ABOUT JMA

The Journal of Microbiology and Antimicrobials (JMA) (ISSN 2141-2308) is published monthly (one volume per year) by Academic Journals.

Journal of Microbiology and Antimicrobials (JMA), is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as Disorders of the immune system, vaccines and antimicrobial drugs, Microbial Metabolism, Protozoology etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMA are peer-reviewed.

Submission of Manuscript

Please read the Instructions for Authors before submitting your manuscript. The manuscript files should be given the last name of the first author

Click here to Submit manuscripts online

If you have any difficulty using the online submission system, kindly submit via this email jma@academicjournals.org.

With questions or concerns, please contact the Editorial Office at jma@academicjournals.org.
Editors

Ass. Prof. Aamer Ikram
Department of Microbiology, Armed Forces Institute of Pathology, Microbiology, Infection Control, Biosafety Pakistan

Prof. Wang Jianhua
Gene Engineering Lab
Feed Research Institute, DNA recombinant, Recombinant protein, peptide expression, Antimicrobial peptide
Chinese Academy of Agricultural Sciences China

Dr. Mohd. Shahid
Antimicrobial Agents & Drug Resistance Researches and Microbial Biotechnology
Department of Medical Microbiology
Jawaharlal Nehru Medical College & Hospital
Aligarh Muslim University, India

Dr. Anil Vyas
Microbial Biotechnology & Biofertilizer Lab.
Department of Botany
J.N.V. University
India

Dr. (Mrs.) Amita Jain
Medical Pathology and Bacteriology
Dept. of Microbiology
King George Medical University, India

Dr. Eduardo Mere
Department of Biochemistry
Genetics, Biochemistry, Molecular Biology
University Federal of Rio de Janeiro, Brazil

Dr. Shwikar Mahmoud Abdel Salam
faculty of medicine, Alexandria University, Egypt

Dr. Gideon Mutie Kikuvi
Institute of Tropical Medicine and Infectious Diseases, Jomo Kentatta University of Agriculture and Technology
Molecular bacteriology and antimicrobial resistance
Pharmacology: Pharmacokinetics
Kenya
Editorial Board

Dr. Manal El Said El Sayed
Bilharz Research Institute (TBRI)
Ministry of Scientific Research
Medical Microbiology and Immunology
Egypt.

Dr. Amber Farooqui
Sardinian Research and Development (SARD)
Porto Conte Research Institute, Alghero,
Italy.

Dr. Chang-Gu Hyun
Applied Microbiology, Biological Science
Laboratory of Bioresources, Jeju Biodiversity
Research Institute (JBRI) & Jeju Hi-Tech Industry
Development Institute (HiDI)
Korea

Dr. Vasant P. Baradkar
Department of Microbiology,
Government Medical College
Aurangabad, Maharashtra

Dr. Manal El Said El Sayed
Medical Microbiology and Infection Control
Egypt.

As. Prof. Ömür Baysal
Turkish Ministry of Agriculture and Rural Affairs
West Mediterranean Agricultural Research Institute
(BATEM)
Plant Pathology and Molecular Biology Departments
Antalya / Turquie

Dr. Nazmul Huda
Molecular biology of microbial drug resistance,
telomere dysfunction
India.

Demelash Biffa
Molecular microbiology and epidemiology
Ethiopia.

Prof. Dr. Omar Abd El-Fattah Mohamed Fathalla
National Research Centre, Dokki, Cairo,
Medicinal Chemistry Department.
Egypt.

Dr. Amber Farooqui
Dept di Scienze Biomediche, Universita di Sassari,
Antimicrobial Chemotherapy, Epidemiology of
Infectious Diseases,
Clinical Microbiology
Italy.

Dr. Kosta V. Kostov
Military Medical Academy,
Department of Pulmonology
Pulmonology, Internal medicine
Bulgaria.

Dr. Antonio Rivera
Benemérita Universidad Autónoma de Puebla
Microbiology, Medical microbiology,
Mycoplasmatology
Mexico.

Dr. Mohammad Rahbar
Dept of Microbiology, Iranian Reference health
Laboratory.
Medical Microbiologist
Iran.

