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Research Article

ANTIBACTERIAL ACTIVITY OF BIOACTIVE *STREPTOMYCES* SPECIES MPPO-02 AGAINST CLINICAL ISOLATES OF BURN, DENTAL CARIES AND URINARY TRACT INFECTIONS

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*Correspondence	ABSTRACT
<p>Prashith Kekuda T.R P.G. Department of Studies and Research in Microbiology, Sahyadri Science College (Autonomous) campus, Kuvempu University, Shivamogga, Karnataka, India</p> <p>DOI: 10.7897/2321-6328.02113</p> <p>Article Received on: 07/01/14 Accepted on: 17/02/14</p>	<p>The present study was conducted to determine antibacterial activity of ethyl acetate extract obtained from the fermentation broth of bioactive <i>Streptomyces</i> species MPPO-02 previously isolated from a rhizosphere soil of Mahishi, Karnataka, India. Agar well diffusion assay was performed to investigate inhibitory effect of ethyl acetate extract against a total of 15 clinical isolates from burn, dental caries and urinary tract infection. The extract was found inhibitory against all test bacteria but to a varied extent. Gram positive bacteria were found more susceptible to extract when compared to Gram negative bacteria. The isolate MPPO-02 can be exploited for the development of agents active against pathogenic microorganisms. Further studies on purification of active principles from solvent extract, their characterization and inhibitory activity are to be carried out.</p> <p>Keywords: Western Ghats, Mahishi, <i>Streptomyces</i>, Antibacterial activity, Burn, Dental caries, Urinary tract infection</p>

INTRODUCTION

In the 20th century, the launching of therapies effective against bacterial infections has completely revolutionized the field of medicine and facilitated the development of modern medicine. The antibiotic therapy cured potentially life-threatening conditions and greatly reduced the incidence of death or disability. These antibiotics had truly become the 'solution' of medicine field and were used to treat even the most common types of infections. However, this overwhelming historical success was challenged by a serious threat to antibiotic therapy i.e., development of resistance against antibiotics. Overuse and abuse of antibiotics are the main factors which are responsible for the development of resistance against antibiotics. Bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, enterococci, *Mycobacterium tuberculosis*, *Escherichia coli*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* are few among the important antibiotic resistant bacteria. Everyday the bacteria, previously sensitive to antimicrobials, are being reported to have developed resistance against different antimicrobials. Initially these antibiotic resistant pathogens have caused significant nosocomial infections and more recently they have spread to community as well and are causing severe illness. Moreover, the ability of microbes to transmit and acquire resistance genes has made the situation even worst. Above all, insufficient investment for antimicrobial research and a scarcity of novel structural classes of antimicrobials to replace ineffective antimicrobials negatively influence successful therapy for infections caused by drug resistant microbes. This situation strongly highlighted need for the discovery of novel antimicrobials and treatment strategies¹⁻⁷.

Actinomycetes are Gram-positive filamentous bacteria possessing high G + C content in their genome. These organisms are widely distributed in soil, water and plants (endophytes) and have provided a vast majority of biologically active compounds (45 %). Among actinomycetes, the species belonging to the genus *Streptomyces* constitute a major portion (50 %) of the total population of soil actinomycetes. The morphological differentiation of *Streptomyces* involves the formation of hyphae that can differentiate into a chain of uninucleated spores by the formation of septa at regular intervals and the process requires a specialized and coordinated metabolism. The soil streptomycetes are known to play an important role in the biodegradation of complex polymers and xenobiotics. Besides, species of *Streptomyces* are well known for producing a variety of metabolites antibiotics, immune modulators, anticancer drugs, antiviral drugs, antioxidants, herbicides, anthelmintics, enzyme inhibitory agents, enzymes and insecticides. *Streptomyces* species are considered as biotechnologically valuable prokaryotes as they have produced > 70 % of bioactive compounds having medical and agricultural importance. Even though a number of antibiotics have been produced from *Streptomyces*, these account for only a small fraction of the repertoire of bioactive compounds produced by them. Hence, isolation of new *Streptomyces* species from natural sources like soil and characterization of their antibiotic activity is a valuable endeavor⁸⁻¹¹. In this study, we report antibacterial activity of bioactive *Streptomyces* species MPPO-02 isolated from a rhizosphere soil of village Mahishi, Karnataka, India against clinical isolates of burn, dental caries and urinary tract infection. In our previous study, the isolate MPPO-02 was

found to display inhibitory activity against a panel of Gram negative and Gram positive bacteria¹².

