonistic activities of *Bacillus* spp. are mainly attributed to the production of hydrolytic enzymes [29], peptide antibiotics [30] and certain volatile extracellular metabolites [31].

Many isolates of *Bacillus* produced cyanogens in the present study and inhibited the mycelial growth of *F. sacchari*. Rhizobacteria are known to produce HCN and protect plants from phytopathogenic fungi [32]. HCN has been implicated in the suppression of different plant pathogens by plant growth promoting rhizobacteria (PGPR) [33, 34]. Further, IAA production by *B. amyloliquefaciens*, *B. subtilis* and *B. safensis* under *in vitro* conditions characterized them as plant growth promoting bacteria. Plant growth promotion by *Bacillus* species was related to the production of phytohormones, like auxins [35], gibberellins [36] and cytokinins [37]. Auxin producing *B. subtilis* strain GSV 3 recovered from groundnut rhizosphere assisted plant growth promotion [38]. In present study, some isolates of *B. amyloliquefaciens* could produce auxins and solubilize phosphate under *in vitro* conditions. However, the *in vivo* effect has to be studied under greenhouse and field conditions. Talboys et al. [39] reported increased root production in *Triticum aestivum* upon seed dressing with *B. amyloliquefaciens* strain FZB42 under low environmental phosphate concentrations.

Bacillus species synthesize various types of AMPs, which have been implicated in the biological control of several plant pathogens. Bacilysin, bacillomycin, fengycin, iturin, surfactin and subtilin are some of the AMPs elaborated by *Bacillus* species and are known to enhance their biocontrol efficacy, as well as fitness in plant environment [21]. Formation of pores in the cell wall and cell membranes of fungi and their subsequent disruption are one of the proposed modes

of action of AMPs [40]. The antagonistic activity of endophytic bacteria isolated from tissue culture plantlets against *F. sacchari* may be attributed to the production of AMPs.

5. Conclusions

Most of the bacteria isolated from tissue culture plantlets of sugarcane are gram positive (*Bacillus* spp.) with an ability to produce protease, cellulase, IAA and HCN, besides their antagonistic potential against *F. sacchari*. Overall, the results of present study suggested the scope and potentiality of sugarcane endophytic bacteria, isolated from tissue culture plantlets, in promoting plant growth and suppression of sugarcane pathogens. However, extensive greenhouse and field studies are essential to utilize these endophytic bacteria for eco-friendly management of sugarcane wilt disease.

