Alexandria Journal of Medicine (2016) **xxx**, xxx–xxx

HOSTED BY

ELSEVIER

Alexandria University Faculty of Medicine

Alexandria Journal of Medicine

http://www.elsevier.com/locate/ajme

Antimicrobial activity of some actinomycetes from Western Ghats of Tamil Nadu, India

Pathalam Ganesan [a], Appadurai Daniel Reegan [a,b], Rajendran Host Antony David [a], Munusamy Rajiv Gandhi [a], Michael Gabriel Paulraj [a], Naif Abdullah Al-Dhabi [c], Savarimuthu Ignacimuthu [a,d,*]

[a] *Entomology Research Institute, Loyola College, Chennai 600 034, Tamil Nadu, India*
[b] *National Vector Borne Disease Control Programme, ROHFW, Govt. of India, Chennai 600 090, India*
[c] *Department of Botany and Microbiology, Addiriyah Chair for Environmental Studies, College of Science, King Saudi University, Riyadh, Saudi Arabia*
[d] *Visiting Professor Programme, Deanship and Scientific Research,College of Science, King Saud University, Riyadh, Saudi Arabia*

Received 4 February 2016; revised 5 March 2016; accepted 15 March 2016

KEYWORDS

Streptomyces rimosus;
Antimicrobial activity;
Disc diffusion;
Zone of inhibition

Abstract *Introduction:* Microbial diseases are increasing year by year and they are becoming a big threat to public health. There are more than 200 known diseases transmitted by bacteria, fungi, viruses, prions, rickettsia and other microbes to humans. The emergence of drug resistance to chemical drugs is the biggest threat in controlling human pathogens. Hence novel antimicrobial agents from actinomycetes are timely needed for the control of several human pathogens.
Aim: The aim was to find some actinomycetes with antimicrobial metabolites.
Methods: Soil samples were collected from Nilgiris district in Western Ghats of Tamil Nadu, India. Actinomycetes were isolated using serial dilution and plating techniques on actinomycetes isolation agar. Streptomycin and ketoconazole (25 μg/disc) were used as reference controls. The active strains were identified by 16S *rRNA* and phylogenetic tree was constructed; the sequences were submitted in the GenBank.
Results: Totally 106 actinomycete strains were isolated and cross streaked against various human microbial pathogens. Only 44 (41.50%) exhibited good antimicrobial activity against different pathogenic microbes. Five isolates (FMS-20, TGH-30, TGH-31, TGH-31-1 and IS-4) were chosen for secondary screening using filtrate. Among them FMS-20 filtrate showed good inhibition on the 16th day against all tested microbial pathogens. Further the intracellular methanol extract of FMS-20 showed maximum zone of inhibition against *A. brasiliensis* (22 mm) at 5 mg/disc. Similarly the extracellular ethyl acetate extract of FMS-20 showed maximum zone of inhibition against *B. subtilis* (25 mm).

* Corresponding author at: Entomology Research Institute, Loyola College, Nungambakkam, Chennai 600 034, Tamil Nadu, India. Tel.: +91 44 2817 8348; fax: +91 44 2817 5566.
E-mail address: eriloyola@hotmail.com (S. Ignacimuthu).

Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2016.03.004

Please cite this article in press as: Ganesan P et al. Antimicrobial activity of some actinomycetes from Western Ghats of Tamil Nadu, India, *Alex J Med* (2016), http://dx.doi.org/10.1016/j.ajme.2016.03.004

Conclusions: The present work revealed that, among 106 actinomycetes screened, *Streptomyces rimosus* (FMS-20) (Accession No-KT827106) showed promising antimicrobial activity against all the tested human microbial pathogens.

1. Introduction

Microbial diseases are increasing year by year and they are becoming a big threat to public health.[1–4] There are more than 200 known diseases transmitted by bacteria, fungi, viruses, prions, rickettsia and other microbes to humans.[5,6] Among the different microbial pathogens, viruses or prions cause 37–44% of diseases, bacteria or rickettsia cause 10–30% of diseases, protozoa cause 10.7% of diseases, fungi cause 6.3% of diseases and helminths cause 3.3% of diseases, leading to millions of death every year.[7–9] Many bacteria excrete through faeces, which can cause undesirable effects in health and environment.[10–13] The emergence of drug and multidrug-resistant pathogen is the biggest threat; consequently, novel antimicrobial agents from natural sources with novel mechanisms of action, are urgently needed in medical and pharmaceutical sectors.

