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Full Length Research Paper

Endophytic bacteria isolated from the pneumatophores of *Avicennia marina*

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Plant-associated bacteria that live inside plant tissues without causing any harm to plants are defined as endophytic bacteria. Endophytic bacteria were isolated from the surface sterilized pneumatophores of *Avicennia marina*. Three isolates were selected based on their colonization and occurrence. The isolates SjAM16101 Gram positive rods, SjAM16102 Gram negative rods and SjAM16103 Gram negative rods were identified using 16S rRNA sequencing and identified as *Bacillus* sp., *Enterobacter* sp. and *Sporosarcina aquimarina,* respectively. The nucleotide sequence has been deposited under accession number GU930357 (*Bacillus* sp.), GU930358 (*Enterobacter* sp.) and GU930359 (*S. aquimarina*). *Bacillus* sp., fixed nitrogen, *S. aquimarina* produced siderophore and all the three strains solubilized phosphate molecule.

Key words: Endophytic bacteria, pneumatophores, *Avicennia marina*, 16S rRNA sequencing.

INTRODUCTION

Plant-associated bacteria that live inside plant tissues without causing any harm to plants are defined as endophytic bacteria. Plants are constantly involved in interactions with a wide range of bacteria. These plant-associated bacteria colonize the rhizosphere (rhizobacteria), the phyllosphere (epiphytes) and the inside of plant tissues (endophytes). Endophytes are sheltered from environmental stresses and microbial competition by the host plant and they seem to be ubiquitous in plant tissues, having been isolated from flowers, fruits, leaves, stems, roots and seeds of various plant species (Kobayashi and Palumbo, 2000). High numbers of diazotrophic endophytes have been observed in the root interior of grasses, such as Kallar grass (Reinhold et al., 1986) and sugar cane (Olivares et al., 1996). They are capable of invading inner tissues including xylem vessels and are of systemic spreading (Hurek et al., 1994; James et al., 1994; Olivares et al., 1997); however, their functions for the plants are still disputed (James and Olivares, 1998; Reinhold-Hurek and Hurek, 1998).

Endophytic bacteria form a range of different relationships including symbiotic, mutualistic, commen-salistic and trophobiotic. Most endophytes appear to originate from the rhizosphere or phyllosphere; however, some may be transmitted through the seed. Some microbes appear actively to penetrate plant tissues through invading openings or wounds, as well as actively using hydrolytic enzymes, such as cellulase and pectinase. Some bacterial endophytes are believed to originate from the rhizosphere or phylloplane commu-nities through penetrating and colonizing root tissue from an access point to the xylem. Endophytic bacteria can promote plant growth and yield and can act as biocontrol agents. Endophytes can also be beneficial to their host by producing a range of natural products that could be harnessed for potential use in medicine, agriculture or industry. In addition to their beneficial effects on plant growth, endophytes have considerable biotechnological potential to improve the applicability and efficiency of phytoremediation (the use of plants and their associated microorganisms to remediate a site).

The microbe-plant interaction in the rhizosphere can be beneficial, neutral, variable or deleterious for plant growth, whereas other microbes support their hosts (Welbaum et al., 2004; Raaijmakers et al., 2008). The rhizosphere is well known to host a variety of Plant growth promoting bacteria (PGPB) which can promote

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plant growth directly or indirectly. Indirect promotion of plant growth is through antibiosis via antibiotic type compounds, through enhanced resistance to pathogenic diseases and or abiotic stresses such as frost damage. It is in the understanding of these mechanisms that promising alternatives for new inoculants are being discovered. Direct methods of increasing plant growth are through phytohormones, such as auxin, cytokinin and gibberellin. Potential growth-promoting metabolites were (i) phosphate solubilizing agents, (ii) siderophores capable of converting ferric iron to soluble forms, and (iii) indole-3-acetic acid (IAA) and auxin analogues.

Mangroves are known for several byproducts of medicinal value and economic importance. Medicinal use of leaves, stems, barks, roots, and fruits of mangrove plant species have been documented by Bandaranayake (1995; 1998). *Avicennia marina*, commonly known as grey mangrove or white mangrove, is a species of mangrove tree classified in the plant family Avicenniaceae (formerly in the Verbenaceae). As with other mangroves, it occurs in the intertidal and core zones of estuarine areas. The high salt tolerance of *A. marina* is a consequence of water use efficiency, which balances the relation between carbon gain, water loss, and ion uptake with the transpiration stream on a low but constant level. As with other *Avicennia* species, it has aerial roots (pneumatophores); these grow to a height of about 20 centimetres, and a diameter of one centimetre. Pneumatophores are originated from an underground cable root. These allow the plant to absorb oxygen, which is deficient in its habitat because of muddy areas. These roots also anchor the plant during the frequent inundation of seawater in the soft substrate of tidal systems.