References

- [1] Government of India (GOI). 2015. *Price Policy for Sugarcane: 2016-17 Sugar Season*. Commission for Agricultural Crops and Prices. Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, New Delhi, 80. <http://cacp.dacnet.nic.in/ViewReports.aspx?Input=2&PageId=41&KeyId=576>.
- [2] Government of India (GOI). 2017. *Price Policy for Sugarcane: 2017-18 Sugar Season*. Commission for Agricultural Crops and Prices. Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Government of India. <http://cacp.dacnet.nic.in/ViewReports.aspx?Input=2&PageId=41&KeyId=595>.
- [3] Omarjee, J., Antwerpen, T. V., Balandreau, J., Kuniata, L., and Rutherford, S. 2004. "Isolation and Characterization of Some Endophytic Bacteria from Papua New Guinea Sugarcane." *Proc. S. Afr. Technol. Ass.* 78: 189-94.
- [4] Azeredo, L. A. I., Gomes, E. A. T., Mendonca-Hagler, L. C., and Hagler, A. N. 1998. "Yeast Communities Associated with Sugarcane in Campos, Rio de Janeiro, Brazil." *Internat. Microbiol.* 1 (3): 205-8.
- [5] Mendes, R., Pizzirani-Kleiner, A. A., Araujo, W. L., and Raaijmakers, J. M. 2007. "Diversity of Cultivated Endophytic Bacteria from Sugarcane: Genetic and Biochemical Characterization of *Burkholderia cepacia* Complex Isolates." *Appl. Environ. Microbiol.* 73 (22): 7259-67.
- [6] Favaro, L. C., Sebastianes, F. L., and Araujo, W. L. 2012. "*Epicoccum nigrum* P16, a Sugarcane Endophyte, Produces Antifungal Compounds and Induces Root Growth." *PLoS ONE* 7 (6): e36826.
- [7] Sinma, K., Nurak, T., and Khucharoenphaisan, K. 2015. "Potentiality of Endophytic Actinomycetes Isolated from Sugarcane." *KMITL Sci. Tech. J.* 15 (2): 88-97.
- [8] Beneduzi, A., Ambrosini, A., and Passagila, L. M. P. 2012. "Plant Growth Promoting Rhizobacteria (PGPR): Their Potential as Antagonists and Biocontrol Agents." *Genet. Mol. Biol.* 35 (4): 1044-51.
- [9] Muangthong, A., Youpensuk, S., and Rerkasem, B. 2015. "Isolation and Characterization of Endophytic Nitrogen Fixing Bacteria in Sugarcane." *Trop. Life Sci. Res.* 26: 41-51.
- [10] Tam, H. M., and Diep, C. N. 2014. "Isolation, Characterization and Identification of Endophytic Bacteria in Sugarcane (*Saccharum* spp. L.) Cultivated on Soils of the Dong Nai Province, Southeast of Vietnam." *Am. J. Life Sci.* 2 (6): 361-8.
- [11] Dong, Z., Canny, M. J., McCully, M. E., Roboredo, M. R., Cabadilla, C. F., Ortega, E., and Rhodes, R. 1994. "A Nitrogen-Fixing Endophyte of Sugarcane Stems." *Plant Physiol.* 105 (4): 1139-47.
- [12] Sambrook, J., and Russel, D. W. 2001. *Molecular Cloning: A Laboratory Manual*. 3rd ed.. New York: Cold Spring Harbor Laboratory Press.
- [13] Normand, P., Courmoyer, B., Simonet, P., and Nazaret, S. 1992. "Analysis of a Ribosomal RNA Operon in the Actinomycete *Frankia*." *Gene* 111 (1): 119-24.
- [14] Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. *Fusarium Species: An Illustrated Manual for Identification*. University Park: Pennsylvania State University Press, 193.
- [15] Dennis, C., and Webster, J. 1971. "Antagonistic Properties of Species Groups of *Trichoderma*: III. Hyphal Interactions." *Trans. Br. Mycol. Soc.* 57 (3): 363-9.
- [16] Pikovaskaya, R. I. 1948. "Mobilization of Phosphorous and Soil in Connection with the Vital Activity of Some Microbial Species." *Mikrobiologii* 17: 362-70.
- [17] Wilson, P. W., and Knight, S. C. 1952. *Experiments in Bacterial Physiology*. Minneapolis, USA: Burgess, 49.
- [18] Bakker, A. W., and Schippers, B. 1987. "Microbial Cyanides Production in the Rhizosphere in Relation to Potato Yield Reduction and *Pseudomonas* spp. Mediated Plant Growth Stimulation." *Soil Microbiol. Biochem.* 19: 451-7.
- [19] Emmyrafedziawati, A. K. R., and Stella, M. 2015. "Hydrolysis of Carboxy Methyl Cellulose (CMC) by *Bacillus* Isolated from Compost." *J. Trop. Agric. and Fd. Sc.* 43 (2): 129-35.
- [20] Shrivastava, U. P., and Kumar, A. 2011. "A Simple and

