Journal of Applied and Natural Science 5 (2): 335-337 (2013)

JANS

Antibacterial activity of actinomycetes against bacterial pathogens of diabetic foot ulcers

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Received: July 10, 2013; Revised received: August 6, 2013; Accepted: August 10, 2013

Abstract: Diabetes mellitus is a serious public health problem worldwide. Diabetic foot ulcers (DFU), a major complication in Type 2 diabetes are one of the major causes of morbidity and mortality around the world. To screen various bacterial pathogens present in diabetic foot ulcers and to determine their antibiotic sensitivity to actinomycetes isolated from various fields of Chandragiri, Tirupati, twenty four actinomycetes isolates were isolated and screened by primary and secondary screening methods to determine potent antibiotic producers by using test organisms. Among 24 isolates, 4 were more potent and they showed varied range of antibacterial activity of pathogens, isolated form diabetic foot ulcers. Four isolates were compared with lenezoid antibiotic. Enterococcus was resistant to lenezoid antibiotic but four actinomycetes inhibited the growth of Enterococi.

Keywords: Actinomycetes, Diabetic foot ulcers, Lenezoid, Antibacterial activity

INTRODUCTION

Diabetes mellitus is a chronic disorder of the endocrine system which plaques approximately 17 million people nationwide (Siva Kumari and Shanthi, 2009; Ozer *et al*., 2010). Diabetic foot ulcers (DFU) are a major complication in Type 2 diabetes are one of the major causes of morbidity and mortality around the world. It occurs in 15% of all patients with diabetes and precedes 84% of all lower leg amputations (Bakker *et al*., 2005). Major increase in mortality among diabetic patients, observed over the past 20 years is considered to be due to the development of macro and micro vascular complications, including failure of the wound healing process. A diabetic foot ulcer involves repeated infections due to aerobes, anaerobes or fungi or in combinations (Siva Kumari and Shanti, 2009). Often these organisms interact with each other and form biofilms which is difficult to treat, enhancing antimicrobial resistance and lead to non-healing ulcer. The microorganisms that occur on foot ulcers generally are *Staphylococcus aureus*, *Streptococcus pyogens* arising from the patient's own skin and *Enterococci* from bowel, *Pseudomonas* sp, *Enterococcus* sp and *Proteus* sp. carry a special role and are responsible for continuing and extensive tissue destruction with the poor blood circulation of the foot (Pathare *et al*., 1998; Anandi *et al*., 2004). The increasing association of multi-drug resistant (MDR) pathogens with diabetic foot ulcers for further compounds the challenge faced by the physician in treating diabetic ulcers without amputation (Yoga *et al*., 2006).

Screening of microorganisms for the production of novel antibiotics has been intensively pursued for many years by scientist (Tara Devi *et al*., 2009; Naikpatil and Rathod, 2011). Antibiotics have been used in many fields including agriculture, veterinary and pharmaceutical industry. Actinomycetes have the ability to synthesize many different biologically active secondary metabolites such as antibiotics, herbicides, pesticides, anti-parasitic and enzymes like cellulose and xylanases used in the waste water treatment. Of these compounds, antibiotics predominate in therapeutic and commercial importance (Lacey, 1973; Mc-Carthy and Williams, 1990; Ouhdouch *et al*., 2001; Saadoun and Gharaibeh, 2003). The present study was aimed to isolate actinomycetes from soil and to test for their antibacterial activity against the bacterial pathogens of the diabetic foot ulcers.

MATERIALS AND METHODS

Isolation of actinomycetes: Soil samples were collected from the various fields of Chandragiri near Titupati of Chittoor District, Andhra Pradesh. All the soil samples were subjected to serial dilution method by using starch casein agar medium (SCA) (Johnson and Curl, 1972). Twenty four isolates were isolated and named as Ms_1, Ms_2 and so on. Various biochemical tests were performed for the identification of the actinomycete isolates. All isolates were screened to select a potent isolate to carry out further work.

