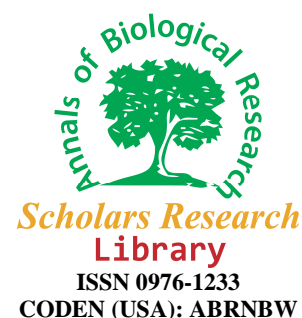




## Scholars Research Library

Annals of Biological Research, 2010, 1 (4) : 240-247  
(<http://scholarsresearchlibrary.com/archive.html>)



ISSN 0976-1233  
CODEN (USA): ABRNBW

# Assessment of endophytic bacterial diversity among mangrove plants and their antibacterial activity against bacterial pathogens

Sundaram Ravikumar<sup>\*1</sup>, Samuel Jacob Inbaneson<sup>1</sup>, Ramasamy Sengottuvel<sup>2</sup> and Andy Ramu<sup>3</sup>

<sup>1</sup>*School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi – 623 409, Ramnathapuram District, Tamil Nadu, India.*

<sup>2</sup>*Department of Microbiology, Vivekanandha College of Arts and Sciences for Women, Elayampalayam, Tiruchengode, Namakkal District, Tamil Nadu, India.*

<sup>3</sup>*School of Chemistry, Department of Inorganic Chemistry, Madurai Kamaraj University, Madurai District, Tamil Nadu, India.*

## ABSTRACT

*Natural products have been the single most productive source of leads for the development of drugs. Natural products from endophytic microbes have been showed many biological activities. The present study was carried out to find out the in vitro antibacterial activity of endophytic bacteria isolated from halophytic plants. A total of 14 endophytic bacterial strains were identified from the leaf tissue of 11 different halophytic plant species. Of them, 2 strains showed broad-spectrum of antibacterial activity against shrimp pathogens which were identified as *Bacillus thuringiensis* (FJ236808) and *Bacillus pumilus* (FJ236809) through 16S rDNA sequencing and deposited in the NCBI GenBank.*

**Keywords:** Antibacterial activity, Endophytes, MBC, MIC, 16s rDNA sequencing

## INTRODUCTION

There is a need to search for new antimicrobial agents because infectious diseases are still a global problem because of the development and spread of drug-resistant pathogens [1,2]. Encouraged by the idea of “Drugs from the Sea”, the chemists have identified lots of bioactive compounds with novel structures from the rich marine bioresource in the recent fifty years [3-5]. Among them, marine derived microbes have contributed an important proportion. Microbes have been known to be a major source of active compounds used in medicine. Endophytic bacteria have been defined as bacteria that can be isolated from surface disinfected plant tissues or extracted from within the plant and additionally, do not visibly harm the plant [6].

The endophytic microbes were well studied in terrestrial plants [7-9] which are found to possess antibacterial [10-12], antifungal [10], anticancer [11,13], antimalarial [11], antiviral [14], antioxidant [15,16] and antidiabetic [17] activities. But, isolation effort of endophytic microorganism from marine plants is limited. In this connection, the present study was made an attempt to explore the endophytic bacteria from the leaves of 11 marine halophytic plant species and to find out the antibacterial activity against poultry, shrimp and human pathogens.

## MATERIALS AND METHODS

### Collection of apical buds

Leaf samples from 11 mangrove halophytic plants viz., *Suaeda monoica*, *Suaeda maritima*, *Salicornia brachiata*, *Lumnitzera racemosa*, *Sesuvium portulacastrum*, *Rhizophora apiculata*, *Rhizophora mucronata*, *Bruguiera cylindrica*, *Ceriops decandra*, *Avicennia marina* and *Aegiceras corniculatum* were collected from Pichavaram mangrove forest (Lat. 11° 27' N and Lon. 79° 47' E) of Tamil Nadu, India and were placed in sealed, unused plastic bags after excess moisture is removed [18]. The authentication of plant species was done by the help of Prof. K. Kathiresan, Centre of Advanced Study in Marine Biology, Annamalai University, Portonovo, Tamil Nadu, India and voucher specimens were deposited in the herbarium facility (Sponsored by the ICMR, New Delhi) in the Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Tamil Nadu, India.