- Rapid Plate Assay for the Screening of Indole-3-Acetic Acid (IAA) Producing Microorganisms." *Int. J. Appl. Biol. Pharm.* 2 (1): 120-3.
- [21] Mora, I., Cabrefiga, J., and Montesinos, E. 2011. "Antimicrobial Peptide Genes in *Bacillus* Strains from Plant Environments." *Int. Microbiol.* 14 (4): 213-23.
- [22] Thomas, P. 2004. "A Three-Step Screening Procedure for Detection of Covert and Endophytic Bacteria in Plant Tissue Cultures." *Curr. Sci.* 87 (1): 67-72.
- [23] Jimtha, J. C., Smitha, P. V., Anisha, C., Deepthi, T., Meekha, G., Radhakrishnan, E. K., Gayathri, G. P., and Remakanthan, A. 2014. "Isolation of Endophytic Bacteria from Embryogenic Suspension Culture of Banana and Assessment of Their Plant Growth Promoting Properties." *Plant Cell Tiss. Org.* 118: 57-66.
- [24] Lian, J., Wang, Z., and Zhou, S. 2008. "Response of Endophytic Bacterial Communities in Banana Tissue Culture Plantlets to Fusarium Wilt Pathogen Infection." *J. Gen. Appl. Microbiol.* 54 (2): 83-92.
- [25] McInroy, J. A., and Kloepper, J. W. 1995. "Survey of Indigenous Bacterial Endophytes from Cotton and Sweet Corn." *Plant Soil* 173: 337-42.
- [26] Souza, A., Cruz, J. C., Susa, N. R., Procopio, A. R. L., and Silva, G. F. 2014. "Endophytic Bacteria from Banana Cultivars and Their Antifungal Activity." *Genet. Mol. Res.* 13 (4): 8661-70.
- [27] Chung, E. J., Hossain, M. T., Khan, A., Kim, K. H., Jeon, C. O., and Chung, Y. R. 2015. "*Bacillus oryzicola* sp. nov., an Endophytic Bacterium Isolated from the Roots of Rice with Antimicrobial, Plant Growth Promoting, and Systemic Resistance Inducing Activities in Rice." *Plant Pathol. J.* 31 (2): 152-64.
- [28] Aydi Ben Abdallah, R., Jabnoun-Khiareddinen, H., Mokni-Tlili, S., Nefzi, A., Medimagh-Saidana, S., and Daami-Remadi, M. 2015. "Endophytic *Bacillus* spp. from Wild Solanaceae and Their Antifungal Potential against *Fusarium oxysporum* f.sp. *lycopercisi* Elucidated Using Whole Cells, Filtrate Cultures and Organic Extracts." *J. Plant Pathol. Microbiol.* 6: 324. doi:10.4172/2157-7471.1000324.
- [29] Fujimoto, A., and Kupper, K. C. 2016. "Production of Antifungal Compounds and Hydrolytic Enzymes by *Bacillus* spp. as Mechanisms of Action against *Phyllosticta citricarpa*." *IOSR-JAVS* 9 (11): 19-27.
- [30] Mannanov, R. N., and Sattarova, R. K. 2001. "Antibiotics Produced by *Bacillus* Bacteria." *Chem. Nat. Compd.* 37 (2): 117-23.
- [31] Podile, A. R., Prasad, G. S., and Dube, H. C. 1987. "Partial Characterization of the Antagonistic Principle of *Bacillus subtilis* AF1." *J. Biol. Control* 1: 60-5.
- [32] Ahmad, F., Ahmad, I., and Khan, M. S. 2008. "Screening of Free-Living Rhizospheric Bacteria for Their Multiple Growth Promoting Activities." *Microbiol. Res.* 163: 173-81.
- [33] Reetha, A. K., Pavani, S. L., and Mohan, S. 2014. "Hydrogen Cyanide Production Ability by Bacterial Antagonist and Their Antibiotics Inhibition Potential on *Macrophomina phaseolina* (Tassi.) Gold." *Int. J. Curr. Microbiol. Appl. Sci.* 3 (5): 172-8.
- [34] Lanteigne, C., Gadkar, V. J., Wallon, T., Novinsack, A., and Fillion, M. 2012. "Production of DAPG and HCN by LBUM300 Contributes to the Biological Control of Bacterial Canker of Tomato." *Phytopathology* 102 (10): 967-73.
- [35] Asghar, H., Zahir, Z., Arshad, M., and Khaliq, A. 2002. "Relationship between *in Vitro* Production of Auxins by Rhizobacteria and Their Growth-Promoting Activities in *Brassica juncea* L." *Biol. Fert. Soils* 35: 231-7.
- [36] Joo, G. J., Kim, Y. M., Kim, J. T., Rhee, I. K., Kim, J. H., and Lee, I. J. 2005. "Gibberellins Producing Rhizobacteria Increase Endogenous Gibberellins Content and Promote Growth of Red Peppers." *J. Microbiol.* 43: 510-5.
- [37] De Salamone, I. E. G., Hynes, R. K., and Nelson, L. M. 2001. "Cytokinin Production by Plant Growth Promoting Rhizobacteria and Selected Mutants." *Can. J. Microbiol.* 47: 404-11.
- [38] Thakur, A., and Parikh, S. C. 2015. "Auxin Hormone Production and Plant Growth Promotion by Phosphate Solubilizing Bacteria of Groundnut Rhizosphere." *Int. J. Innov. Res. Sci. Eng. Technol.* 4 (9): 8539-48.
- [39] Talboys, P. J., Owen, D. W., Healey, J. R., Withers, P. J., and Jones, D. L. 2014. "Auxin Secretion by *B. amyloliquefaciens* FZB42 Both Stimulates Root Exudation and Limits Phosphorous Uptake in *Triticum aestivum*." *BMC Plant Biol.* 14 (51): 1-9.
- [40] Sumi, C. D., Yang, B. W., Yeo, I. C., and Hahm, Y. T. 2015. "Antimicrobial Peptides of the Genus *Bacillus*: A New Era for Antibiotics." *Can. J. Microbiol.* 61 (2): 93-103.