Dr. Chang-Gu Hyun
Jeju Biodiversity Research Institute (JBRI) and Jeju Hi-
Tech Industry Development Institute (HiDI)
S Korea Advanced Cosmetics, Bioactive Natural
Products Chemistry
Korea.

Dr. Abd El-Latif Hesham
Genetics Department, Faculty of Agriculture,
Assiut University,
Microbial Genetics, Biotech, biodegradation, Meta-
Genomics
Egypt.

Dr. Samuel Sunday Taiwo
Dept Med. Microbiology and Parasitology,
College of Health Sciences,
Clinical and Molecular Bacteriology
Nigeria.
Dr. Najia Dar-Odeh  
University of Jordan,  
Oral Medicine  
Jordan.

Prof. Dr. Asiye Meric  
Anadolu Univ, Fac Pharmacy,  
Dept. Pharm. Chem.,TÜRKİYE (TR)

Prof. Salah M. Azwai  
AlFateh University.  
Microbiologist  
Libya.

Prof. Dr. Abdel Salam Ahmed  
Department of Microbiology,  
Faculty of Medicine  
Alexandria University,  
Egypt.

Dr. Kuldeep Kumar Shivalya  
Indian Veterinary Research Institute,  
Izatnagar, Bareilly, PU,  
Biotechnology and Microbiology  
India.

Prof. Viroj wiwanitkit  
Wiwanitkit House, Bangkhae, Bangkok  
Clinical Medicine, Laboratory Medicine, Tropical Medicine,  
Thailand.

Dr. Hafizah Chenia  
School of Biochemistry,  
Genetics, Microbiology,Plant Pathology,University of KwaZulu-Natal  
Durban.

Dr. Gholamreza Salehi Jouzani  
Microbial Biotechnology and Biosafety Dept,  
Agric Biore institute of Iran ABRII  
Iran.

Dr. Wilson Parawira  
Institute of Food, Nutrition and Family Sciences,  
University, Zimbabwe.

Dr. Subhash C Mandal  
Division of Pharmacognosy,  
Department of Pharmaceutical Technology  
Jadavpur University  
India.

Dr. Adesemoye AO  
Department of Plant Pathology,  
Centre for integrated Plant Systems,  
Michigan State University  
Phytobacteriology, Plant Growth Promoting Rhyzobacteria  
and soil borne Plant Pathogen/soil Microbiology  
USA.

Dr. Giselli Fernandes Asensi  
Universidade Federal do Rio de Janeiro Brazil  
Microbiology, Food Microbiology  
Brazil.

Prof. Hongyue Dang  
Centre for Bioengineering and Biotech, China Univ. of Petroleum china  
Microbial Ecology and Biotechnology  
China.

Dr. Babu Joseph  
Acharya”s Bangalore School  
Microbial Biotechnology  
India.

Dr. Aamer Ali Shah  
Faculty of Biological Sci,  
Quaid-i-Azam Univ, Islamabad, Pakistan

Dr. Tadele Tolosa  
Jimma University, College of Agriculture and Veterinary Medicine,  
Ethiopia.

Dr. Urveshkumar D. Patel  
Department of Pharmacology and Toxicology,  
Veterinary College,  
Anand Agricultural University, Pharmacology and Toxicology  
(Research in Antimicrobial Therapy)  
India.

Dr. Saeed Zaker Bostanabad  
Islamic Azad University,  
Tehran Medical and Parand Branch,  
Iran.

Dr. Rakesh Kumar Singh  
Florida State University, College of Medicine  
Molecular Microbiolgy, Biochemistry, Chromatin and Genomic stability  
USA.
Ass Prof. Vintila Iuliana  
Dunarea de Jos University,  
Food Science & Technology  
Romania.

Dr. Saganuwan Alhaji Saganuwan  
University of Agriculture,  
Dept. of Physiology,  
Makurdi, Nigeria.

Dr. Eskild Petersen  
Dept. of Infectious Diseases,  
Aarhus University Hospital  
London.

Dr. Shobha  
Melaka Manipal Medical College (Manipal Campus)  
Microbiologist (Bacteriologist)  
India.

Dr. Elpis Giantsou  
Cambridge University Hospitals.  
Respiratory Medicine-Intensive Care,  
England.