The only report stated that the genus *Rivularia* (heterocystous cyanobacterium) was commonly occurring on mangrove pneumatophores (Charles and Birgitta, 2002). But some nitrogen-fixing bacteria inhabiting barks of mangrove trees has been isolated and characterized by Uchino et al. (1984). However, there were no published reports on endophytic bacteria isolated from the pneumatophores of *A. marina*. Therefore, the present study was aimed to isolate and to identify the endophytic bacteria from the pneumatophores of *A. marina.*

MATERIALS AND METHOD

Isolation of entophytic bacterium from pneumatophores) of *A. marina*

The pneumatophores (5 to 7 cm in length) of *A. marina* were collected from the intertidal zone of Vellar estuary. The roots were washed with tap water and then distilled water to remove the soil particles. They were excised and subjected to three steps of surface sterilization procedure; Step 1: Washed with 70% ethanol for 1 min and subsequently with distilled water. Step 2: Soaked in 0.1% mercuric chloride for 3 min and washed with distilled water for 2 times. Step 3: Soaked in 70% ethanol for 30 s and washed for 5 to 7 times with distilled water. The additional step was followed in this sterilization procedure, proposed by Gagne et al. (1987). The surface sterilized roots were aseptically sectioned into small pieces (0.1 cm thickness). Totally 300 sections were made and placed on to the plates containing isolation medium (Nutrient agar), followed by incubation at 26°C for 48 h. The bacterial growths were associated with root sections and were purified by repeated plating on nutrient agar. The cultures were maintained as spore suspension by freezing in 20% (v/v) glycerol.

Phenotypic characterization

The isolate was gram-stained and examined microscopically for its morphological characteristics. A set of 10 physiological characteristics, including acid production from sugars (TSI), sodium citrate utilization, urease production, starch, gelatin hydrolysis and voges-proskauer reaction was carried out using standard procedure. Casein hydrolysis was detected after incubation of strain for 3 days 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 day. It was also estimated at selected temperatures (26, 28, 30, 32, 40 and 50ºC) and pH (4.5, 5.5, 6.5, 7 and 9) in slants incubated for 3 days.

Plant growth promoting activities (*in vitro*)

Indoleacetic acid (IAA) production

Indoleacetic acid produced by bacteria was assayed calorimetrically using ferric chloride-perchloric acid reagent ($FeCl_3$ – $HClo_4$) (Gordon and Weber, 1951). The indole compounds produced by the strains were estimated by the medium containing L-tryptophan (Frankenberger and Poth, 1988). The test was repeated for three times. The amount of IAA produced was read in UV – spectrophotometer at 530 nm absorbance and was calculated using standard curve.

Siderophore production

Siderophore production was tested qualitatively using chrome azurol S (CAS) agar as described by Alexander and Zuberer (1991). The plating was repeated for three times. Orange halos around the colonies after overnight incubation indicated siderophore-production.

Nitrogen fixation

Fixation of atmospheric nitrogen by the strains was tested qualitatively using Burk's N- free medium (Subba, 1999). The plating was repeated for three times. Halos around the colonies after overnight incubation indicated positive result for nitrogen fixation.

Phosphate solubilization

Phosphate solubilization test was conducted qualitatively by plating the strains in agar containing precipitated tricalcium phosphate, modified from Pikovskaya medium (Subba, 1999). Bacterial culture was streaked on the surface of replicated agar plates. The plating was repeated for three times. The presence of clearing zone around bacterial colonies after overnight incubation indicated positive phosphate solubilization.

Sulphur reduction

Reduction of sulphur by the strains was tested qualitatively by

Table 1. Phenotypic characteristics of the isolates.

Tests	SjAM16101	SjAM16102	SjAM16103
Morphological characteristics			
Shape	Rod	Rod	Rod
Motility	Positive	Negative	Positive
Gram's staining	Positive	Negative	Negative
Physiological characteristics			
Temperature	26ºC - Positive	28ºC - Positive	25ºC - Positive
pH (7)	7 - Positive	6.5 - Positive	7 - Positive
Salinity (5%)	Negative	Negative	Positive
Exoenzyme activities			
Starch hydrolysis	Positive	Negative	Negative
Gelatin hydrolysis	Positive	Positive	Positive
Casein hydrolysis	Positive	Negative	Negative
Endoenzyme activities			
Catalase production	Negative	Positive	Positive
Urease production	Negative	Negative	Positive
Oxidase production	Positive	Negative	Positive
Voges-proskauer	Positive	Positive	Negative

Sulphate API agar (Subba, 1999). In which the strains reduce sulphur compounds, the plating was repeated for three times. The presence of colonies after overnight incubation indicated positive result.

Determination of cellular fatty acid composition

Cellular fatty acid composition of endophytic bacterium was analyzed using the Sherlock system (MiDi Company, USA) and according to the manufacturer's instructions.

Sequence and analysis of 16S rRNA genes

Genomic DNA was isolated from pure culture (Sambrook et al., 1989). A large fragment of 16S rRNA was amplified by polymerase chain reaction (PCR) using primers 5' -TGA GGA AGA TAA TGA CGG -3' and 5' -CCT 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 min, 30 amplification cycles of 94°C for 1 min (denaturation), 57°C for 1 min (annealling), 72°C for 2 min (extension) and final polymerization at 72°C for 4 min. PCR product was purified and sequenced. Searches in the Gen Bank/EMBL/DDBJ/PDB data libraries were performed using BLAST (blastn) search algorithm (Altschul et al., 1997) in order to establish the identity of the isolate. Sequences of the close relatives were retrieved and aligned with the newly determined sequences. Multiple alignments were performed with CLUSTALX. The neighbor-joining program NEIGHBOR (Saitou and Nei, 1987) contained in the phylogenetic program package PHYLIP version 3.573 (Felsenstein, 1993) was used to infer phylogenetic relationships.