Many research works have been carried out to control the pathogens and to identify new antimicrobial agents.[14–16] Microbes from soils are the most important natural sources exhibiting strong biological activity against a wide range of pathogens.[17] Generally microbes produce bioactive molecules which are unnecessary for their growth and development but useful in defence mechanism.[18] Soil microorganisms in particular are intensively exploited.[19]

Actinomycetes are Gram positive, filamentous bacteria with 55% of guanine and cytosine in their DNA.[20–23] Actinomycetes represent one of the most important classes of bacteria for their ability to produce a wide range of biologically active secondary metabolites, which are very effective against microbial pathogens.[20,23] More than 70–80% of all known antibiotics have been isolated from actinomycetes and are used in medicine and agriculture.[24] The genus *Streptomyces* is the biggest producer of antibiotics. Several microbial secondary metabolites are reported to be rich sources of therapeutic drugs.[25,26]

The present study aimed to evaluate some actinomycetes from different soils for antimicrobial activity against human pathogens.

2. Materials and methods

2.1. Isolation of actinomycetes from soil samples

Soil samples were collected from five different places in Western Ghats of Tamil Nadu India viz., Topslip greenhouse (TGH), Fishery mountain, (FMS), near dam mountain (NDM), Iduhatty (IS) and Kothagiri (KS). The soil samples were collected from 15 cm depth using sterile technique as per the method of Valan Arasu et al.[20] and transported to the laboratory. These soil samples were air-dried for 34 h at 45 °C, crushed, and sieved prior to use for isolation following established method.[27] The isolation of actinomycetes was done by standard serial dilution method. One gram of soil was suspended in 9 ml of sterile double-distilled water. The dilution was carried out up to 10^{-5} dilutions. Aliquots (0.1 ml) of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were spread on the actinomycetes isolation agar (AIA) medium containing nalidixic acid (100 mg/l) and ketoconazole (30 mg/l) and incubated at 30 °C for 7–10 days.[20,28–31] Based on the colony morphological characterization, the actinomycetes were selected and purified on ISP-2 (International Streptomyces project medium No. 2).

2.2. Morphological characterization of isolates

Morphological features of active isolates were observed with a light microscope.[18,20,30,32–34] Morphological features were observed in various media such as Actinomycetes isolation agar (AIA), Starch casein agar (SCA), Yeast peptone glucose agar (YPG), Bennet medium (BENNET), M3 medium (M3), Modified nutrient glucose agar medium (MNGA), ISP-International Streptomyces Project No. 2 (ISP-2), ISP-International Streptomyces Project No. 4 (ISP-4), ISP-International Streptomyces Project No. 6 (ISP-6), and ISP-International Streptomyces Project No. 7 (ISP-7). The results were recorded after incubation at 30 °C for 7–10 days.

2.3. Preliminary screening for antimicrobial activity

The antimicrobial activities of isolated actinomycetes were performed by cross streak method.[35] AIA plates were prepared and inoculated with isolates by single streak in the centre of petri plate and incubated at 30 °C for 10 days. The plates were then inoculated with the test organism by a single streak at 90° angles to the actinomycetes strain and incubated at 37 °C overnight. Then the antagonism of test organism was recorded. The human bacterial pathogenic organisms such as *Staphylococcus aureus* (MTCC-96), *Micrococcus luteus* (MTCC-106), *Enterococcus faecalis* (MTCC-439), *Bacillus subtilis* (MTCC-441), *Staphylococcus epidermidis* (MTCC-3615), *Klebsiella pneumonia* (MTCC-109), *Enterobacter aerogenes* (MTCC-111), *Vibrio parahaemolyticus* (MTCC-451), *Yersinia enterocolitica* (MTCC-840), *Saccharomyces cerevisiae* (MTCC-251), *Shigella flexneri* (MTCC-1457), *Proteus vulgaris* (MTCC-1771), *Pseudomonas mendocina* (MTCC-11808) and human pathogenic candidal strains like *Candida albicans* (MTCC-4748), *Candida krusei* (MTCC-9215), *Candida tropicalis* (MTCC-4370) and *Candida parapsilosis* (MTCC-1965) and human pathogenic fungal strains like *Trichophyton mentagrophytes* (MTCC-8476), *Scopulariopsis* sp. (MTCC-3553), *Aspergillus niger* (MTCC-10180), *Botyritis cinerea* (MTCC-2880), *Epidermo floccosum* (MTCC-613), *Aspergillus tubingenis* (MTCC-961) and *Aspergillus brasiliensis* (MTCC-1344) were used in the present study.

Please cite this article in press as: Ganesan P et al. Antimicrobial activity of some actinomycetes from Western Ghats of Tamil Nadu, India, *Alex J Med* (2016), http://dx.doi.org/10.1016/j.ajme.2016.03.004