Ass Prof. Emana Getu Degaga  
Addis Ababa University  
Ethiopia.

Dr. Subramanian Kaviarasan  
Dept of Molecular Medicine, University Malaya,  
Kuala Lumpur,  
India.

Ass Prof. Nongyao Kasatpibal  
Faculty of Nursing, Chiang Mai University  
Epidemiology, Infection control  
Thailand

Dr. Praveen Rishi  
Panjab University  
India

Prof. Zeinab Nabil Ahmed Said  
Microbiology & Immunology Dept,  
Faculty of Med Al-Azhar Univ.  
Egypt.

Dr. Sumit Dookia  
Ecology and Rural Development Society  
Wildlife Biology, Microbial Ecology  
India

Ass. Prof. Abdulaziz Zorgani  
Medical School, Edinburgh University

Dr. Adenike Adedayo Ogunshe  
University of Ibadan,  
Nigeria.

Prof. Itzhak Brook  
Pediatrics and Medicine, Georgetown University  
Infectious Diseases  
USA.

Dr Md. Shah Alam Sarker  
School Agric and Rural Development,  
Bangladesh Open University  
Aquaculture Nutrition and Feed Technology  
Bangladesh.

Dr. Ramnik Singh  
Khalsa College of Pharmacy  
Pharmaceutics  
Amritsar.

Prof. Amita Jain  
CSM Medical University  
Tuberculosis, Drug resistance, Virology  
India.

Prof. Yulong Yin  
Institute of Subtropical Agriculture,  
The Chinese Academy of Science  
China.

Prof. Mohan Karuppayil  
School of life sciences, Srtm university, Maharashtra  
India.

Dr. Seyedeh Seddigheh Fatemi  
Iran.

Dr. Sunil Gupta  
National Centre for Disease Control  
India.

Dr. Zakaria  
Ministry of Health, Palestinian Authority  
El Astal.
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Mustafa Gul</td>
<td>Kahramanmars Sutcuimam University, Faculty of Medicine, Department of Microbiology and Clinical Microbiology</td>
<td>Turkey</td>
</tr>
<tr>
<td>Dr. Nese Karaaslan Biyikli</td>
<td>Anadolu Medical Center Pediatric Nephrology</td>
<td>Turkey</td>
</tr>
<tr>
<td>Dr. Johnson Afonie</td>
<td>Department of Pharmacology, College of Health Sciences, Nnamdi Azikiwe University</td>
<td>Nigeria</td>
</tr>
<tr>
<td>Dr. Giri Bhoopander</td>
<td>Department of Botany, Microbial Biotechnology</td>
<td>India</td>
</tr>
<tr>
<td>Dr. Zafar Iqbal</td>
<td>Dept Plant Pathology, Univ Coll. Agriculture, Habil., Andras Fodor</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Ass Prof. Habil András Fodor</td>
<td>Department of Plant Protection, Georgikon Fac., Pannonia Univ</td>
<td>Hungary</td>
</tr>
<tr>
<td>Dr. Neelam Mewari</td>
<td>Department of Botany, University of Rajasthan, Rajasthan, Jaipur</td>
<td>India</td>
</tr>
<tr>
<td>Dr. Sanjib Bhattacharya</td>
<td>Bengal School of Tech. Pharmacy, India</td>
<td>India</td>
</tr>
<tr>
<td>Dr. Habibur Rahman</td>
<td>PSG College of Pharmacy, India</td>
<td>India</td>
</tr>
<tr>
<td>Md. Elisa Bassi</td>
<td>Department of Dermatology, Delmati Hospital</td>
<td>Italy</td>
</tr>
<tr>
<td>Iheanyi Omezuruike Okonko</td>
<td>University of Ibadan, Nigeria</td>
<td>Nigeria</td>
</tr>
<tr>
<td>Ass. Prof. Weihua Chu</td>
<td>Tongjiaxiang, Dept. of Microbiology, School of Life Science &amp; Technology, China Pharmaceutical University</td>
<td>China</td>
</tr>
<tr>
<td>Dr. Mat Yamage</td>
<td>World Organization for Animal Health (OIE)</td>
<td>Japan</td>
</tr>
<tr>
<td>Dr. Ali Abbas Qazilbash</td>
<td>United Nations Industrial Development Organization</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Dr. Kulachart Jangpataratongsa</td>
<td>Department of Clinical Microbiology, Med Tech, Mahidol University</td>
<td></td>
</tr>
<tr>
<td>Dr. Nasrin Ghasemi</td>
<td>Research and Clinical Centre for Infertility, Yazd SSU of Medical Sciences</td>
<td></td>
</tr>
<tr>
<td>Dr. Branka Vasiljevic</td>
<td>Institute of Molecular Genetics and Genetic Engineering Serbia</td>
<td></td>
</tr>
<tr>
<td>Dr. Mehmet Ulug</td>
<td>BSK Anadolu Hospital Infectious Diseases and Clinic Microbiology</td>
<td>Turkey</td>
</tr>
<tr>
<td>Dr. Vimala</td>
<td>Gitam University</td>
<td>India</td>
</tr>
<tr>
<td>Dr. Pooja Jain</td>
<td>University of California, Department of Pathology; Irvine, California</td>
<td>USA</td>
</tr>
<tr>
<td>Dr. Chellaiah Edward Raja</td>
<td>Cancer Biology Unit, School of Biological Sciences, M.K.University</td>
<td>India</td>
</tr>
</tbody>
</table>
Prof. Zeinab Nabil Ahmed Said
Fac. of Medicine (for girls) Al-Azhar University
Egypt