RESULTS

Three isolates (SjAM16101, SjAM16102 and SjAM16103) differing in colony morphology on isolation medium were evaluated in terms of their ability in occurrence and colonization. The isolate SjAM16101 was identified as Gram positive rod shaped 2.5 × 1µm and motile bacterium (Figure 4A). The optimum growth was at 26°C and pH 7. The isolate SjAM16102 was identified as Gram negative rod shaped 1.5 × 0.5 µm and non-motile bacterium (Figure 4B). The optimum growth was at 28°C and pH 6.5. The isolate SjAM16103 was identified as Gram positive rod shaped 2.0 × 1.0 µm and motile bacterium (Figure 4C). The optimum growth was at 25°C and pH 7. Distinguishing phenotypic characteristics of the isolates are given in Table 1.

Bacillus sp., produced 3.28 µmol/ml, *Sporosarcina aquimarina* produced 2.37 µmol/ml and *Enterobacter* sp., produced 1.97 µmol/ml of IAA. The strain SjAM16103, *S. aquimarina* produced siderophore whereas other strains could not produce siderophore. All the three strains could solubilize phosphate molecule. Starins SjAM16101 was reduced sulphur compounds. Whereas, SjAM16102 and SjAM16103 could not reduce sulphur compounds. Plant growth promoting activities of the isolates are given Table 2.

Using BLAST the isolates SjAM16101, SjAM16102 and SjAM16103 are identified as *Bacillus* sp*., Enterobacter* sp*.,* and *S. aquimarina,* respectively. Blast sequences of *Bacillus* sp., *Enterobacter* sp., and *S. aquimarina* are given in Figures 5, 6 and 7, respectively. The nucleotide sequence has been deposited in the Genbank/EMBL/DDBJ/PDB under accession number GU930357 (*Bacillus* sp.), GU930358 (*Enterobacter* sp.) and GU930359 (*S. aquimarina*).

The cellular fatty acids compositions of the isolates are given.

Table 2. Plant growth promoting activities (*in vitro*) of the isolates.

Strains ID	IAA production (µmol/ml)	P – solubilization	N – fixation	S – reduction	Siderophore production
SjAM16101	3.28	Positive	Positive	Positive	Negative
SjAM16102	1.97	Positive	Positive	Negative	Negative
SjAM16103	2.37	Positive	Negative	Negative	Positive

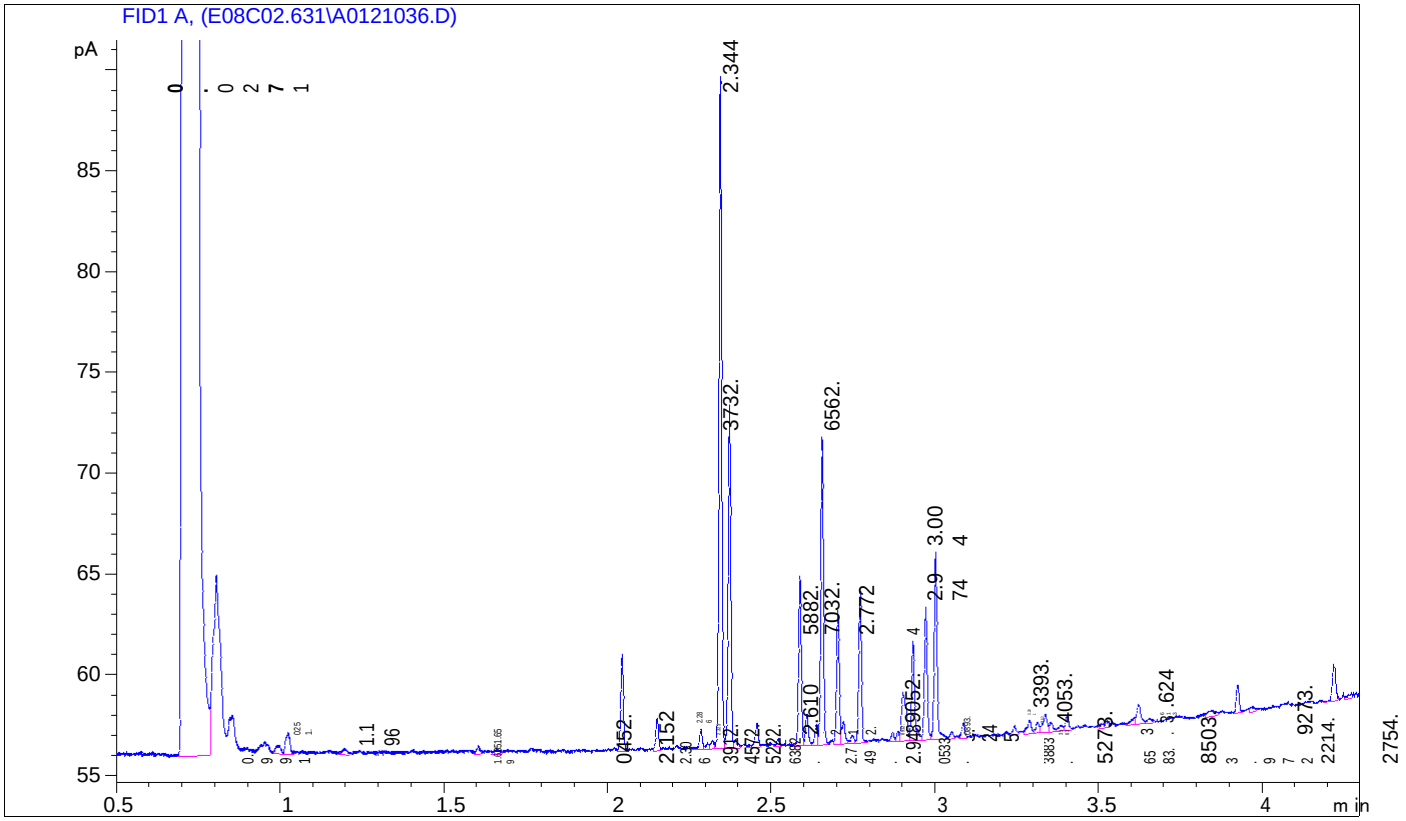


Figure 1. Gas chromatographic methyl ester profiles of *Bacillus* sp.,(SjAM16101).

in Table 3. Gas chromatographic methyl ester profiles of *Bacillus* sp., *Enterobacter* sp., and *S. aquimarina* are given in Figures 1, 2 and 3, respectively.