### Isolation of endophytic bacteria

Collected leaf samples were thoroughly surface sterilized with 70% ethanol and air dried under a laminar-flow hood to eliminate surface contaminating microbes. The outer tissues were removed from the samples with a sterile knife blade and the inner tissues were excised and macerated with sterile distilled water by using mortar and pestle. The macerated samples were serially diluted and plated on to Zobell marine agar 2216 medium (HiMedia Laboratories Pvt. Limited, Mumbai, India) in triplicates and incubated at 37±2°C for 24 h in a thermostat incubator. After attaining visible growth, the colonies were counted using colony counter (Subra Scientific Co., India). Morphologically different heterotrophic bacterial colonies were selected and restreaked on to a Zobell marine agar 2216 slants for further use.

### Test organisms

The chicken pathogens viz., *Escherichia coli*, *Staphylococcus aureus*, *Salmonella sp.*, *Klebsiella sp.*, *Haemophilus parasalarum*, *Pasterulla multocida*, shrimp pathogens viz., *Bacillus subtilis*, *Serratia sp.*, *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Aeromonas hydrophila* and human bacterial pathogens viz., *Klebsiella sp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Streptococcus sp.*, *Proteus morganii* were used for *in vitro* antibacterial assay. All the culture collections from different sources were identified and maintained in the Division of Marine Microbiology and Infectious Medicine, Alagappa University, Thondi Campus, Tamil Nadu, India.

### Antibacterial sensitivity assay

Single streak of endophytic bacteria were streaked on the surface of Mueller Hinton agar medium (HiMedia Laboratories Pvt. Limited, Mumbai, India) plates followed by the overnight culture (10<sup>8</sup> cells/ml) of pathogenic bacteria were streaked at perpendicular to the original streak and incubated at 37±2°C. The inhibition was observed after 24 h and triplicates were maintained for each analysis [19]. Endophytic bacterial strains (MB4 and MB8) which showed maximum zone of inhibition were further inoculated into 100 ml of sterile nutrient broth (HiMedia Laboratories Pvt. Limited, Mumbai, India) and kept at 37±2°C for 24 h with continuous shaking.

Then 20 ml of grown culture was transferred into 1000 mL of sterile nutrient broth and incubated at  $37\pm 2^{\circ}\text{C}$  for 5 days under continuous shaking at 200 rpm/min. Mass cultivated cultures were filtered by using muslin cloth and the supernatant was mixed with equal volume of ethyl acetate (1:1) in a separating funnel and after vigorous shaking, the organic phase was collected and subjected for evaporation under reduced pressure using rotary evaporator (Superfit, India) and the weight of the extracts were measured [20] and expressed as percentage of extraction using the following formula: % of extraction = Quantity of the extract (g)/Quantity of endophytic bacterial biomass (g) X 100

Minimum inhibitory concentration (MIC) was carried out with the extracts from MB4 and MB8. 500  $\mu\text{l/ml}$  of various concentration of extracts (31, 62, 125, 250, 500, 1000  $\mu\text{g/ml}$ ) was prepared with dimethyl sulphoxide (DMSO) and mixed with 500  $\mu\text{l/ml}$  of nutrient broth and 50  $\mu\text{l}$  of 24 h old bacterial culture ( $10^8$  cells/ml) and allowed to grow at  $37^{\circ}\text{C}$  for 48 h. To calculate the MIC, turbidity due to bacterial growth was observed in each concentration. To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds, the minimum bactericidal concentration (MBC) was determined by subculturing the MIC dilutions on to the sterile agar plates. The lowest concentration of the extracts which inhibits the growth of tested bacteria are observed and tabulated [21].

### **16S rRNA sequencing of promising endophytic bacteria**

#### **Genomic DNA extraction**

The promising strains MB4 and MB8 were inoculated in nutrient broth separately and incubated overnight at  $28^{\circ}\text{C}$ . The culture was spun at 7000 rpm for 3 min. The pellet was resuspended in 400  $\mu\text{l}$  of sucrose TE buffer. Lysozyme was added to a final concentration of 8 mg/ml and incubated for 1 h at  $37^{\circ}\text{C}$ . To the tube, 100  $\mu\text{l}$  of 0.5 M EDTA (pH 8.0), 60  $\mu\text{l}$  of 10% SDS and 3  $\mu\text{l}$  (20 mg/ml) of proteinase K (Amersham Biosciences, USA) were added and incubated at  $55^{\circ}\text{C}$  overnight. Extracted with equal volume of phenol: chloroform (1:1), centrifuged (10000 rpm: 10 min) and the supernatant was transferred to a sterile tube. The supernatant was extracted twice with phenol: chloroform and once again with chloroform: isoamylalcohol (24:1) and ethanol precipitated. The DNA pellet was resuspended in sterile distilled water and stored at  $4^{\circ}\text{C}$  for immediate use and  $-20^{\circ}\text{C}$  for further use [22].