Prof. Manal Mohammad Baddour
Alexandria University, Faculty of Medicine,
Dept. of Microbiology and Immunology, Azarita
Egypt

Dr. Bechan Sharma
Department of Biochemistry
Coordinator: Centre for Biotechnology
University of Allahabad
Allahabad-India

Ass Prof. Ravichandran Veerasamy
Faculty of Pharmacy, AIMST University,
Pharmaceutical Chemistry, Medicinal Chemistry,
Phyto Chemistry
Malaysia

Dr. Mohammad Ibrahim
Programa de Pós-Graduação em Bioquímica
Toxicológica,
Centro de Ciências Naturais e Exatas, Universidade
Federal de Santa Maria, Brazil Biochemical
Toxicology.
Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The cover letter should include the corresponding author’s full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author’s surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review. Decisions will be made as rapidly as possible, and the journal strives to return reviewers’ comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJFS to publish manuscripts within weeks after submission.
Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors’ experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author’s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author’s name should be mentioned, followed by ‘et al’. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ‘a’ and ‘b’ after the date to distinguish the works.

Examples:


References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:


Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.
Fees and Charges: Authors are required to pay a $650 handling fee. Publication of an article in the Journal of Microbiology and Antimicrobials is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Copyright: © 2015, Academic Journals. All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Disclaimer of Warranties

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the JMA, whether or not advised of the possibility of damage, and on any theory of liability.

This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.
Antimicrobial activity of endophytic fungi isolated and identified from salt marsh plant in Vellar Estuary
Indira Kalyanasundaram, Jayaprabha Nagamuthu and Srinivasan Muthukumaraswamy
Antimicrobial activity of endophytic fungi isolated and identified from salt marsh plant in Vellar Estuary

Indira Kalyanasundaram*, Jayaprabha Nagamuthu and Srinivasan Muthukumaraswamy

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai – 608 502, Tamil Nadu, India.

Received 10 December, 2014; Accepted 23 January, 2015

The purpose of this work was to evaluate the antimicrobial potential of endophytic fungi isolated from leaves and stems of Suaeda maritima and Suaeda monoica. Many endophytes were isolated by using potato dextrose agar (PDA) medium. All the endophytic isolates were identified by using standard taxonomic keys and monographs. From a total of 16 isolates, nine potent strains were taken for further study. The fungal culture was extracted with ethyl acetate and used as a crude extract for checking antimicrobial activities by well diffusion method. The crude extract showed different inhibitory activity against all pathogens, the zones of inhibition obtained was between 2 and 12 mm. SM-EF 3 crude extract showed high zone of inhibition of 11.6±0.57 mm against Salmonella typhi, 8.3±1.52 mm against Trichophyton rubrum, respectively. The present findings concludes that SM-EF 3, SM-EF 7 and SM-EF 9 showed comparatively higher antimicrobial activity against all the human pathogens. From the present work, it is possible to conclude that these microorganisms could be promising source of bioactive compounds and warrant further study.