DISCUSSION

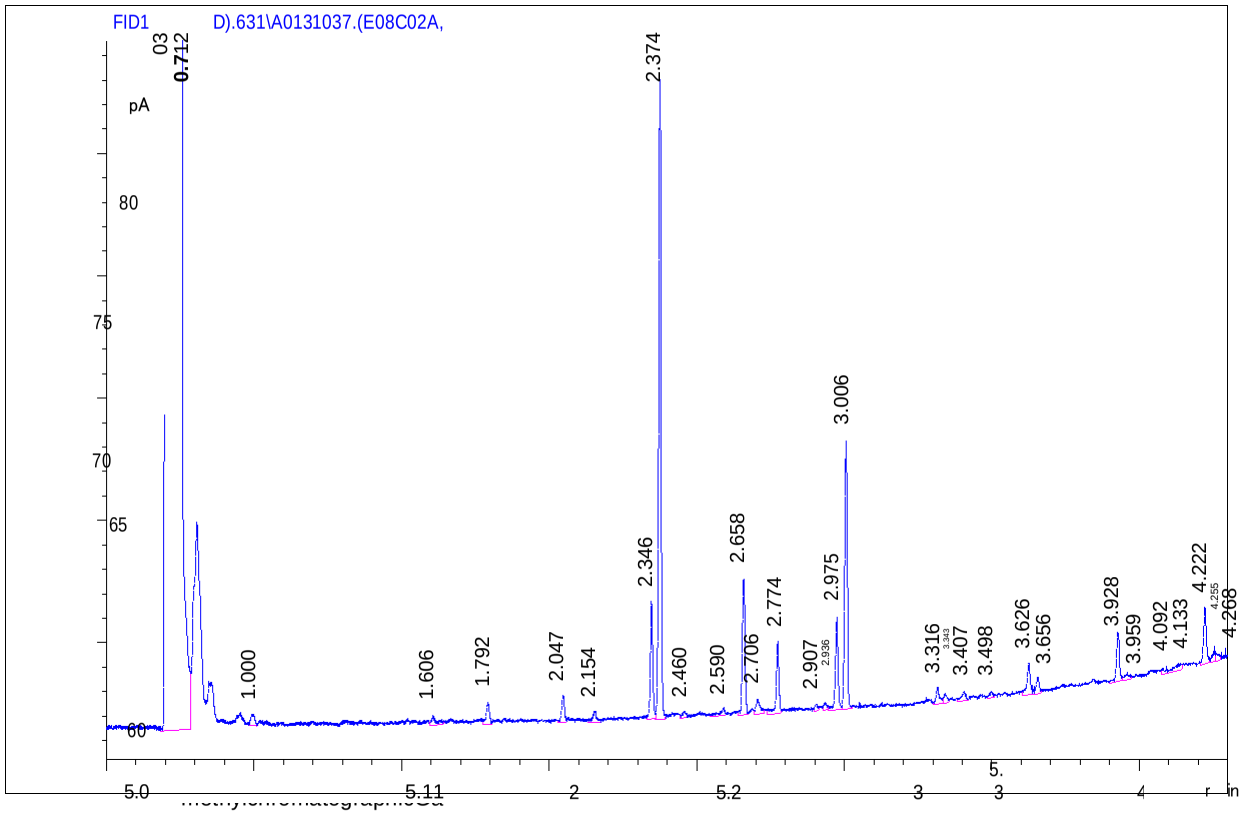
In present study, three endophytic bacteria were isolated from the pneumatophores of *A. marina*. The isolate SjAM16101 has been identified as *Bacillus* sp., using morphological, physiological and biochemical methods as well as 16S rRNA sequencing. *Bacillus* species have been reported as biocontrol agents effective against numerous root pathogens (Maplestone and Campbell, 1989; Kim et al., 1997; Sari et al., 2006).

The isolate SjAM16102 has been identified as *Enterobacter* sp., using morphological, physiological and biochemical methods as well as 16S rRNA sequencing. *Enterobacter* species have been reported as plant growth promoting rhizobacteria (PGPR) which has potential role in developing sustainable systems for crop production (Mauricio, 2009).