MATERIALS AND METHODS

Isolation of *Streptomyces* species MPPO-02

The isolate MPPO-02 (Figure 1) was recovered on Starch casein nitrate (SCN) agar from a rhizosphere soil of Mahishi by serial dilution-plating method in our previous study. The isolate was identified as a species of *Streptomyces* on the basis of microscopic, cultural and biochemical characteristics¹².

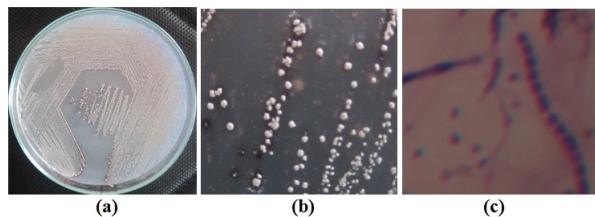


Figure 1: Culture [(a) and (b)] and spore arrangement [(c)] of isolate MPPO-02

Fermentation

Erlenmeyer flask containing 250 ml sterile SCN broth medium was aseptically inoculated with spore suspension of well sporulated culture of *Streptomyces* species MPPO-02. The flask was aerobically incubated at 28°C for 10 days. After incubation, the fermentation broth was aseptically filtered through sterile Whatman No. 1 filter paper¹³.

Solvent extraction

The culture filtrate obtained was centrifuged and the supernatant obtained was subjected to solvent extraction procedure. Equal volume (1:1) of culture filtrate and ethyl acetate (EtOAc) were taken in a sterile separation funnel and agitated well for about 30 minutes. EtOAc layer was separated and the aqueous portion was extracted twice with EtOAc. The EtOAc were pooled, evaporated to dryness at 40°C and used for antibacterial studies¹³.

Test bacteria

A panel of 15 clinical isolates was used to screen their susceptibility to EtOAc extract of MPPO-02. The test bacteria comprised of 5 isolates of *Staphylococcus aureus* (Sa-01 to Sa-05) from burn infection, 5 isolates of *Streptococcus mutans* (Sm-01 to Sm-05) from dental caries and 5 isolates (*Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*) from urinary tract infection.

Preparation of bacterial inocula

The cultures of isolates from burn and urinary tract infection were inoculated into test tubes containing sterile Nutrient broth (HiMedia, Mumbai, India) and the isolates from dental caries were inoculated into test tubes containing sterile Brain heart infusion broth (HiMedia, Mumbai, India). The tubes were incubated at 37°C for 24 hours. The broth cultures thus obtained were used for antibacterial studies.

Antibacterial activity of EtOAc extract

Agar well diffusion assay was employed to screen the inhibitory effect of EtOAc extract of MPPO-02 against clinical isolates. The Nutrient broth cultures of isolates from

burn and urinary tract infection were swabbed aseptically on sterile Nutrient agar (Hi Media, Mumbai, India) plates and the Brain heart infusion broth cultures of isolates from dental caries were aseptically swabbed on sterile Brain heart infusion agar (Hi Media, Mumbai, India) plates using sterile cotton swabs. Using a sterile cork borer, wells of 6 mm diameter were punched in the inoculated plates. 100 µl of EtOAc extract (5 mg/ml) and reference (standard) antibiotic (Chloramphenicol, 1 mg/ml) was transferred into labeled wells. The plates were incubated aerobically at 37°C for 24 hours in upright position. The zones of inhibition formed around the wells were measured after incubation¹³.

Statistical analysis

The experiment was performed in triplicates. The results are represented as Mean ± Standard deviation (SD).