#### **Amplification of 16S rRNA genes**

The universal eubacterial primers of F 5' AGAGTTTGATCCTGGCTCAG 3' and R 5' ACGGCTACCTTGTTACGACTT 3' were used for PCR amplification of highly variable regions within the 16S rRNA gene [22]. Polymerase chain reaction was performed in a 50  $\mu\text{L}$  reaction mixture containing 2  $\mu\text{l}$  (10 ng) of DNA as the template, each primer at a concentration of 0.5  $\mu\text{mol}$ , 1.5 mmol  $\text{MgCl}_2$  and each deoxynucleoside triphosphate at a concentration of 50  $\mu\text{mol}$ , as well as 1 U of *Taq* polymerase and buffer (MBI Fermentas). After the initial denaturation for 3 min at  $95^{\circ}\text{C}$ , there were 40 cycles consisting of denaturation at  $95^{\circ}\text{C}$  for 1 min, annealing at  $55^{\circ}\text{C}$  for 1 min and extension at  $72^{\circ}\text{C}$  for 2 min and then a final extension step consisting of 5 min at  $72^{\circ}\text{C}$  in Mastercycler Personal (Eppendorf, Germany). The amplification of 16S rDNA was confirmed by running the amplification product in 1% agarose (Amersham, USA) gel in 1 X TAE (Tris-Acetic Acid – EDTA [Ethylene Diamine Tetraacetic Acid]) buffer.

#### **Cloning and sequencing of 16S rRNA gene**

The amplified product (1,500-bp) was purified using HiYield<sup>TM</sup> Gel/PCR DNA Extraction Kit (RBC Biotech Corporation) according to manufacturer's instruction. The 16S rDNA amplicon was cloned in pTZ57R/T vector according to the manufacturer's instruction (InsT/Aclone<sup>TM</sup> PCR Product Cloning Kit #K1214, MBI Fermentas). Sequencing of the 16S rRNA gene (about 1500-

bp) for both marine isolates were carried out using M13F (-20) primer 5' GTAAACGACGGCCAGT 3', M13RpUC (-40) 5' CAGGAAACAGCTATGAC 3' and 16SMP (-20) 5' GCCACATTGGGACTGAGACA 3' in ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems).

