

Research Article

Plant Growth Promoting of Endophytic *Sporosarcina aquimarina* SjAM16103 Isolated from the Pneumatophores of *Avicennia marina* L.

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Endophytic *Sporosarcina aquimarina* SjAM16103 was isolated from the inner tissues of pneumatophores of mangrove plant *Avicennia marina* along with *Bacillus* sp. and *Enterobacter* sp. Endophytic *S. aquimarina* SjAM16103 was Gram variable, and motile bacterium measured 0.6–0.9 μm wide by 1.7–2.0 μm long and light orange-brown coloured in 3-day cultures on tryptone broth at 26°C. Nucleotide sequence of this strain has been deposited in the GenBank under accession number GU930359. This endophytic bacterium produced 2.37 $\mu\text{Mol/mL}$ of indole acetic acid and siderophore as its metabolites. This strain could solubilize phosphate molecules and fix atmospheric nitrogen. Endophytic *S. aquimarina* SjAM16103 was inoculated into four different plants under *in vitro* method to analyse its growth-promoting activity and role inside the host plants. The growth of endophytic *S. aquimarina* SjAM16103 inoculated explants was highly significant than the uninoculated control explants. Root hairs and early root development were observed in the endophytic *S. aquimarina* SjAM16103 inoculated explants.

1. Introduction

The genus *Sporosarcina*, which belongs to the family Bacillaceae, was created by Kluver and van Neil [1] to accommodate bacteria that have spherical or oval-shaped cells, low DNA G+C content (40–42 mol%), and MK-7 as the major menaquinone. *Sporosarcina* species can be differentiated from other members of the Bacillaceae, by their coccoid or rod-shaped cells, motility, sporulation, and possession of MK-7 as the major menaquinone and A4 α as the peptidoglycan variant. The genus *Sporosarcina* originally comprised two species, *S. ureae* and *S. halophila* [2, 3]. However, three species of the genus *Bacillus*, namely, *Bacillus globisporus* [4], *B. psychrophilus* [5], and *B. pasteurii* [6], which belonged to rRNA group 2 [7] and contain L-lysine in their cell wall, have recently been transferred to the genus *Sporosarcina* [8] as *S. globispora*, *S. psychrophila*, and *S. pasteurii* also identified a novel species, *S. aquimarina* isolated from seawater. However, there were no published reports

on endophytic *S. aquimarina* isolated from the living cells. Therefore, the present study was aimed to investigate the role and effects of endophytic *S. aquimarina* isolated from the pneumatophores of *A. marina*.

Endophytic bacteria are living inside the plant tissue without eliciting symptoms of disease, common to a large number of plant species. Endophytic bacteria can promote the plant growth and yield and can act as a biocontrol agent [9]. In recent years, much attention has been paid to natural methods of crop growing in expectation of moving toward agriculturally and environmentally sustainable development [10]. Endophytic bacteria promote plant growth due to their abilities in nitrogen fixation [11], phytohormone production [12], solubilization of phosphorus [13], and disease control [14, 15].

Endophytic microbial inocula, primarily bacteria, are used as propagule priming agents, both as *in vitro* cocultures and transplanting [16]. It is an emerging trend in biotechnological approach aimed at reducing chemical

input in plant production, while increasing plant fitness, productivity, and resistance to diseases in the context of sustainable horticulture.

In the present study, endophytic bacteria were isolated from the surface sterilized pneumatophores of *A. marina*. The isolates were identified phenotypically and genotypically. The isolate *S. aquimarina* was taken for further investigation. This endophytic bacterium was inoculated into four different plants, two fresh water plants (*Bacopa monnieri* and *Eupatorium triplinerve*) and two mangrove plants (*Excoecaria agallocha* and *A. marina*) to analyse its growth-promoting efficacy and role as endophyte. These plants were selected based on their needs in the society, because they have high medicinal and economical values. Medicinally, they are used for curing skin diseases, even for leprosy [17], HIV [18], fungal diseases [19], mental disorders [20, 21], and economically as fire wood, match boxes, paper pulp [17], and used as fodder in India and in Australia.

2. Materials and Methods

2.1. Isolation of Strains and Growth Condition. Endophytic bacterium was isolated from the inner tissues of healthy pneumatophores. Parts of pneumatophores about 1 cm of diameter were sterilized with 70% ethanol and 0.1% mercuric chloride [22]. Sterilized parts were excised with a sterile scalpel blade. Slices (0.1 cm thickness) were placed on nutrient agar plates and incubated at 26°C for 48 h. Bacterial growth associated with the pneumatophore sections was purified by repeated plating on nutrient agar, and cultures were maintained as spore suspensions by freezing in 20% (V/V) glycerol.

2.2. Phenotypic Characterization. The isolate was Gram-stained and examined microscopically for its morphological characteristics. Some set of physiological characteristics include acid production from sugars (TSI), sodium citrate utilization, urease production, starch, gelatin hydrolysis, and voges-proskauer reaction was carried out using standard protocols described by Gordon et al., [23]. Casein hydrolysis was detected after 3 days of incubation on nutrient agar supplemented with 2% skimmed milk. Growth in the presence of NaCl (2%, 3%, 5%, 7%, and 9%) was determined in nutrient broth as the basal medium during incubation at 28°C for 3 days. It also was estimated at selected temperatures (2, 4, 6, 8, 10, 26, 32, 40, and 50°C) and pH (4.5, 5.5, 7.0, and 9.0) in agar slants incubated at 28°C for 3 days.