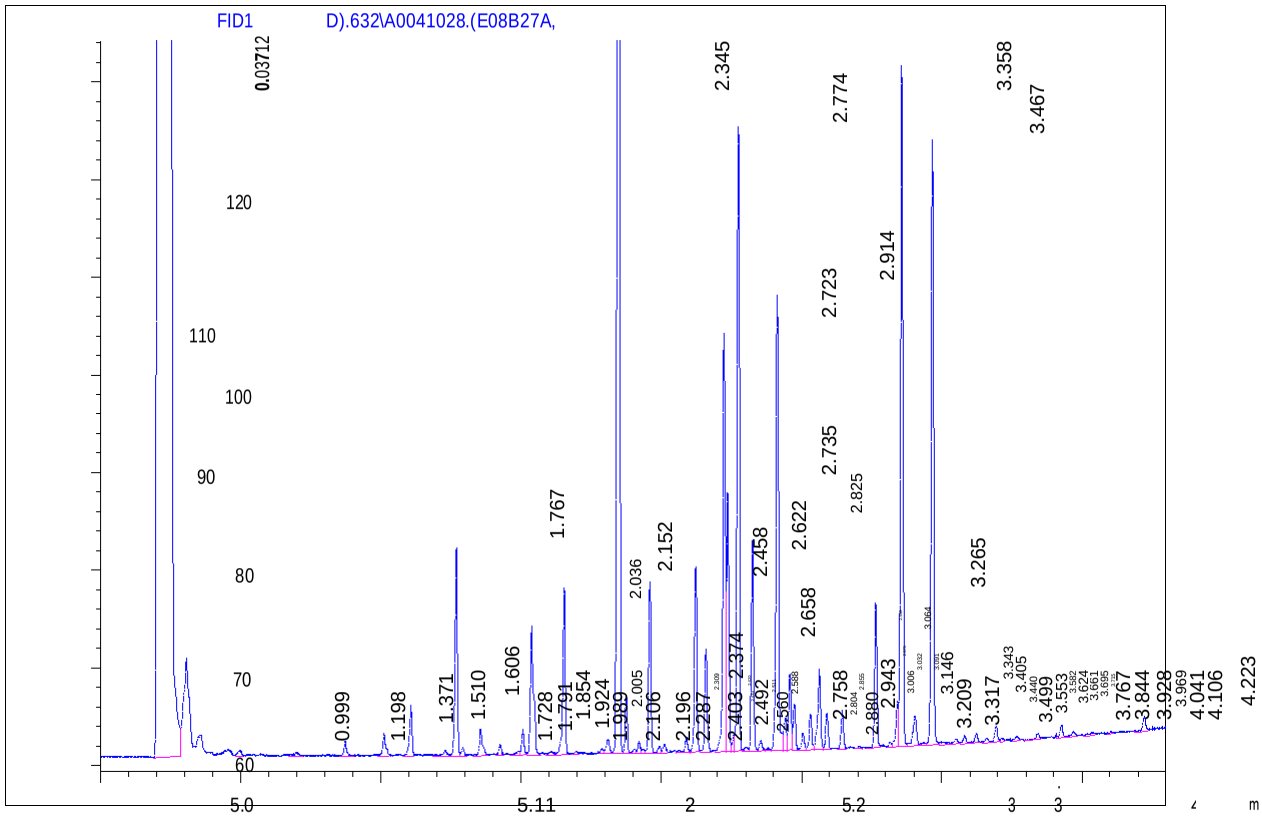
The isolate SjAM16103 has been identified as *S. aquimarina* using morphological, physiological and biochemical methods as well as 16S rRNA sequencing. *S. aquimarina* have been isolated from seawater in Korea (Jung-Hoon et al., 2001). This is the first report on *S. aquimarina* isolated from the inner tissue of the plant.

Bacillus sp., (SjAM16101) could fix nitrogen; this implies that the activity is on the same order of magnitude reported by Seldin et al. (1984) for plant bacteria of genus Bacillus isolated from roots and soil of grasses and wheat that was considered an efficient nitrogen fixing bacteria.

All the four strains could solubilize phosphate, phosphorus is the least accessible macronuntrient in many



.2Figure s ester profiles of *Enterobacter* ,.sp (SjAM16102).



.3Figure methylchromatographicGas ester profiles of .*S aquimarina* .(SjAM16103)