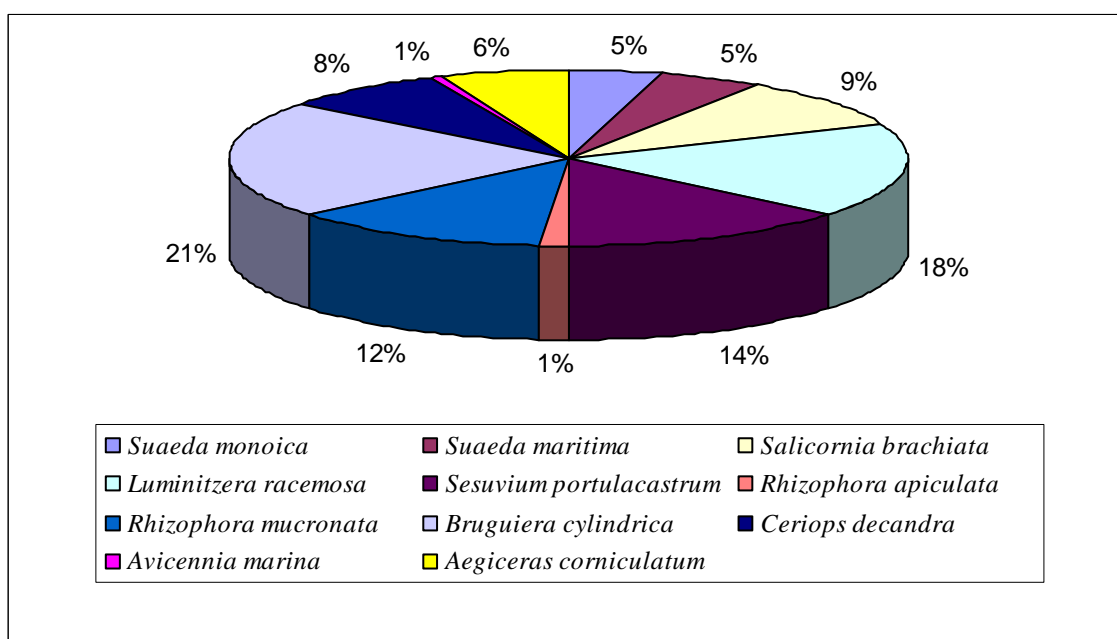
### Phylogenetic inferences

Sequence analysis was performed with sequences in the NCBI database using BLAST and sequences were aligned by using the Clustalw program. The nearest described species with the highest similarity identified.

## RESULTS AND DISCUSSION

The endophytic bacteria isolated from the leaf sample of 11 halophytic plant species belonging to 6 families. It reveals that, the counts were found maximum in *Bruguiera cylindrica* (21%) and found minimum in *Avicennia marina* (1%) (Fig.1). Fourteen endophytic bacterial strains were selected based on the morphological characteristics like form, elevation, margin and colour of the colony (Table.1), which were subjected for the antibacterial sensitivity against chosen bacterial pathogens. It shows that, the MB4 strain showed antibacterial sensitivity against 3 shrimp pathogens such as *Bacillus subtilis*, *Vibrio harveyi* and *Aeromonas hydrophila* and the MB8 strain showed antibacterial sensitivity against *Bacillus subtilis* and *Serratia sp.* However, all the strains (MB1-MB14) did not showed antibacterial sensitivity against 6 poultry pathogens and 6 human pathogens (Table.2). In MIC assay, the MB4 crude extracts showed high sensitivity against *Bacillus subtilis*, *Serratia sp.* (250 µg/ml) and *Aeromonas hydrophila* (500 µg/ml). However, the MB8 crude extracts showed high sensitivity (250 µg/ml) against *Bacillus subtilis* and *Serratia sp.* (Table.3). Based on the sequence analysis, the query sequences of MB4 and MB8 having high similarity alignment with *Bacillus sp.* and the MB4 is identified as *Bacillus thuringiensis* and MB8 is identified as *Bacillus pumilus*. Both the nucleotide sequences of 16S rDNA of rRNA gene partial sequences were deposited in the GenBank under the accession number FJ236808 and FJ236809 respectively.

Fig.1. Percentage occurrence and distribution of endophytes between halophytes



Values are found significant among the plant species ( $P > 0.01$ )

Table 1. Morphological characteristics of endophytic bacterial strains isolated from mangrove leaves

Strain no	Colony morphology			Colour of the colony	Gram staining	Shape
	Forms	Elevation	Margin			
MB1	Circular	Flat	Entire	Yellow	Positive	Cocci
MB2	Circular	Flat	Entire	Translucent	Positive	Cocci
MB3	Circular	Raised	Entire	Orange	Positive	Cocci
MB4	Irregular	Flat	Undulate	White	Positive	Rod
MB5	Irregular	Flat	Erose	Orange	Negative	Rod
MB6	Circular	Convex	Entire	yellow	Negative	Cocci
MB7	Circular	Convex	Entire	Yellow	Negative	Rod
MB8	Circular	Raised	Entire	White	Negative	Rod
MB9	Circular	Raised	Entire	White	Negative	Rod
MB10	Circular	Raised	Entire	Pale yellow	Positive	Rod
MB11	Irregular	Flat	Lobate	Dark yellow	Negative	Rod
MB12	Irregular	Flat	Undulate	Dry white	Negative	Rod
MB13	Irregular	Raised	Lobate	Orange yellow	Negative	Cocci
MB14	Irregular	Flat	Lobate	pale white	Positive	Rod

Table 2. Antibacterial sensitivity of endophytic bacteria against various bacterial pathogens