2.3. 16S rRNA Gene Sequence Analysis. Genomic DNA was isolated from pure culture [24]. A large fragment (800–1100 bp) of 16S rRNA was amplified by PCR using primers 5'-TGA GGA AGA TAA TGA CGG-3' and 5'-CCCT CTA TCC TCT TTC CAA CC-3'. The 50 µL PCR reaction mixtures contained 100 ng of DNA extract, 1 × Taq reaction buffer, 20 pmol of primers, 200 µM dNTPs, and 1.5 U of Taq DNA polymerase (Promega). The thermocycling conditions consisted of an initial denaturation at 94°C for 3 mins, 30 amplification cycles of 94°C for 1 min (denaturation), 57°C for 1

minute (annealing), 72°C for 2 mins (extension), and final polymerization at 72°C for 4 mins. PCR product was purified and sequenced. Searches in the GenBank/EMBL/DDBJ/PDB data libraries were performed using BLAST (blastn) search algorithm [25] in order to establish the identity of the isolate.

2.4. Determination of Cellular Fatty Acid Composition. Cellular fatty acids composition of endophytic bacterium was analyzed using the Sherlock system (MiDi Company, USA) and according to the manufacturer's instructions.

2.5. Plant Growth Promoting Activities

2.5.1. IAA Production. Indoleacetic acid (IAA) produced by bacterium was assayed colorimetrically using FeCl₃-HClO₄ [26]. The bacteria were grown in modified nutrient broth M26 for 24 hours on a gyratory shaker (150 rpm) at room temperature as seed culture. The medium contained (in 1000 mL distilled water) 5 g NaCl, 10 g peptone, and 10 g of beef extract. After overnight incubation, 100 µL of culture was inoculated to 10 mL minimal salt (MS) medium amended with 5 mM tryptophan [27] and grown again for 48 hours on the shaker. The MS medium contained (in 1000 mL distilled water) 1.36 g KH₂PO₄, 2.13 g Na₂HPO₄, 0.2 g MgSO₄·7H₂O, and trace elements. The pH of MS medium was adjusted to 7.0 before autoclaving. L-Tryptophan solution was prepared as stock solution containing (in 100 mL distilled water) 10 g glucose, 1 g glucose, 1 g L-Tryptophan, and 0.1 g yeast extract. The stock solution was filtered through a sterile 0.2 µm membrane filter (Millipore). 1.5 mL bacterial broth culture was centrifuged at 12,000 rpm for 5 mins. One millimeter of the supernatant was added to 2 mL FeCl₃-HClO₄ reagent. After 25 mins (after color density reaches its maximum), the mixture was read in UV spectrophotometer at 530 nm absorbance. The amount of IAA produced per milliliter of culture was estimated using a standard curve. The number of bacterial population in the culture expressed in colony forming unit (CFU) was estimated by the Miles and Misra drop plate method.

2.5.2. Phosphate Solubilization. Phosphate solubilization test was conducted qualitatively by plating the bacterium in agar containing precipitated tricalcium phosphate. The medium was a modification of Pikovskaya medium [28].

2.5.3. Nitrogen Fixation. Fixation of atmospheric nitrogen by the bacterium was tested qualitatively using Burk's N-free medium [28].

2.5.4. Sulphur Reduction. Reduction of sulphur by the bacterium was tested qualitatively by sulphate API agar [28].

2.5.5. Siderophore Production. Siderophore production was tested qualitatively using chrome azurol S (CAS) agar as described by Alexander and Zuberer [29]. The bacterial culture was spread on the CAS agar plates with three replications. Orange halos around the colonies after overnight incubation indicated siderophore production.

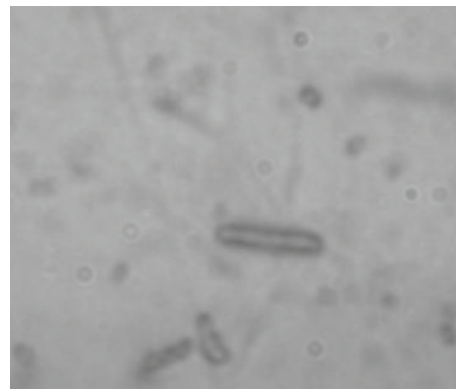
TABLE 1: Phenotypic characteristics of *Sporosarcina aquimarina* SjAM16103.

Morphological characteristics	
Shape	Bacilli (with flagellum)
Motility	Motile
Gram's staining	Gram variable
Physiological characteristics	
Temperature	
Min 2–10°C	Negative
Max 26–50°C	Positive
pH	
4.5	Negative
5.5	Negative
7.0	Positive
9.0	Positive
Salinity	
2%	Positive
3%	Positive
5%	Positive
7%	Positive
9%	Positive
Exoenzyme activities	
Starch hydrolysis	Negative
Gelatin hydrolysis	Positive
Casein hydrolysis	Negative
Endoenzyme activities	
Catalase production	Positive
Urease production	Positive
Oxidase production	Positive
Voges-proskauer	Negative

2.6. *Inoculation of Explants with Endophytic Bacterium.* Nodal segments (length: 0.5 cm) of four different plants (*Bacopa monnieri*, *Eupatorium triplinerve*, *Excoecaria agallocha*, and *Avicennia marina*) were disinfected by sonicating in water for 20 min and dipping in 70% ethanol for 1 min, followed by 25 min of sodium hypochlorite (25%)/Tween 80 (0.01%) solution and rinsed three times with distilled and sterile water [30].