Key words: Endophytic fungi, antimicrobial activity, salt marsh plant, secondary metabolite.

INTRODUCTION

Endophytic microorganisms colonize living, internal tissues of the plants without causing any immediate, overt negative effects (Bacon and White, 2000; Wasser, 2002). Endophytes have proved to be the promising sources of biologically active products which are of interest for specific medicinal applications (Strobel, 2002). Recent investigations have been intensified by the potentialities of endophytic fungal strains in the production of pigments, bioactive metabolites, immune-suppressants, anticancer compounds and biocontrol agents (Wang et al., 2002; Stinson et al., 2003; Strobel and Bryn, 2003; Selvin et al., 2004; Strobel et al., 2004). Presently, research groups have identified more than hundreds of endophytic isolates from South Indian medicinal plants that showed promising activity against anti-tumour and antimicrobial agents (Gangadevi and Muthumary, 2007, 2009).

The development of drug resistance in human patho-
genic bacteria has prompted a search for more and better antibiotics, especially as diseases caused by pathogenic microorganisms now represent a clear and growing threat to world health (Raviglione et al., 1995; Pablosmendez et al., 1997). The increase of microbial resistance to antibiotics threatens public health on a global scale as it reduces the effectiveness of treatments and increases morbidity, mortality and health care costs (Coast et al., 1996). Evolution of highly resistant bacterial strains has compromised the use of newer generations of antibiotics (Levy, 2002; Levy and Marshall, 2004). Although, the active constituents may occur in lower concentrations, endophytic fungal pigments may be a better source of antimicrobial compounds than synthetic drugs. Therefore, the investigations of the antimicrobial activity of natural products have opened new ways for drug development in the control of antibiotic resistant pathogens. The researchers are currently paying more attention to the drug development from the endophytic fungi isolated from medicinal plants (Tan and Zou, 2001). The purpose of the present study was to extract, explore and characterize antimicrobial activity produced by the endophytic fungi isolated from salt marsh plant leaves and stems of *Suaeda maritima* and *Suaeda monoica*.

**MATERIALS AND METHODS**

**Sampling**

Healthy (showing no visual disease symptom) and mature plants were carefully chosen for sampling. Leaves and stem of each plant were randomly collected from Vellar estuary in Porto Novo (also known as Parangipettai Lat. 11° 29’ N; Long. 79° 47’ E). The plant material was brought to the laboratory in sterile bags and processed within a few hours after sampling. Fresh plant materials were used for isolation work to reduce the chance of contamination.

**Isolation of endophytic fungi**

Endophytic fungi were isolated from the leaves and stems of *S. maritima* and *S. monoica*. Isolation of endophytic fungi was carried out according to the method described by Suryanarayanan et al. (2003). The plant samples were rinsed gently in running water to remove dust and debris. After proper washing, stems and leaves were cut into pieces 0.5-1 cm long (150 bits per tissue/season), under aseptic conditions. Surface sterilization was done by 1-13% Sodium hypochlorite according to the type of tissues (for example higher concentration was used for leaf samples). Each set of plant material was treated with 75% ethanol for 1 min followed by immersion in sodium hypochlorite and again in 75% ethanol for 30 s. Later, the segments were rinsed three times with sterile distilled water. The plant pieces were blotted on sterile blotting paper. In each Petri dish, 8-10 segments were placed on potato dextrose agar (PDA) amended with chloramphenicol 150 mg/l. The dishes were sealed with parafilm and incubated at 27 ± 2°C for four to six weeks in dark room. The Petri dishes were monitored frequently to check the growth of endophytic fungal colonies.

**Identification of endophytic fungi**

For characterisation of the morphology of fungal isolates, slides prepared from cultures were stained with lactophenol cotton blue reagent and examined with a bright-field and phase-contrast microscope. The taxa were assigned to genera following Barnett and Hunter (1998).