Name of the pathogens	Name of the isolated endophytic strains													
	MB1	MB2	MB3	MB4	MB5	MB6	MB7	MB8	MB9	MB10	MB11	MB12	MB13	MB14
<b>Chicken pathogens</b>														
<i>Escherichia coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Salmonella sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Haemophilus parasalinarum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pasterulla multocida</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Shrimp pathogens</b>														
<i>Bacillus subtilis</i>	-	-	-	+	-	-	-	+	-	-	-	-	-	-
<i>Serratia sp.</i>	-	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>Vibrio parahaemolyticus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Vibrio harveyi</i>	-	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Aeromonas hydrophila</i>	-	-	-	+	-	-	-	-	-	-	-	-	-	-
<b>Human bacterial pathogens</b>														
<i>Klebsiella sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Streptococcus pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Streptococcus sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Proteus morganii</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : Sensitivity ; - : No Sensitivity



Table 3. MIC and MBC ( $\mu\text{g/ml}$ ) of crude extracts of MB4 and MB8 against shrimp pathogens

Name of the endophytes	<i>Bacillus subtilis</i>		<i>Serratia sp.</i>		<i>Vibrio harveyi</i>		<i>Aeromonas hydrophila</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
MB4	250	500	-	-	250	500	500	500
MB8	250	500	250	500	-	-	-	-

Values are found significant among the shrimp pathogens ( $P>0.01$ ) and among the endophytic ( $P>0.05$ )

Endophytes are a poorly investigated group of microorganism that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural, and industrial arenas [23]. The mechanisms through which endophytes exist and respond to their surroundings must be better understood in order to be more predictive about which higher plants to seek study and spend time for isolating microfloral components. This may facilitate the product discovery processes.

The mangrove plant species are a valuable source of useful metabolites and their endophytes have gained more importance [24]. Hence, the present study was made an attempt to find out the biodiversity of endophytic microorganisms in marine halophytic plants. Mangrove and mangrove associates are specially adopted group of woody plants found in between land and sea. They have special adaptation such as salt excreting gland, stilt root, prop root, pneumatophores, high content of phenolic compounds and more UV-absorbing compounds [25]. Among the plant species, the *Bruguiera cylindrica* harboured maximum counts of endophytic bacteria and *Avicennia marina* harboured minimum counts. Contrastingly the endophytic actinomycetes were found maximum in the salt marsh plant species *Suaeda monoica* and *Salicornia brachiata* (unpublished data). The presence of maximum counts in *B. cylindrica* might be due to the reason that, the plant species always inhabited in water logged condition but the *A. marina* prefer to inhabit in less water habitat and salt marshes grow in the water less habitat. (Field observation).

The abundance and diversity of endophytic populations averaged  $10^3$  and  $10^5$  CFU/g (fresh wt.) for the endosphere and endorhiza respectively, which were lower than those for the ectophytic microenvironments, with  $10^5$  and  $10^7$  CFU/g (fresh wt.) for the phyllosphere and rhizosphere, respectively [26]. A total of 853 endophytic strains were isolated from aerial tissues of four agronomic crop species and 27 plant species. Host range greenhouse studies demonstrated that, 26 of 29 endophytes were recoverable from at least one host other than corn and sorghum at levels of up to  $5.8 \log_{10}$  CFU/g (fresh weight) [27].

Recently, it has been demonstrated that bacterial endophytes may have beneficial effects on host plants, such as growth promotion and biological control of pathogens [28-32]. In the present study, 14 endophytic bacterial strains were isolated from mangrove plants of these two strains (MB4 and MB8) showed promising antibacterial activity. The preliminary study for antibacterial activity by cross streak method indicated that 16 isolates have excellent antagonistic properties [33]. A group of researchers reported seven endophytes of 35 isolated strains were found to antagonize bacterial and fungal pathogens [10]. The strain MB4 has MIC values at  $250 \mu\text{g/ml}$  against *Bacillus subtilis*, *Vibrio harveyi* and the strain MB8 showed MIC value of  $250 \mu\text{g/ml}$  against *Bacillus subtilis* and *Serratia sp.* Our previous reports reveals that, the endophytic strain ENS3 from the seagrass *Syringodium isoetifolium* and the endophytic strain ENC5 from the seagrass *Cymodocea serrulata* posses MIC values of  $125 \mu\text{g/ml}$  against *Pseudomonas aeruginosa* [21]. The MIC of *M. jodocodo* against *E. coli* was  $2.75 \text{ mg/ml}$  while that of *T.*

*robustus* against *M. bourtardi* was 15.75 mg/ml[34]. Comparatively, the MIC value was found to be minimum in the present study which showed the economic feasibility of the antibacterial drug development from the marine halophytic endophytes.