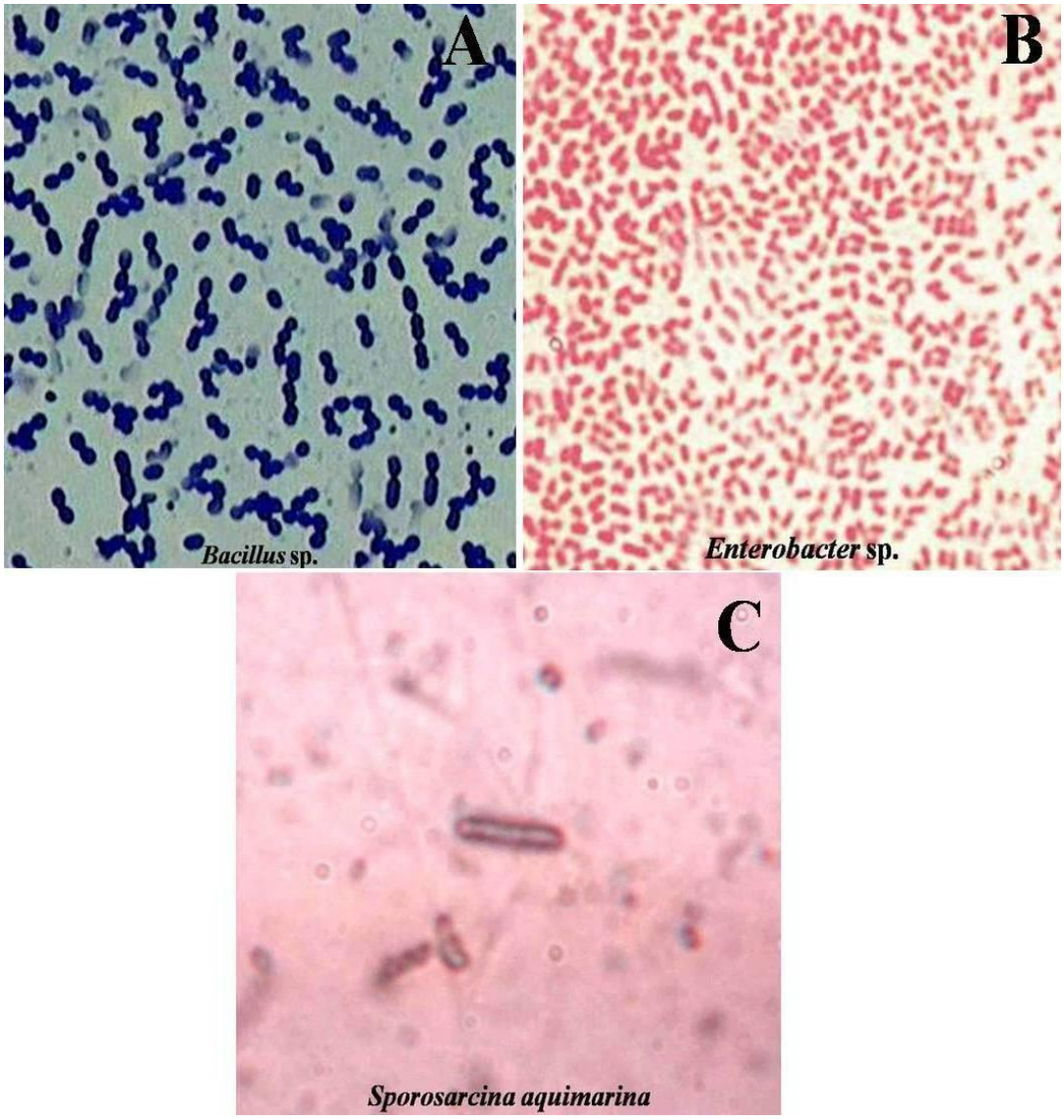


Figure 4. Microscopical identification of endophytic bacteria.

ecosystems and its low availability is often limiting to plant growth (Raghothama, 1999). Altomare et al. (1999) reported three possible mechanisms by which the strains might convert phosphate to a soluble form (i) acidification, (ii) production of chelating metabolites, and
(iii) redox activity, concluding that chelation was the more likely mechanism for P- solubilization by the endophytic bacteria.

The strain SjAM16103, Sporosarcina aquimarina can produce sideraphore as its metabolite. 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 (Renshaw et al., 2002). This element is assimilated by root cells in the reduced form (Fe II); however, especially in sufficiently aerated soils, the oxidized state (Fe III) is predominant and needs to be reduced to be taken up by plants. *S. aquimarina*, can reduce Fe III through chelators such as siderophores, and plants can take up chelated iron by reductases on the plasma membrane (Altomare et al., 1999; Jalal et al., 1986, 1987).

The colonization of pneumatophores by endophytic bacteria enhances growth of the entire plant, increasing productivity and the yield of reproductive organs. Intermediate steps in this interaction may be realized by different strains, by increasing nutrient availability in the plant rhizosphere, by production of plant hormones analogues or the activation of local or systemic acquired resistance to disease (Yedidia et al., 1999).There is increasing interest in developing the potential biotechnological applications of endophytes for improving phytoremediation and the sustainable production of non-

Length=1441

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Sbjct 40 AGCTTGCTCCCTGAGATCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAA 99

Query 88 CTCTGGGATAAGTCTGGGAA-CCGGGTCTAATACTGGATACGACG--TCCTACCGCATGG 144
Sbjct 100 GACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACTCAGTTCCT--CGCATGA 157

Query 145 TGAACTGTTGGAAAG-GGTTTTACTGGCTACCACTTACAGATGGACCCGCGGCGCATTAG 203
Sbjct 158 GGAACTGTTGAAAGGTGGCTTT--TAGCTACCACTTACAGATGGACCCGCGGCGCATTAG 215

Query 204 CTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCAACCTGAGAGGGTGAT 263
Sbjct 216 CTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGAT 275

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Sbjct 276 CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCT 335

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Sbjct 396 GTAAAACTCTGTTGTTAGGGAAGAACAAGTGCCGTTCGAATAGGGCGGCGCCTTGACGGT 455

Query 444 ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA 503
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Query 504 AGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTG 563
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Query 564 AAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAAAA 623
Sbjct 576 AAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAG 635

Query 624 GAAA 627
Sbjct 636 GAAA 639

Figure 5. Nucleotide sequence of *Bacillus sp.* (SjAM16101) using BLAST search algorithm.

Length=1371

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Sbjct  516  TGAGCCC  510
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Figure 6. Nucleotide sequence of *Enterobacter sp.* (SjAM16102) using BLAST search algorithm.