**Cultivation for screening and isolation of secondary metabolites**

For small scale fermentation, each fungal strain was inoculated into a 1000 ml Erlenmeyer flask containing 300 ml of liquid PDA medium. For this purpose, a strain that nearly covered the surface of a Petri dish (after one to two weeks growth on PDA medium) was cut into small pieces and these were transferred to an Erlenmeyer flask containing the sterilised medium. Cultivation was performed at room temperature under static conditions and daylight. Depending on the fungal growth, cultures on liquid medium were incubated for three to four weeks. The fermentation was brought to an end by adding 250 ml EtOAc to the culture flask and standing closed for at least 24 h.

**Extraction of fungal liquid cultures**

**Total extraction of culture media and mycelia**

250 ml EtOAc were added to each Erlenmeyer flask containing 300 ml culture medium and left overnight to stop cell growth. Culture media and mycelia were then extracted in the Ultraturrax for 10 min for cell destruction and filtered under vacuum using a Buchner funnel. The mycelium was discarded and the culture filtrate transferred to a separation funnel. The EtOAc and H2O phases were separated and the aqueous phase extracted two more times with 300 ml EtOAc each. All obtained extracts were taken to dryness under reduced pressure at 40°C. After evaporating the solvent, the residue was mixed with the same solvent, these crude extracts were subjected to antimicrobial assays.

**Pathogens used for antimicrobial activity**

 Totally eight human bacterial pathogens used were *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Salmonella paratyphi*, *Vibrio cholera*, *Klebsiella oxytoca*, *Klebsiella pneumonia* and *Staphylococcus aureus*. The stock culture was maintained on nutrient agar medium at 4°C. Five human fungal pathogens namely *Candida albicans*, *Epidermophyton floccosum*, *Micosporum canis*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* were also used. The stock culture was maintained on PDA medium at 4°C. These bacterial and fungal strains were isolated and obtained from the Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

**Screening for antimicrobial activity**

**Antagonistic assay for endophytic fungal extract against bacterial pathogens**

Antagonistic assay was done by an agar-well diffusion method in aerobic condition. Isolated endophytic fungal extract were tested for the antibacterial activity. Bacterial pathogens were spread on MHA plates. Then wells were made and 50 μL crude extract of each strain was inoculated into a separate well. Antagonistic activity was detected after an incubation of 24 to 48 h at 35°C. The presence of zone clearance on agar plates was used as an indicator for the antibacterial activity. The strains which showed the maximum zone of clearance was chosen for further study. The presence of zone of
clearance on agar plates was used as an indicator of bioactive potential of the strain (Portrait et al., 1999).

RESULTS

*S. maritima* and *S. monoica* were collected from Vellar estuary in Southeast coast of India. Totally, 1200 segments (150 leaves, 150 stem per season) were screened for isolating endophytic fungi. From 1200 tissue segments, 433 isolates were produced in culture of *S. maritima* grouped into 15 taxa. 422 isolates were produced in the culture of *S. monoica* grouped into 14 taxa. All the endophytic isolates were identified by using standard taxonomic keys and monographs and are shown in Figures 1 to 16. In the present investigation, a
total of nine endophytic fungi were selected for screening of antimicrobial activity against human pathogens. The selected endophytic fungi are shown in Table 1.

Endophytic fungal crude extracts were tested against the bacterial and fungal pathogens by well diffusion method. Totally, 13 microorganisms which consisted of 8 bacteria and five fungi were tested. The ethyl acetate extracts were assayed against the test organisms, the zones of inhibition obtained was between 2 and 12 mm. The results of preliminary screening tests are summa-
Figure 12. Light microscopic observation of fungi. 
Meyerozyma sp.

Figure 13. Light microscopic observation of fungi. 
Penicillium sp. 1.

Figure 14. Light microscopic observation of fungi. 
Penicillium sp. 2.

Figure 15. Light microscopic observation of fungi. 
Phoma sp.

Figure 16. Light microscopic observation of fungi. 
Ulocladium sp.

Table 1. The selected endophytic fungi.