These potential strains were identified as *Bacillus thuringiensis* (MB4) and *Bacillus pumilus* (MB8) by using 16S rRNA sequencing. A group of researchers isolated endophytic bacteria from bean nodules and identified as *Bacillus subtilis* by using 16rRNA sequence analysis [35]. Many researchers were identified endophytic bacteria by 16S rRNA sequencing [35-37]. The partial sequencing of the 16S rRNA genes of isolated endophytes revealed a broad phylogenetic spectrum of bacteria, including members of the alpha, beta, and gamma subgroups of the Proteobacteria, high and low-G+C-content gram-positive organisms and microbes belonging to the Flexibacter-Cytophaga-Bacteroides group [38].

It is concluded from the present study, the endophytic bacterial strains isolated from the leaves of mangrove plants have promising antibacterial activity against shrimp pathogenic bacteria. Further study is in progress for the identification of active chemical classes responsible for the antibacterial activity.

### Acknowledgments

The authors are thankful to the authorities of Alagappa University for providing required facilities and also to Indian Council of Medical Research, New Delhi for financial assistance.

### REFERENCES

- [1] D. Pillay, M. Zambon, *Br. Med. J.*, **1998**, 317, 660–662.
- [2] M.A. Espinel, A. Laszlo, L. Simonsen, F. Boulahbal, S.J. Kim, A. Reniero, S. Hoffner, H.L. Rieder, N. Binkin, C. Dye, R. Williams, M.C. Raviglione, *N. Eng. J. Med.*, **2001**, 344, 1294–1303.
- [3] D.J. Faulkner, *Nat. Prod. Rep.*, **2000**, 17, 1-6.
- [4] P. Proksch, R.A. Edrada, R. Ebel, *Appl. Microbiol. Biotechnol.*, **2002**, 59, 125-134.
- [5] E. Marris, *Nat.*, **2006**, 443, 904-905.
- [6] J. Hallmann, A. Quadts-Hallmann, W.F. Mahaffee, J.W. Kloepper, *Can. J. Microbiol.*, **1997**, 43, 895–914.
- [7] O. Petrini, Fungal endophytes of tree leaves. In microbial ecology of leaves, eds. Andrews JH and Hirano SS, Springer-Verlag, New York, **1991**, 179–197.
- [8] K. Saikkonen, S.H. Faeth, M. Helander, T.J. Sullivan, *Annu. Rev. Ecol. Syst.*, **1998**, 29, 319–343.
- [9] R.X. Tan, W.X. Zou, *Nat. Prod. Rep.*, **2001**, 18, 448–459.
- [10] A. Sessitsch, B. Reiter, G. Berg, *Can. J. Microbiol.*, **2004**, 50(4), 239-249.
- [11] S. Wiyakrutta, N. Sriubolmas, W. Panphut, N. Thongon, K. Danwisetkanjana, N. Ruangrunsi, V. Meevootisom, *Wor. J. Microbiol. Biotechnol.* **2004**, 20, 265–272.
- [12] H.H. Long, N. Furuya, D. Kurose, M. Takeshita, Y. Takanami, *J. Fac. Agr.*, **2003**, 48(1-2), 21-28.
- [13] G.A. Strobel, A. Stierle, D. Stierle, W.M. Hess, *Taxomyces andreanae* a proposed new taxon for a bulbilliferous hyphomycete associated with Pacific yew. *Mycotaxon*, **1993**, 47, 71–78.
- [14] B. Guo, J. Dai, S. Ng, Y. Huang, C. Leong, W. Ong, B.K. Carte, *J. Nat. Prod.*, **2000**, 63, 602–604.
- [15] J.K. Harper, E.J. Ford, G.A. Strobel, A. Arif, D.M. Grant, J. Porco, D.P. Tomer, K. Oneill, *Tetrahedron*, **2003**, 59, 2471–2476.