Pathogens of DFU: Pure cultures of bacterial pathogens

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of diabetic foot ulcers were collected from the Department of Microbiology of S.V. Medical College, Tirupati, Andhra Pradesh. Pathogens include *Staphylococcus aureus*, *Escherichia coli*, *Primilidium*, *Enterococci*, *Pseudomonas* and *Klebsiella*.

Screening of actinomycetes for antibacterial activity: Screening was performed in two parts - primary screening and secondary screening.

Primary screening: Twenty four isolates were subjected to primary screening by perpendicular streak method using *Bacillus*, *Staphylococcus*, *Escherichia coli*, *Klebsiella* and *Pseudomonas* as test organisms to select a potent antibiotic producing isolate. Among the 24 isolates, 11 isolates showed maximum inhibition of test organisms compared to others.

Secondary screening: Eleven isolates were separately inoculated to starch casein broth and incubated at 37^{0}C under continuous shaking for 7 days. After incubation period, the content of the flask filtered through Whatman No. 1 filter paper. Then the filtrate used as a crude antibiotic extract. The test organisms were spread over the nutrient agar medium separately. Then wells were made by using cork borer and 50µl of the crude extract was added to the each well and incubated at 37^{0}C for 24 hrs. Among the seven isolates 4 (Ms_1, Ms_2, Ms_3 and Ms_4) isolates showed maximum inhibition of the test organisms compared to others and they were selected for further study.

Extraction of antibacterial metabolites: 250 ml of starch casein broth was inoculated with above four isolates separately and incubated at 37^{0}C for 7 days on continuous shaking. After incubation, the content of the flasks were filtered through Whatman No.1 filter paper. An antimicrobial compounds were extracted from the culture filtrate by solvent extraction method of Westly *et al* (1986). Ethyl acetate was added to the filtrate in the ratio of 1:1 (v/v) and shaken vigorously for 1 hour for complete extraction. The organic phase was separated and evaporated to dryness by keeping at 80-90^{0}C on water bath. The residue was dissolved in 1 ml of ethyl acetate.

Antimicrobial activity of the actinomycetes against DFU bacterial pathogens: Antimicrobial activity was performed in Muller-Hinton agar medium by using well method (Sen *et al.*, 1995). After medium solidified, microbial pathogens were spread on the medium and wells were made with the help of the cork borer and they were sealed to prevent the leakage of the extract. To this wells the above extract was loaded and incubated at 37^{0}C for 24 hrs. All the above antimicrobial extracts were compared with the linezolid antibiotic which is commonly used in the treatment of DFU.

RESULTS AND DISCUSSION

The prevalence of the diabetes is increasing in India faster than the other countries in the world. There are about 33 million diabetics mainly from the urban population.