<table>
<thead>
<tr>
<th>Strain code</th>
<th>Strain name</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM-EF 1</td>
<td>Alternaria alternate</td>
</tr>
<tr>
<td>SM-EF 2</td>
<td>Aspergillus flavus</td>
</tr>
<tr>
<td>SM-EF 3</td>
<td>A. terreus</td>
</tr>
<tr>
<td>SM-EF 4</td>
<td>A. niger</td>
</tr>
<tr>
<td>SM-EF 5</td>
<td>Cladosporium sp.</td>
</tr>
<tr>
<td>SM-EF 6</td>
<td>Fusarium sp.</td>
</tr>
<tr>
<td>SM-EF 7</td>
<td>Penicillium sp.</td>
</tr>
<tr>
<td>SM-EF 8</td>
<td>Sterile mycelium</td>
</tr>
<tr>
<td>SM-EF 9</td>
<td>Meyerozyma sp.</td>
</tr>
</tbody>
</table>

In antibacterial activity, SM-EF 3 crude extract showed high zone of inhibition of 11 mm against S. typhi, 10 mm against S. aureus and V. cholera, 9 mm against E. coli and K. pneumonia, 8 mm against S. paratyphi, low activity of 5 mm against K. oxytoca. SM-EF 7 crude extract showed a zone of inhibition of 11 mm against S. typhi, 10 mm against V. cholera, 9 mm against E. coli, 8
mm against *K. oxytoca*, 7 mm against *S. paratyphi*, 5 mm against *K. pneumonia*. SM-EF 9 crude extract showed a zone of inhibition of 11 mm against *E. coli*, 10 mm against *S. paratyphi*, 8 mm against *P. mirabilis* and *S. aureus*, 7 mm against *S. typhi* and *V. cholera* and 6 mm against *K. pneumonia* (Table 2).

In antifungal activity, SM-EF 3 crude extract showed a zone of inhibition of 6 mm against *T. rubrum*, 6 mm against *M. canis*, 4 mm against *C. albicans*, 3 mm against *T. mentagrophyte*. SM-EF 7 crude extract showed only a zone of inhibition of 6 mm against *M. canis*, 5 mm against *C. albicans*, 4 mm against *E. floccosum* and 3 mm against *T. rubrum*. SM-EF 9 crude extract showed only a zone of inhibition of 7 mm against *M. canis*, 6 mm against *E. floccosum* and *T. rubrum*, 4 mm against *T. mentagrophyte* (Table 3).

**DISCUSSION**

In the present study, endophytic fungi of halophytes were investigated on two plant species (*S. maritima* and *S. monoina*). 433 isolates were produced in culture of *S. maritima* grouped into 15 taxa, 422 isolates were produced in culture of *S. monoica* grouped into 14 taxa. Suryanarayanan and Kumaresan (2000) isolated endophytic fungi of some halophytes from an estuarine mangrove forest. Kumaresan and Suryanarayanan (2001) reported the fact that the species diversity of the endophytes varied in different mangrove hosts indicating that a selection mechanism was operating in constituting the endophyte assemblages.

Discovery of endophytic fungi in plant tissues opened up new possibilities in search for metabolically active compounds. Cuomo et al. (1995) examined a large number of terrestrial and marine fungal isolates and found a higher number of anti-microbially active species among marine isolates. Endophytes or any type of fungus are capable of producing novel secondary metabolites as the reports says many of the endophytes are still unknown and the compound are produced by the respective fungus are still unknown. So with this view, the salt marsh endophytic fungus is taken for testing its production for secondary metabolites.

---

**Table 2. Zone of inhibition produced by endophytic fungal strains on human bacterial pathogens.**

<table>
<thead>
<tr>
<th>Endophytic fungi</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>SM-EF 1</td>
<td>5.6±0.57</td>
</tr>
<tr>
<td>SM-EF 2</td>
<td>-</td>
</tr>
<tr>
<td>SM-EF 3</td>
<td>9.3±1.52</td>
</tr>
<tr>
<td>SM-EF 4</td>
<td>4±1</td>
</tr>
<tr>
<td>SM-EF 5</td>
<td>6.6±0.57</td>
</tr>
<tr>
<td>SM-EF 6</td>
<td>4.3±0.57</td>
</tr>
<tr>
<td>SM-EF 7</td>
<td>9.6±0.57</td>
</tr>
<tr>
<td>SM-EF 8</td>
<td>-</td>
</tr>
<tr>
<td>SM-EF 9</td>
<td>11.3±0.57</td>
</tr>
</tbody>
</table>

Values are average of three replicates ± SE.