- [16] G.A. Strobel, E. Ford, J. Worapong, J.K. Harper, A.M. Arif, D.M. Grant, P.C.W. Fung, K. Chan, *Phytochem.*, **2002**, 60, 179–183.
- [17] B. Zhang, G. Salituro, D. Szalkowski, Z. Li, Y. Zhang, I. Royo, D. Vilella, M. Dez, F. Pelaez, C. Ruby, R.L. Kendall, X. Mao, P. Griffin, J. Calaycay, J.R. Zierath, J.V. Heck, R.G. Smith, D.E. Moller, *Sci.*, **1999**, 284, 974–981.
- [18] K. Shrestha, G.A. Strobel, S. Prakash, M. Gewali, *Planta Med.*, **2001**, 67, 374–376.
- [19] K. Sivakumar, M.K. Sahu, K. Kathiresan, *Asi. J. Microbiol. Biotechnol. Environ. Sci.*, **2005**, 7, 457–464.
- [20] R. Balagurunathan, A. Subramanian, *Ad. Bios.*, **2001**, 20(11), 71–76.
- [21] S. Ravikumar, N. Thajuddin, P. Suganthi, S. Jacob Inbaneson, T. Vinodkumar, *J. Environ. Biol.*, **2010**, 31, 387–389.
- [22] T. Ganesh Babu, P. Nithyanand, E. Kannapiran, A. Veer Ravi, S. Karutha Pandian, Molecular identification of bacteria associated with the coral reef ecosystem of Gulf of Mannar Marine Biosphere Reserve using 16S rRNA sequences. Proceedings of MBR 2004 National Seminar on Frontiers in Marine Bioscience Research, Chennai, **2004**, 47–53.
- [23] G. Strobel, B. Daisy, *Microbiol. Mol. Biol. Rev.*, **2003**, 67(4), 491–502.
- [24] K. Ananda, K.R. Sridhar, *Can. J. Microbiol.*, **2002**, 48(10), 871–878.
- [25] K. Kathiresan, B.L. Bingham, *Adv. Mar. Biol.*, **2001**, 40, 81–251.
- [26] A. Krechel, A. Faupel J. Hallmann, A. Ulrich, G. Berg, *Can. J. Microbiol.*, **2002**, 48(9), 772–786.
- [27] D.K. Zinniel, P. Lambrecht, N.B. Harris, Z. Feng, D. Kuczmarski, P. Higley, C.A. Ishimaru, A. Arunakumari, R.G. Barletta, A.K. Vidaver, *Appl. Environ. Microbiol.*, **2002**, 68(5), 2198–2208.
- [28] M.I. Frommel, J. Nowak, J. Lazarovits, *Plant Physiol.*, **1991**, 96, 928–936.
- [29] C. Chen, E.M. Bauske, G. Musson, R. Rodríguez-Cabaña, J. Kloepper, *Biol. Con.*, **1995**, 5, 83–91.
- [30] A.V. Sturz, B.R. Christie, B.G. Matheson, *Can. J. Microbiol.*, **1997**, 44, 162–167.
- [31] K.J. Downing, J.A. Thomson, *Can. J. Microbiol.*, **2000**, 46, 363–369.
- [32] T.B. Adhikari, C.M. Joseph, G. Yang, D.A. Phillips, L.M. Nelson, *Can. J. Microbiol.*, **2001**, 47, 916–924.
- [33] P. Ellaiah, K.V. Raju, K. Adinarayana, G. Adinarayana, V. Saisha, S. Madhavi, J. Premkumar, *Hind. Antibiot. Bull.*, **2002**, 44(1-4), 8–16.
- [34] J. Gbolagade, L. Kigigha, E. Ohimain, *J. Agric. Environ. Sci.*, **2007**, 2, 364–368.
- [35] K.D. Lee, Y. Bai, D. Smith, H.S. Han, Supanjani, *Res. J. Agric. Biol. Sci.*, **2005**, 1(3), 232–236.
- [36] P. Garbeva, L.S. Van Overbeek, J.W.L. Van Vuurde, J.D. Van Elsas, *Microb. Ecol.*, **2001**, 41, 369–383.
- [37] X. Tian, L. Cao, H. Tan, W. Han, M. Chen, Y. Liu, S. Zhou, *Microb. Ecol.*, **2007**, 53, 700–707.
- [38] B. Reiter, A. Sessitsch, *Can. J. Microbiol.*, **2006**, 52(2), 140–149.