The sterile explants of *Bacopa monnieri* and *Eupatorium triplinerve* were cultured in a hormone-free Murashige-Skoog (MS) medium [31] with the addition of 200 µL of endophytic *Sporosarcina aquimarina* SjAM16103. The sterile explants of *Excoecaria agallocha* and *Avicennia marina* were cultured in a hormone-free X medium (M S Swaminathan Research Foundation, India) with the addition of 200 µL of endophytic *Sporosarcina aquimarina* SjAM16103.

Then, these explants were cultured under a photoperiod of 16 h of light and 8 h of dark under an irradiance of 52 mmol m⁻² seg⁻¹. The explants without endophytic *Sporosarcina aquimarina* SjAM16103 were marked as control explants.

FIGURE 1: *Sporosarcina aquimarina* SjAM16103.

2.7. *Statistical Analysis.* The whole experiment was set up in the randomized design with 10 replicas. All the data collected from these experiments were subjected to an analysis of variance (ANOVA) using SPSS statistical tool. The significant level ($P > 0.05$) was evaluated between various growth parameters (shoot length, number of roots, and root length).

3. Results

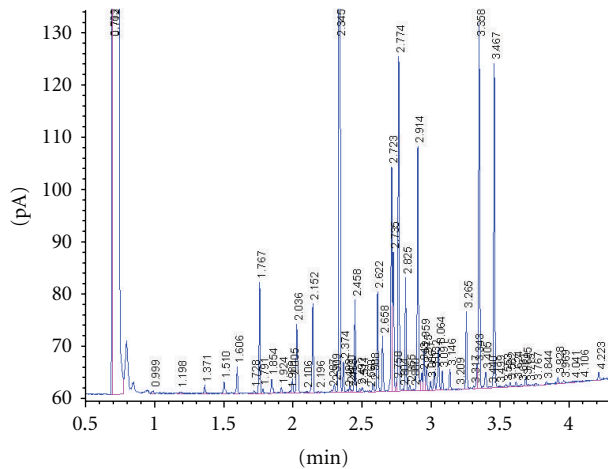
3.1. *Morphology.* During this study, 13 bacterial strains were isolated from the pneumatophores of *A. marina L.* Among them, four strains (SjAM16101, SjAM16102, SjAM16103, and SjAM16104) were genotypically analysed as *Bacillus* sp., *Enterobacter* sp., *Sporosarcina aquimarina*, and *Bacillus cereus*, respectively. Strain SjAM16103 was Gram variable, and motile bacterium measured 0.6–0.9 µm wide by 1.7–2.0 µm long (Figure 1) and light orange-brown coloured in 3-day cultures on tryptone broth at 26°C.

3.2. *Phenotypic Characteristics.* Phenotypic characteristics of strain SjAM16103 are given in Table 1. The optimal growth temperature was 32°C. Strain SjAM16103 grew at 26–50°C but not at 2–10°C. The optimal pH for growth was 7.0, and growth was inhibited at pH values below 5.0. Strain SjAM16103 grew in the presence of 2–9% NaCl. Gelatin was hydrolysed and showed catalase, urease, and oxidase activities. Acid was produced in the triple sugar iron test. Fatty acids compositions of strain SjAM16103 are given in Table 3. Gas chromatographic methyl ester profiles of strain SjAM16103 are given in Figure 2.

3.3. *16S rRNA Gene Sequences.* The 16S rRNA of strain SjAM16103 was directly sequenced following PCR amplification, and its partial nucleotide sequence was determined. The 16S rRNA sequence of strain SjAM16103 was 971 bp long (Table 2) and was identified as *Sporosarcina aquimarina* with highest similarity value of 98%. The nucleotide sequence of *S. aquimarina* SjAM16103 has been deposited in the GenBank/EMBL/DDBJ/PDB under accession number GU930359.

TABLE 2: Nucleotide sequence of *Sporosarcina aquimarina* SjAM16103.

AAGTGAGGACGCCGCTCTCGGAAGGTTAAGCTTACTACTTCTTTTGAACCCACTCCCATGGTGT
 GACGGGCGGTGTGTACAAGGCCCGGAACGTATTCACCGTGGCATTCTGATCCACGATTACTAGCG
 ATTCCGACTTCATGGAGTTCGAGTTGCAGACTCCAATCCGGACTACGACATACTTTATGAGGTCCGC
 TTGCTCTCGGAGGTCGCTTCTCTTTGTATATGCCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGG
 GCCATGATGACTTGACGTCATCCCACCTTCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCC
 GGCCGACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTACA
 ACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACGGTTCCCGAAGGACTAAGGCATCTCTGC
 CGAATTCGGTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCC
 ACCGCTTGTGCGGGCCCCGTCATTCATTTAGTTTAACTTGC GGCCGACTCCCCAGGCGGT
 CGACTTAACGCGTTAGTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTAC
 GGCGTGGACTACCAGGTATCTAATCCTGTTTGTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTT
 GTCCAGGGGGCCGCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTACCAGTACACCTGG
 AATTCACCCCCCTCTACAAGACTCAAGCCTGCCAGTTTCGAATGCAGTTCACGAGTTGAGCCC

FIGURE 2: Gas chromatographic methyl ester profiles of endophytic *Sporosarcina aquimarina* SjAM16103.