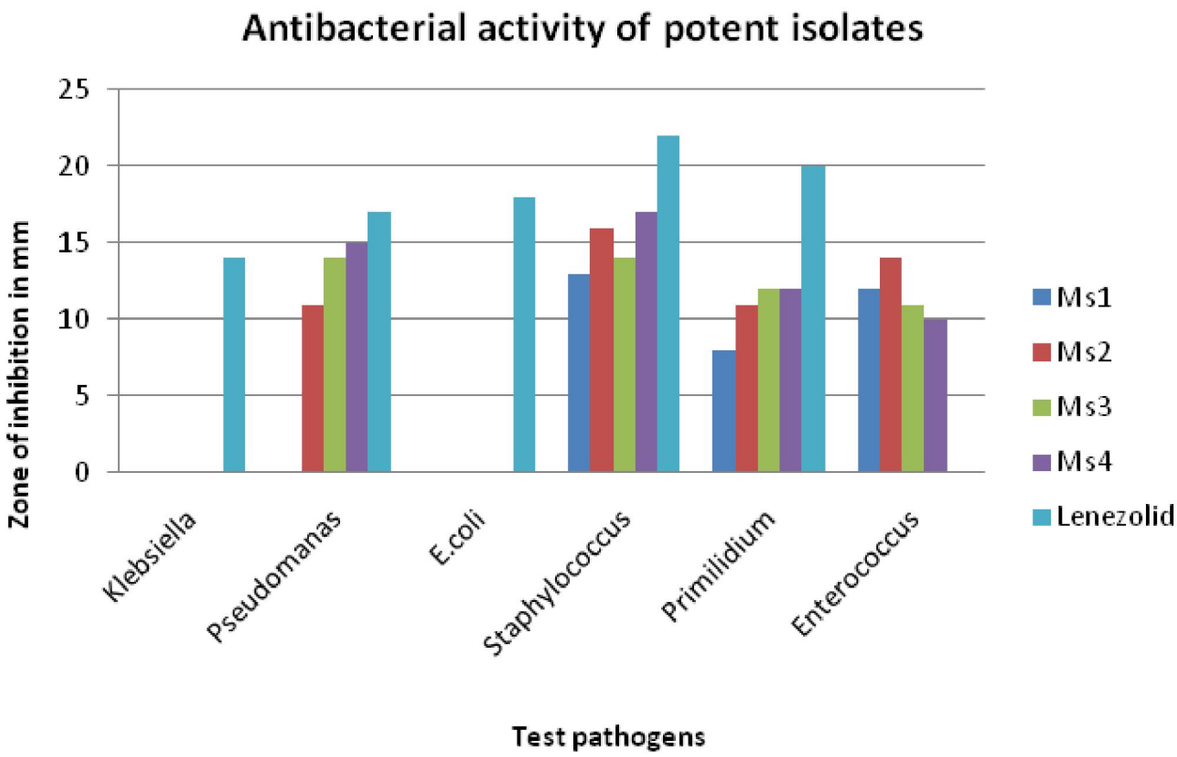

Fig 1. *Antibacterial activity of different isolates of actinomycetes.*

Diabetes affects many other organs of the body but our study was carried out only on the diabetic foot infections of the affected patients in Tirupati, India. Peripheral vascular disease and neuropathy and other risk factors promotes the development of the diabetic foot ulcers with infection that can spread rapidly and lead to tissue destruction and subsequently amputation (Edmonds and Foster, 2004).

Twenty four isolates were isolated from the soil and were subjected to primary and secondary screenings to select potent antibiotic producers. Among the 24 isolates, 4 isolates showed maximum inhibition of test cultures like *Bacillus, Staphylococcus, Escherichia coli, Klebsiella* and *Pseudomonas*, respectively (Oskay *et al.*, 2004; Dhananjeyan *et al.*, 2010; Ozer *et al.*, 2010). Four isolates (Ms_1, Ms_2, Ms_3 and Ms_4) inhibited the growth of the pathogens of diabetic foot ulcers. Among the four isolates, Ms_4 isolate showed maximum inhibition of all the pathogen, except *E.coli* compared to others and followed by Ms_3, Ms_2, and Ms_1, respectively (Fig. 1). *E.coli* and *Klebsiella* were resistant to all the actinomycetes, but they are controlled by linezolid antibiotic. *Enterococci* were resistant to linezolid antibiotic, but controlled by the antimicrobial extracts of the all the four actinomycetes isolates.

The search for novel metabolites especially from actinomycetes requires a large number of isolates (over thousands) in order to discover a novel compound of pharmaceutical interest. The search will be more promising if divers actinomycetes are sampled and screened. For this reason, soils were specifically collected under identified farming soils. This is based on the hypothesis that actinomycetes diversity may be influenced by the diversity of cultivated plant species as these bacteria grow profusely in the humus and leaf litter layer. Furthermore, different plants produce different type of secondary metabolites and some of these chemical compounds are toxic to soil microorganisms including actinomycetes. However, adaptation has in turn leaded the actinomycetes to produce their own secondary metabolites.

It was concluded that among 24 isolates, isolated from soil of fields of Chandragiri near Titupati of Chittoor District, four isolates were potent. Enterococcus was resistant to lenezoid antibiotic while four actinomycetes inhibited the growth of Enterococi.

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