---

**Table 3. Zone of inhibition produced by endophytic fungal strains on human fungal pathogens.**

<table>
<thead>
<tr>
<th>Endophytic fungi</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td>SM-EF 1</td>
<td>-</td>
</tr>
<tr>
<td>SM-EF 2</td>
<td>2.3±0.57</td>
</tr>
<tr>
<td>SM-EF 3</td>
<td>4.6±0.57</td>
</tr>
<tr>
<td>SM-EF 4</td>
<td>-</td>
</tr>
<tr>
<td>SM-EF 5</td>
<td>-</td>
</tr>
<tr>
<td>SM-EF 6</td>
<td>-</td>
</tr>
<tr>
<td>SM-EF 7</td>
<td>5.3±1.15</td>
</tr>
<tr>
<td>SM-EF 8</td>
<td>3.3±0.57</td>
</tr>
<tr>
<td>SM-EF 9</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are average of three replicates ± SE.
Antimicrobial activities of compounds biosynthesised by the plant endophytes have been reported only by few researchers (Nishioka et al., 1997; Huang et al., 2001; Strobel et al., 2001; Harper et al., 2003). The bioassay method is very useful for applying the screening for antimitotic and antifungal activities of secondary metabolites from various natural sources and it is quick and easy method (Jagessar, 2007; Kobayashi et al., 1996).

In the present study, nine crude extracts of endophytic fungal species were tested against the human bacterial and fungal pathogens by well diffusion method. All crude extracts of endophytic fungal species showed activity against human bacterial and fungal pathogens. Guimarães et al. (2008) screened extracts from 39 endophytic fungi isolated from Viguiera arenaria and Tithonia diversifolia, and reported 5.1% active extracts against S. aureus and 25.6% active extracts against E. coli. Similarly, several metabolites of the marine isolate, A. niger showed anti-bacterial and anti-fungal potential.

In this study, 70% of endophytic fungi showed antibacterial and antifungal activity against at least one of the test human pathogens, which was coincidence with our previous report (Liu et al., 2001). The ethyl acetate extracts were assayed against the test organisms, the zones of inhibition obtained in this study was between 2 and 12 mm. The maximum zone of inhibition was 11.6±0.57 mm against S. typhi. Selim et al. (2011) reported ethyl acetate crude extract of 55 endophytic isolates (55.5%) of 99 screened strains, exhibited significant inhibitory activity against a wide range of pathogenic test microorganisms, with diameters of inhibition zones ranging from 9 to 27 mm for the test bacteria, and from 8 to 31 mm for test Candida on disc diffusion assay.

Antimicrobial activity of A. terreus, Meyerozyma sp. and Penicillium showed significant effect on different Gram positive and negative bacteria and on different fungi. These endophytes can reduce the growth of the harmful bacteria or fungi by different mode of action. Our results correlated with the findings of other reports (Verma et al., 2009; Wiyakrutta et al., 2004; Corrado and Rodrigues, 2004; Li et al., 2008; Ramasamy et al., 2010) which they reported the antimicrobial activity of endophytes. Similarly, Prabavathy and Valli Nachiyar (2012) studied the crude extract from culture filtrate of the endophytes from Plumbago zeylanica, Aegle marmelos and Ficus carica that showed considerable activities, the mycelial as well as culture filtrate extract of endophyte isolated from Ficus carica alone showed appreciable antibacterial activities against Pseudomonas aeruginosa.

Conclusion

Salt marsh plants harbours diverse species of endophytic fungi and some of these isolates exhibited significant inhibitory activity on human pathogenic microorganisms. The study revealed the presence of good antibacterial activity for the crude extracts SM-EF 3, SM-EF 7 and SM-EF 9 could be a good source for bioactive compounds and the isolated compounds may be further checked in in vivo model as antimicrobial agents.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

We thank the authorities of Annamalai University for providing the necessary facilities and the first author thank the Department of Science and Technology (DST)-Promotion of University Research and Scientific Excellence (PURSE) Programme for their financial support.

REFERENCES