3.4. Plant Growth Promoting Activities. Endophytic *S. aquimarina* SjAM16103 produced $2.37 \mu\text{Mol/mL}$ of IAA. This strain could solubilize phosphate molecules and fix atmospheric nitrogen. Orange halos around *S. aquimarina* SjAM16103 colonies were observed. This confirmed that *S. aquimarina* SjAM16103 was a siderophore producing bacteria (Table 4).

3.5. Inoculation of Explants with *S. aquimarina* SjAM16103. Growth parameters were measured to assess the growth promotion capability of endophytic *S. aquimarina* SjAM16103 in four different explants. The numbers of roots were counted. Shoot length and root length were measured in centimeters. The measurements were recorded; for 30–35 days in fresh water plants *B. monnieri* and *E. triplinerve* and for 35–45 days in mangrove plants *E. agallocha* and *A. marina*.

3.5.1. In *B. monnieri* L. The shoot length and root length of the inoculated explants were increased day by day when compared with control explants. The length of shoot and

root of inoculated explants were higher than the control explants 30 days after incubation (Figure 3). Maximum significant ($P > 0.05$) value was observed in the shoot and root length of inoculated explants (Figure 4). Nonsignificant values were observed in control explants. The growth observed in the *S. aquimarina* SjAM16103 inoculated explants was highly significant than the control explants (Table 5). The number of roots counted in the *S. aquimarina* SjAM16103 inoculated explants was highly significant than the control explants (Figure 4).

3.5.2. In *E. triplinerve* Vahl. Growth of shoot in the inoculated explants was increased gradually, and its length was determined 5 days after incubation. The roots were developed after 3 days incubation, and their length was determined 5 days after incubation. The length of shoot and roots of *S. aquimarina* SjAM16103 inoculated explants were higher than the control explants (Figure 5). The shoot and root length of inoculated explants determined were highly significant ($P > 0.05$) than the value of control explants (Table 5).

The numbers of roots were counted with the addition of secondary roots. The numbers of roots of *S. aquimarina* SjAM16103 inoculated explants were highly significant ($P > 0.05$) than the control explants. Root hairs were observed 10 days after incubation in *S. aquimarina* SjAM16103 inoculated explants (Figure 6), whereas root hairs were absent in control explants. This observation provided special concentration for further investigation.

3.5.3. In *E. agallocha*. The shoot length of *S. aquimarina* SjAM16103 inoculated explants, and control explants, were measured 10 days after incubation. Shoot length of inoculated explants was highly significant ($P > 0.05$) than the control explants (Figure 7). Early root development was observed in the *S. aquimarina* SjAM16103 inoculated explants (Figure 8). Roots were developed 15 days after incubation, whereas in control explants roots were developed 30 days after incubation. Root length and number of roots of *S. aquimarina* SjAM16103 inoculated explants were highly significant ($P > 0.05$) than the control explants (Table 5).

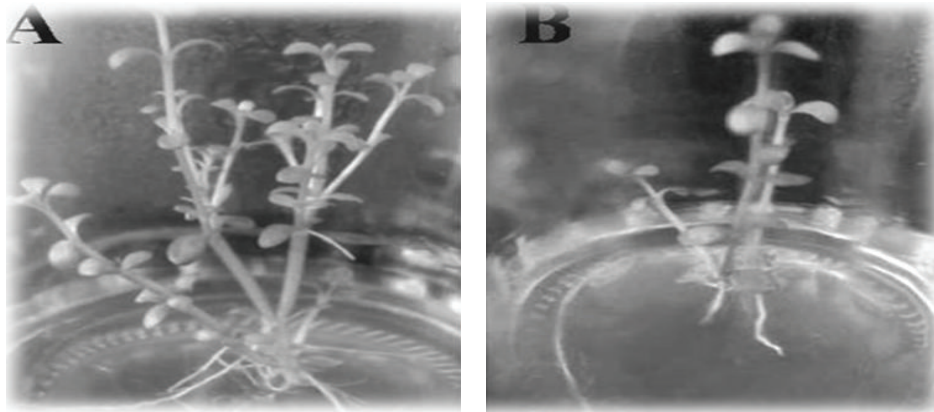


FIGURE 3: Inoculation of endophytic *S. aquimarina* SjAM16103 in *B. monnieri* (in vitro). (A) *S. aquimarina* SjAM16103 inoculated explants; (B) control explants.