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Query  480 TCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGT 539
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Sbjct  452 TCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGT 393

Query  540 GCGGGTCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTG  599
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  392 GCGGGTCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTG  333

Query  600 CTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGT 659
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  332 CTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGT 273

Query  660 TTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAG  719
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  272 TTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAG  213

Query  720 CGTCAGTTACAGACCAGAAAGCCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCAT 779
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  212 CGTCAGTTACAGACCAGAAAGCCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCAT 153

Query  780 TTCACCGCTACACGTGGAATTCCGCTCTCCTCTCCTGCACTCAAGTCCCCCAGTTTCCAA 839
            |||||||||||||||||||||||| |||||| ||| ||||||||||||||||||||||
Sbjct  152 TTCACCGCTACACGTGGAATTCCGCTTTCCTCTTCTGTACTCAAGTCCCCCAGTTTCCAA 93

Query  840 TGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGGGACCGC 890
            |||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  92   TGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAAGGACCGC 42
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Figure 7. Nucleotide sequence of *S. aquimarina* (SjAM16103) using BLAST search algorithm.

Table 3. Cellular fatty acid profiles of the isolates.

Fatty acids	SjAM16101	SjAM16102	SjAM16103
	Saturated fatty acids		
$C_{10:0}$	0.44	-	0.17
$C_{12:0}$	0.38	1.41	0.86
$C_{14:0}$	1.27	1.19	2.63
$C_{15:0}$	-	-	-
$C_{16:0}$	5.72	5.23	8.99
$C_{17:0}$	-	-	0.54
$C_{18:0}$	-	1.02	0.68
$C_{19:0}$	-	-	0.06
	Unsaturated fatty acids		
$C_{11:0}$ 3OH	-	-	0.15
$C_{12:0}$ 2OH	-	-	0.19
$C_{12:0}$ 3OH	-	-	0.41
$C_{15:0}$ 2OH	-	-	0.19
$C_{15:0}$ 3OH	-	-	0.58
$C_{15:1}$ w5	-	-	0.19
$C_{15:1}$ w6	-	-	0.17
$C_{15:1}$ w8	-	-	0.08
$C_{16:0}$ 3OH	-	-	2.15
$C_{16:0}$ N alcohol	0.74	-	-
$C_{16:1}$ w5	0.37	-	0.06
$C_{16:1}$ w7 alcohol	6.22	1.09	0.25
$C_{16:1}$ w11	5.09	1.64	-
$C_{17:0}$ 2OH	-	0.59	0.11
$C_{17:0}$ 3OH	-	-	0.13
$C_{17:1}$ w6	0.36	-	-
$C_{17:1}$ w8	-	-	0.58
$C_{18:1}$ w9	-	0.73	0.62
$C_{20:1}$ w7	-	-	0.12
$C_{20:1}$ w9	-	0.74	-
	Branched fatty acids		
$C_{13:0}$ iso	-	-	3.15
$C_{13:0}$ anteiso	-	1.87	0.14
$C_{14:0}$ iso	3.75	2.03	--
$C_{15:0}$ iso	24.48	7.82	28.39
$C_{15:0}$ anteiso	-	40.55	0.78
$C_{15:0}$ iso 3OH	-	-	3.00
$C_{15:1}$ iso F	0.90	-	0.07
$C_{15:1}$ anteiso A	-	-	-
$C_{16:0}$ iso	10.99	8.95	1.61
$C_{16:0}$ iso 3OH	-	-	0.59
$C_{16:1}$ iso H	1.22	-	-
$C_{17:0}$ iso	5.06	6.62	0.68
$C_{17:0}$ anteiso	7.16	17.39	0.27
$C_{17:0}$ cyclo	-	-	1.42
$C_{17:0}$ iso 3OH	-	-	8.96
$C_{17:1}$ iso w10	-	0.51	-
$C_{17:1}$ anteiso w9	0.43	-	-
$C_{17:1}$ iso w10	2.78	0.51	-
$C_{19:0}$ cyclo w8	-	-	0.25

Table 3 Contd.

	Summed feature		
Summed Feature 1	0.24	-	-
Summed Feature 2	-	-	2.87
Summed Feature 3	0.93	-	6.40
Summed Feature 4	3.71	0.62	-
Summed Feature 8	-	-	9.92
Summed Feature 9	-	-	7.25
			14.502*****
Unknown fatty acids	-	-	13.591*****
			16.586*****

Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system; * represents peak name.

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REFERENCES

Alexander DB, Zuberer DA (1991). Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere baceria. Biol. Fertil. Soils. 2: 39-45.

Altomare C, Norvell WA, Bjorkman T, Harman G (1999). Solubilization of phosphates and micronutrients by the plant growth-promoting and biocontrol fungus Trichoderma harzianum Rifai 1295-22. Appl. Environ. Microbiol., 65: 2926-2933.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped blast and psi-blast:a new generation of protein database search programs. Nucleic. Acids Res., 25: 3389-3402.

Bandaranayake WM (1995). Survey of mangrove plants from Northern Australia for phytochemical constituents and UV-absorbing compounds. Curr. Top Phytochem., 14: 69-78.

Bandaranayake WM (1998). Traditional and medicinal uses of mangroves. Mangroves Salt Marshes. 2: 133–148.

Charles L, Birgitta B (2002). Biological N2-fixation on Mangrove Pneumatophores: Preliminary Observations and Perspectives. AMBIO. 31(7): 612-613.

Felsenstein J (1993). Phylip: phylogeny inference package (version 3.573). Department of Genetics, University of Washington, Seattle, WA, USA.

Frankenberger WT, Poth M (1988). L – tryptophan transaminase of a bacterium isolated from the rhizosphere of Festuca octoflora (Gramineae). Soil Biol. Biochem., 20: 299-304.

Gagne S, Richard C, Roussean H, Antoun H (1987). Xylem-residing bacteria in *alfalfa* roots. Can. J. Microbiol., 33: 996-1000.