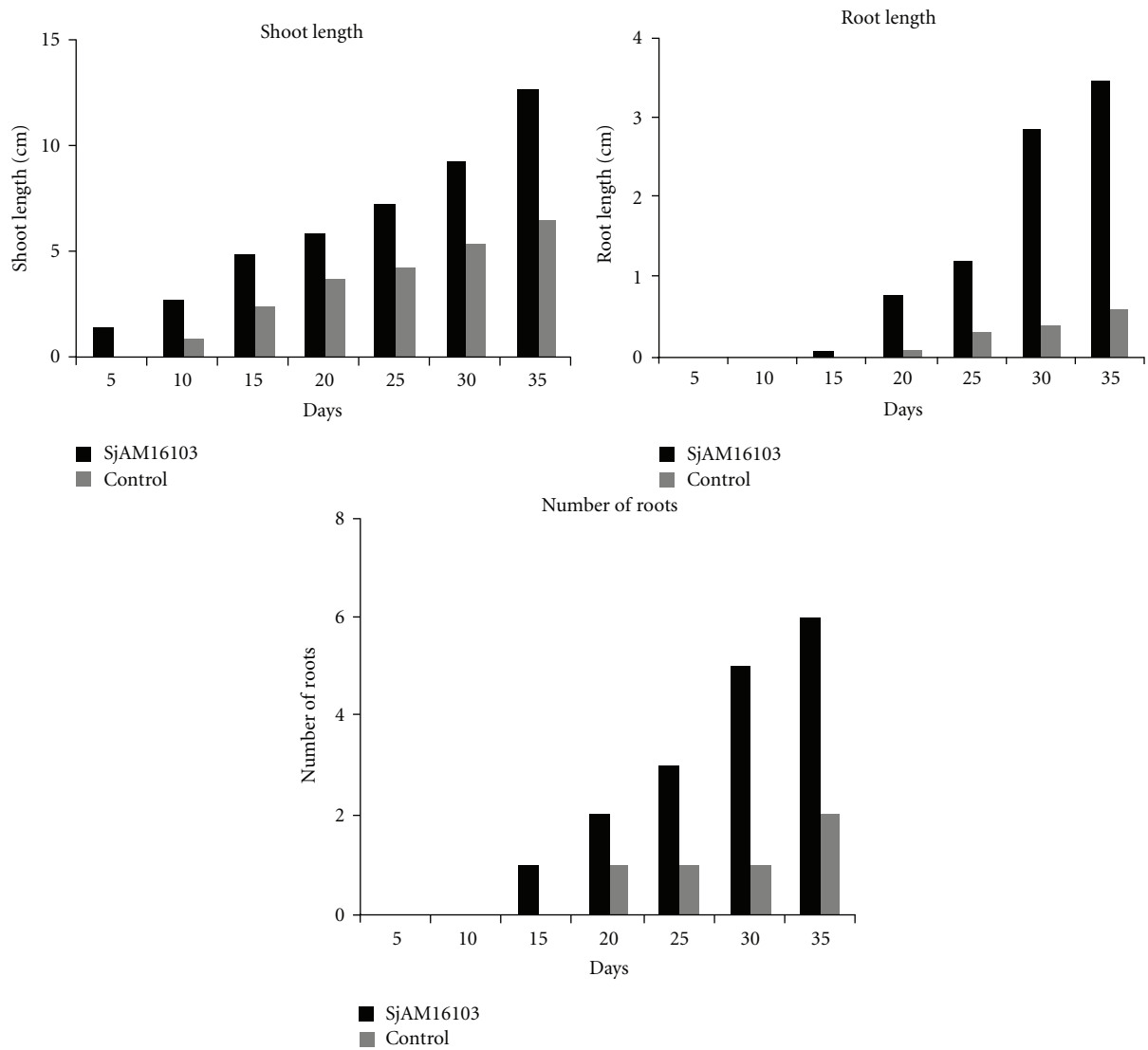


FIGURE 4: Inoculation of endophytic *S. aquimarina* SjAM16103 in *B. monnieri*.

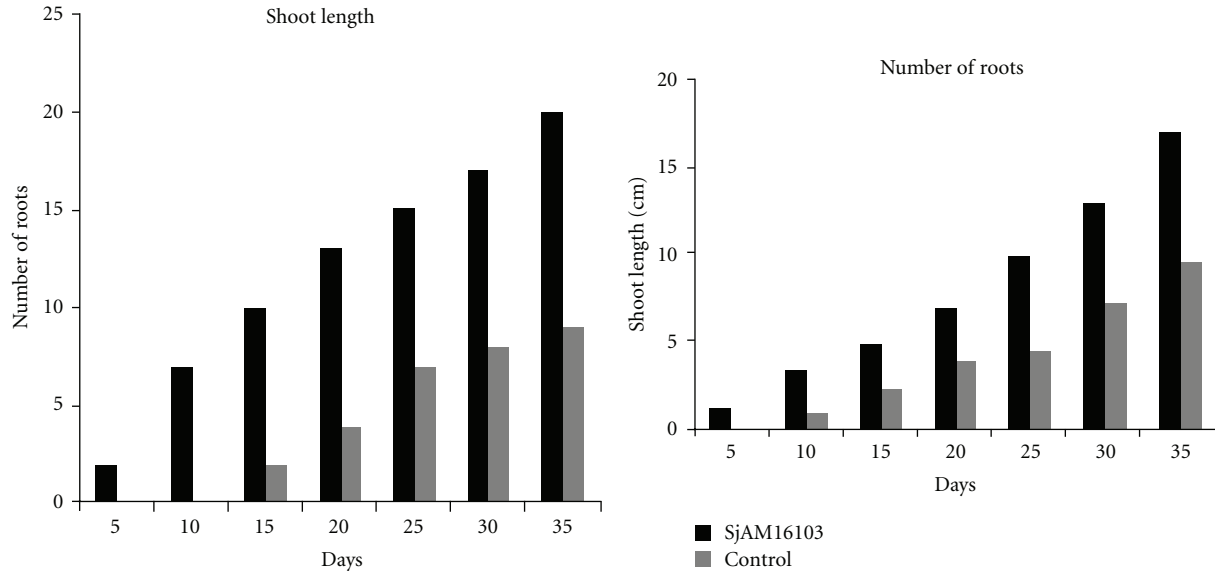


FIGURE 5: Inoculation of endophytic *S. aquimarina* SjAM16103 in *E. triplinerve*.

TABLE 3: Fatty acid composition of *Sporosarcina aquimarina* SjAM16103.

Saturated fatty acids		Unsaturated fatty acids		Branched fatty acids		Summed feature	
C _{10:0}	0.17	C _{11:0} 3OH	0.15	C _{13:0} iso	3.15	1	—
C _{12:0}	0.86	C _{12:0} 2OH	0.19	C _{13:0} anteiso	0.14	2	2.87
C _{14:0}	2.63	C _{12:0} 3OH	0.41	C _{14:0} iso	—	3	6.40
C _{15:0}	—	C _{15:0} 2OH	0.19	C _{15:0} iso	28.39	4	—
C _{16:0}	8.99	C _{15:0} 3OH	0.58	C _{15:0} anteiso	0.78	8	9.92
C _{17:0}	0.54	C _{15:1} w5	0.19	C _{15:0} iso 3OH	3.00	9	7.25
C _{18:0}	0.68	C _{15:1} w6	0.17	C _{15:1} iso F	0.07		
C _{19:0}	0.06	C _{15:1} w8	0.08	C _{15:1} anteiso A	—		
		C _{16:0} 3OH	2.15	C _{16:0} iso	1.61		
		C _{16:0} N alcohol	—	C _{16:0} iso 3OH	0.59		
		C _{16:1} w5	0.06	C _{16:1} iso H	—		
		C _{16:1} w7 alcohol	0.25	C _{17:0} iso	0.68		
		C _{16:1} w11	—	C _{17:0} anteiso	0.27		
		C _{17:0} 2OH	0.11	C _{17:0} cyclo	1.42		
		C _{17:0} 3OH	0.13	C _{17:0} iso 3OH	8.96		
		C _{17:1} w6	—	C _{17:1} iso w10	—		
		C _{17:1} w8	0.58	C _{17:1} anteiso w9	—		
		C _{18:1} w9	0.62	C _{17:1} iso w10	—		
		C _{20:1} w7	0.12	C _{19:0} cyclo w8	0.25		
		C _{20:1} w9	—				
Unknown fatty acids		11.543					

3.5.4. In *A. marina* Vierh. Shoot growth of *S. aquimarina* SjAM16103 inoculated explants was observed 5 days after incubation and measured 10 days after incubation. Whereas, in control explants, the shoot growth was observed 10 days after incubation and measured 15 days after incubation. Shoot length of *S. aquimarina* SjAM16103 inoculated explants was highly significant ($P > 0.05$) than the control

explants (Figure 9). Root growths were observed 20 days after incubation in *S. aquimarina* SjAM16103 inoculated explants (Figure 10). Early leaves were observed in the *S. aquimarina* SjAM16103 inoculated explants. Root length of *S. aquimarina* SjAM16103 inoculated explants was significantly ($P > 0.05$) higher than the root length of control explants (Table 5).

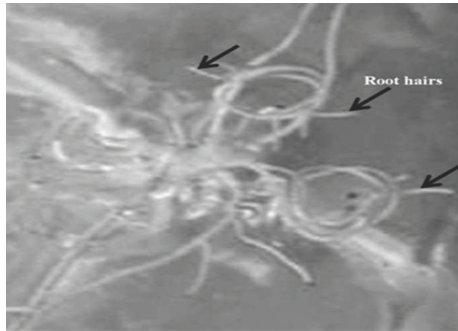


FIGURE 6: Growth of root hairs in *S. aquimarina* SjAM16103 inoculated explants of *E. triplinerve* (*in vitro*).

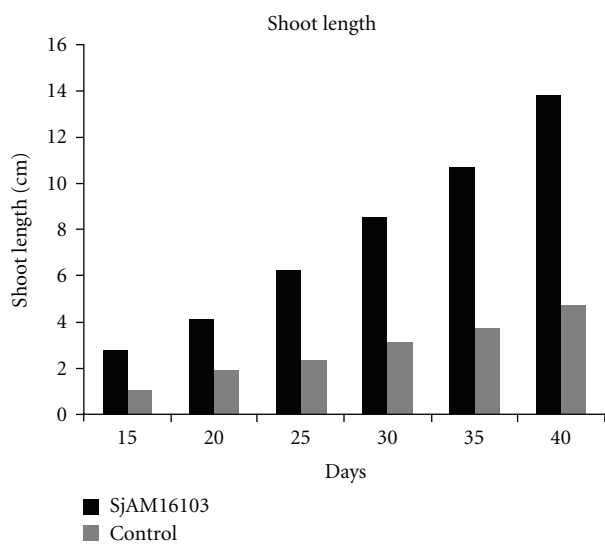


FIGURE 7: Inoculation of endophytic *S. aquimarina* SjAM16103 in *E. agallocha*.

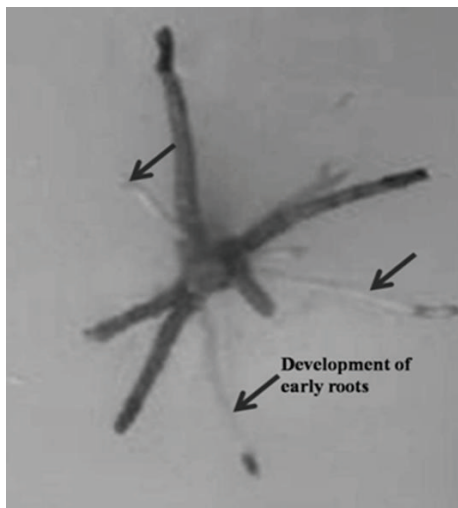


FIGURE 8: Development of early roots in *S. aquimarina* SjAM16103 inoculated explants of *E. agallocha* (*in vitro*).