Gordon SA, Weber RP (1951). Colorimetric estimation of indoleacetic acid. Plant Physiol., 26: 192-197.

Hurek T, Reinhold-Hurek B, Van Montagu M, Kellenbberger E (1994). Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. J. Bacteriol., 176: 1913-1923.

Jalal MAF, Love SK, Van der Helm D (1987). Siderophore mediated iron (III) uptake in *Gliocladium virens* (*Trichoderma virens*). 2. Role of ferric mono- and dihydroxamates as iron transport agents. J. Inor. Biochem., 29: 259-267.

Jalal MAF, Love SK, vanderHelm D (1986). Siderophore mediated iron (III) uptake in Gliocladium virens (Trichoderma virens). 1. Properties of cis-fusarinine, transfusarinine, dimerum acid, and their ferric complexes. J. Inor. Biochem., 28: 417-430.

James EK, Olivares FL (1998). Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. Crit. Rev. Plant Sci., 17: 77-119.

James EK, Reis VM, Olivares FL, Baldandi JI, Dobereiner J (1994). Infection of sugar came by the nitrogen-fixing bacterium *Acetobacter diazotrophicus.* J. Exp. Bot., p. 45-757.

Jung-Hoon Yoon, Keun-Chul Lee, Norbert Weiss, Yung Hee Kho, Kook Hee Kang, Yong-Ha Park (2001). *Sporosarcina aquimarina* sp. nov., a bacterium isolated from seawater in Korea, and transfer of *Bacillus globisporus* (Larkin and Stokes 1967), *Bacillus psychrophilus* (Nakamura 1984) and *Bacillus pasteurii* (Chester 1898) to the genus *Sporosarcina* as *Sporosarcina globispora* comb. nov., *Sporosarcina psychrophila* comb. nov. and *Sporosarcina pasteurii* comb. nov.,and emended description of the genus *Sporosarcina***.** In. J. Sys. Evol. Microbiol., 51: 1079-1086.

Kim DS, Cook RJ, Weller DM (1997). *Bacillus* sp. L324-92 for biological control three root diseases of wheat grown with reduced tillage. Phytopathology. 87: 551-558.

Kobayashi DY, Palumbo JD (2000). Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon, C.W. and White, J.F. (Eds.) Microbial endophytes. Marcel Dekker, Inc. N.Y., New York. pp. 199-233.

Maplestone PA, Campbell R (1989). Colonization of roots of wheat seedlings by Bacilli proposed as biocontrol agents against take-all. Soil Biol. Biochem., 21: 543-550.

Mauricio S, Ribaudo CM, Pardo MA, Cantore ML, Luigi Ciampi, Cura JA (2009). Plant growth promoting properties of a strain of *Enterobacter ludwigii* isolated from Loliumperenne rhizosphere. Soil Biol. Biochem., 41: 1768-1774.

Olivares FL, BaldanI VLD, Reis VM, Baldani JI, Dobereiner J (1996). Occurrence of the endophytic diazotrophs *Herbaspirillum* spp. in roots, stems, and leaves, predominantly of Gramineae. Biol. Fertil. Soils. 21: 197-200.

Olivares FL, James EK, Baldani JI, Dobereiner J (1997). Infection of mottled stripe disease-susceptible and resistant sugar cane varieties by the endophytic diazotroph *Herbaspirillum.* New Phytol., 135: 723-737.

Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moenne-Loccoz Y (2008). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil. 321: 341-361.

Raghothama KG (1999). Phosphate acquisition. Ann Rev Plant Physiol. Mole. Biol. 50: 665-693.

Reinhold B, Hurek T, Niemann EG Fendrik I (1986). Close association of *Azospirillum* and diazotrophic rods with different root zones of Kallar grass. Appl. Environ. Microbiol., 52: 520-529.

Reinhold Hurek B, Hurek T (1998). Life in grasses diazotrophic endophytes. Trends Microbiol., 6: 139-144.

Renshaw J, Robson G, Trinci A, Wiebe M, Livens F, Collison D, Taylor R (2002). Fungal siderophores: structure, functions and applications. Mycol. Res., 106: 1123-1142.

Saitou N, Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Sambrook J, Fritsch EF, Maniatis T (1989). Molecular Cloning, A

Laboratory Manual, 1989 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
Sari E, Etebarian HR, Roustaei A, Aminian H, (2006). Biological control of *Gaeumannomyces graminis* on wheat with *Bacillus* spp. Plant Pathol J. 5: 307-314.
Seldin L, Van Elsas JD, Penido E (1984). *Bacillus azotofixans* sp. Nov. a nitrogenfixing specie from Brazilian soils and grass roots. Int. J. Syst. Bacterial., 34: 451-456.
Subba Rao NS (1999). Soil microbiology (Fourth edition of soil microorganisms and plant growth). Science publishers, Inc.USA.
Uchino F, Hambali GG, Yatazawa M (1984). Nitrogen-fixing bacteria from warty lenticellate bark of a mangrove tree, *Bruguiera gymnorrhiza (L.)* Lamk. Appl. Environ. Microbiol., 47: 44-48.
Welbaum G, Sturz AV, Dong Z, Nowak J (2004). Fertilizing soil microorganisms to improve productivity of agroecosystems. Crit. Rev. Plant Sci., 23: 175-193.
Yedidia I, Benhamou Y, Chet I (1999). Induction of defense responses in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol., 65: 1061-1070.