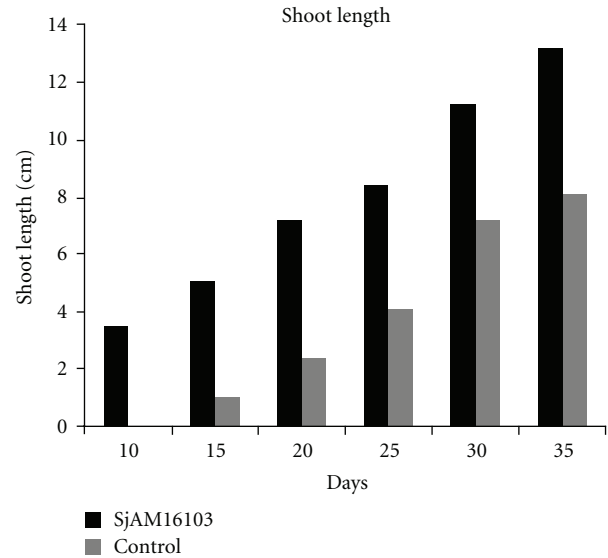


FIGURE 9: Inoculation of endophytic *S. aquimarina* SjAM16103 in *A. marina*.

4. Discussion

Endophytic bacteria are well known in crop plants [9, 32] but largely have not been investigated in mangrove plants. In the present study, thirteen bacterial strains were isolated from the pneumatophores and four strains (SjAM16101, SjAM16102, SjAM16103, and SjAM16104) were selected for further studies based on their morphology and colonization. The strain SjAM16103 was identified as *Sporosarcina aquimarina* using 16s rRNA and was confirmed using biochemical tests and fatty acids profiling. The results obtained in this research are perfectly coincide with reports on endophytes from other hosts in which generally a large number of species can be isolated from a given host, but only very few species are present in a significant number [33]. *S. aquimarina* had been isolated from seawater in Korea [8]. However, this was the first report on *S. aquimarina* isolated from the inner tissue of the plant.

The strain SjAM16103, *S. aquimarina* could produce siderophore and IAA. Siderophores are iron chelating ligands which can be beneficial to plants by increasing the solubility of ferric iron (Fe III), which otherwise is unavailable for plant nutrition [34]. The production of IAA enhances root growth of the plants by stimulating plant cell elongation or cell division [35]. The colonization of pneumatophores by endophytic *S. aquimarina* SjAM16103 enhances growth of the entire plants.

The results indicated that reintroduction of naturally occurring endophytic bacteria into tissue culture can lead to improve plant growth and yield. The present study revealed that the endophytic *S. aquimarina* SjAM16103 isolated from the pneumatophores of *A. marina* was not host specific. Some endophytic genera, however, exhibit no host specificity and are invariably recovered from plants

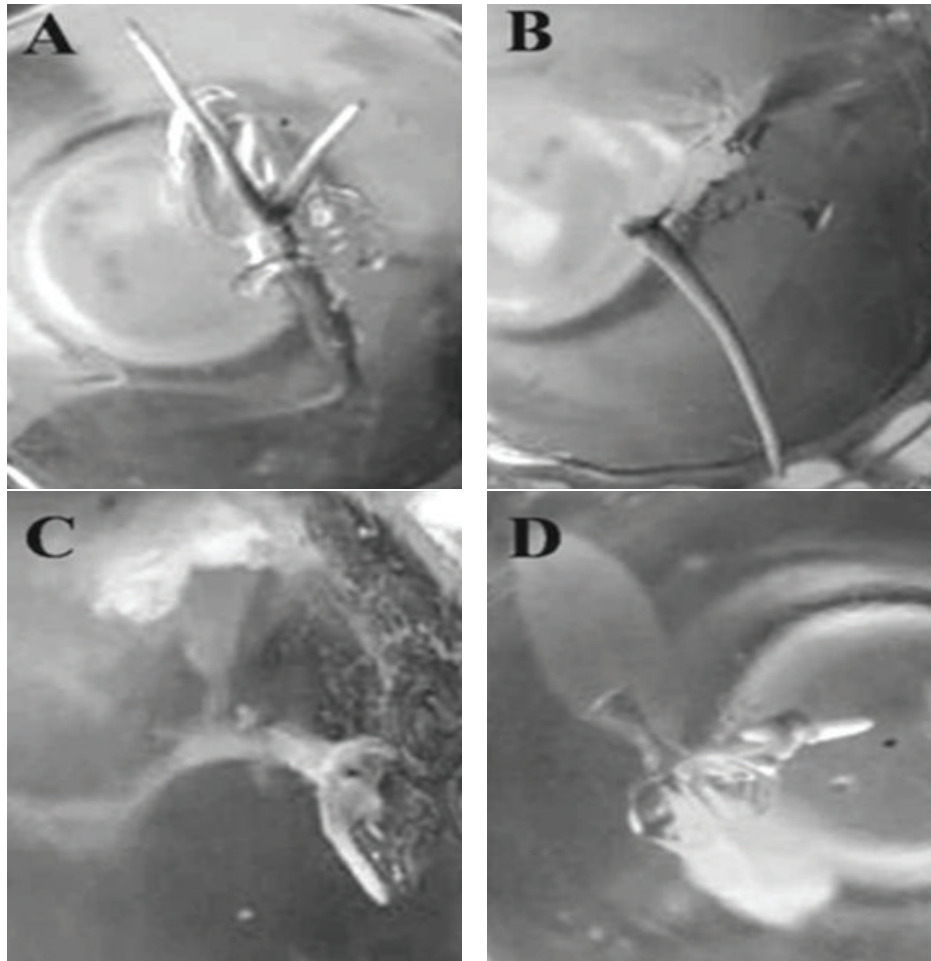


FIGURE 10: Inoculation of endophytic *S. aquimarina* SjAM16103 in *A. marina* (root growth) (*in vitro*). (A and B) Endophytic *S. aquimarina* SjAM16103 inoculated explants; (C and D) control explants.

belonging to different groups and growing in different geographical locations [36, 37].

In the present study, another interesting observation was the growth of root hairs developed in the endophytic *S. aquimarina* SjAM16103 inoculated explants, root hairs could fix atmospheric nitrogen [38]. Endophytic N_2 -fixing bacteria seem to constitute only a small proportion of total endophytic bacteria [39, 40], and increasing N_2 -fixing populations in plants has been considered as a possibility to increase nitrogen fixation. This was the first report on *S. aquimarina* isolated from the living tissue and could produce IAA and fixes atmospheric nitrogen.

This research has been directed to find endophytic *S. aquimarina* SjAM16103 that could significantly increase the yields in four different plants (*B. monnieri*, *E. triplinerve*, *E. agallocha*, and *A. marina*) after their inoculation. Growth of *S. aquimarina* SjAM16103 inoculated explants of *B. monnieri*, *E. triplinerve*, *E. agallocha*, and *A. marina* was highly significant than their control explants. The development of leaves in *S. aquimarina* SjAM16103 inoculated explants was much earlier than control explants. Leaves were developed in *S. aquimarina* SjAM16103 inoculated explants of *A. marina*

TABLE 4: Plant growth-promoting activities of *Sporosarcina aquimarina* SjAM16103.

Growth promoting activities	Result
IAA production	2.37 ($\mu\text{Mol/mL}$)
P-solubilization	Positive
S-reduction	Negative
N-fixation	Positive
Siderophore production	Positive

IAA: Indole acetic acid production; P: phosphate solubilization; S: sulphur reduction.

5 days after incubation, whereas, in control explants, leaves were developed 13 days after incubation. Inoculant seems to be successful in this micropropagated plants, as there were few or no other microorganisms with which to compete. There could be enormous benefits to be gained through the inoculation of microorganisms into soilless mixes in which plants are transplanted at an early stage in their growth. The natural condition of plants seems to be in a close interaction with endophytes.

TABLE 5: Two-way ANOVA for the differences in shoot length, root length, and number of roots of inoculated and control explants.

Plants	Shoot length	Root length	Number of roots
<i>B. monnieri</i> control explants	0.011	-0.034	-0.012
<i>B. monnieri</i> inoculated with endophytic bacterium	0.791*	0.556*	0.798*
<i>E. triplinerve</i> control explants	0.009	-0.193	-0.174
<i>E. triplinerve</i> inoculated with endophytic bacterium	0.907*	0.628*	0.904*
<i>E. agallocha</i> control explants	0.010	-0.818	-0.928
<i>E. agallocha</i> inoculated with endophytic bacterium	0.936*	0.083 [#]	0.258*
<i>A. marina</i> control explants	0.003	-0.545	-0.367
<i>A. marina</i> inoculated with endophytic bacterium	0.863*	0.074 [#]	0.279*

Highly significant ($P > 0.5$) represented by * and significant ($P = 0.5$) represented by [#].

Roots were developed earlier in the *S. aquimarina* Sjam16103 inoculated explants than the control explants. *S. aquimarina* Sjam16103 inoculated explants of *E. triplinerve* showed the growth of root hairs, whereas this characteristic feature was absent in the control explants of *E. triplinerve*. Early roots were observed in the *S. aquimarina* Sjam16103 inoculated explants of *E. agallocha*. Endophytic *S. aquimarina* Sjam16103 seems promising to increase crop yields, produced IAA and siderophore, fixed nitrogen, and solubilized phosphate. The distribution and biological activity of this endophytic bacterium deserve to be explored to make full use of their habitation inside the plants. The present study reveals that endophytic *S. aquimarina* Sjam16103 can be used as a biofertilizer, which can subsequently be used by the plant, thereby improving plant growth.

5. Conclusion

In the present study, mangrove was chosen because mangrove ecosystems are known for high productivity. At the same time, pneumatophores were chosen for this research due to its mechanisms (anaerobic respiration), which taken up gases directly from the atmosphere and various other nutrients, like iron, from the inhospitable soil. This report states that endophytic *S. aquimarina* Sjam16103 promotes the plant growth and produces plant growth promoting substances probably by means similar to plant growth-promoting rhizobacteria (PGPR). Plant growth promoting bacteria were environmentally friendly alternative to chemical fertilizers and pesticides, the use of which was regulated and sometimes forbidden, the market for bioinoculants is still expanding. Therefore, a better understanding of endophytic *S. aquimarina* Sjam16103 may help to elucidate its function and potential role more effectively in developing sustainable systems in agricultural field.

Abbreviations

μm:	Micrometer
μMol/mL:	Micromoler per milliliter
mol %:	Moler percentage
ng:	Nanogram
pMol:	Pico mole.

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