RESULTS

Table 1 represents the inhibitory potential of EtOAc extract against *S. aureus* isolates recovered from burn infection. The extract was shown to inhibit all isolates with zone of inhibition ranging 1.5 to 2.2 cm. Reference antibiotic caused higher inhibition of bacterial isolates when compared with extract.

Table 1: Inhibitory effect of EtOAc extract against *S. aureus* from burn

Test bacteria	Zone of inhibition in cm	
	EtOAc extract	Standard
Sa-01	1.5 ± 0.0	4.2 ± 0.2
Sa-02	1.7 ± 0.1	3.4 ± 0.1
Sa-03	2.2 ± 0.0	4.0 ± 0.2
Sa-04	1.6 ± 0.2	3.5 ± 0.2
Sa-05	1.8 ± 0.0	3.4 ± 0.2

The result of anti caries potential of EtOAc extract against *S. mutans* isolates recovered from dental caries subjects is shown in Table 2. All isolates were found susceptible to extract with zone of inhibition ranging 1.8 to 2.1 cm. Inhibitory activity of standard was higher than that of EtOAc extract.

Table 2: Inhibitory effect of EtOAc extract against *S. mutans* from dental caries

Test bacteria	Zone of inhibition in cm	
	EtOAc extract	Standard
Sm-01	1.8 ± 0.0	3.3 ± 0.2
Sm-02	2.0 ± 0.2	3.5 ± 0.0
Sm-03	1.9 ± 0.1	3.0 ± 0.1
Sm-04	2.1 ± 0.3	3.8 ± 0.2
Sm-05	2.0 ± 0.2	3.5 ± 0.0

Table 3 shows the result of inhibitory effect of EtOAc extract against pathogens of urinary tract infections. The extract was found inhibitory to varied extent against all bacteria. It was observed that Gram positive bacteria displayed higher susceptibility to extract than Gram negative bacteria. *S. aureus* and *P. aeruginosa* were found susceptible to higher extent among Gram positive and Gram negative bacteria respectively. Inhibitory effect of reference antibiotic was higher than that of EtOAc extract.

Table 3: Inhibitory effect of EtOAc extract against urinary tract pathogens

Test bacteria	Zone of inhibition in cm	
	EtOAc extract	Standard
<i>E. faecalis</i>	1.6 ± 0.0	3.0 ± 0.2
<i>S. aureus</i>	1.8 ± 0.1	3.8 ± 0.1
<i>P. aeruginosa</i>	1.4 ± 0.2	3.5 ± 0.3
<i>K. pneumoniae</i>	1.3 ± 0.2	3.0 ± 0.2
<i>E. coli</i>	1.2 ± 0.2	3.4 ± 0.2

DISCUSSION

Burns wounds are highly vulnerable to microbial infections are often suitable sites for multiplication of pathogenic bacteria. These wounds are ideal sources of infection because of the larger area involved and longer duration of patient stay in the hospital. Burn infection remains the major cause of morbidity and mortality in hospitalized burn patients and accounts for more than 75 % of deaths following burn. Burn infections are usually poly-microbial in nature i.e., involvement of more than one microbial strain and the most common pathogens being isolated from burn wounds are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and various *Coliform bacilli*. *Staphylococcus aureus* is frequently isolated in both community and hospital practices and remains the dominant cause of nosocomial infection in burn patients. Most strains of *S. aureus* have acquired resistance to a number of antibiotics viz., penicillin, methicillin, vancomycin and others. Infection of burn wounds by multidrug resistant pathogens makes the treatment complicated¹⁴⁻¹⁹. Hence there is need for development of antimicrobial agents which can combat burn infections. Actinomycetes and their metabolites have been found to possess inhibitory activity against *S. aureus* isolates including resistant strains. Haste *et al.*²⁰ observed marked inhibitory effect of etamycin produced by a marine actinomycete against community and hospital associated methicillin resistant *S. aureus*. Satheer and Jebakumar²¹ found marked inhibitory activity of five mangrove actinomycetes against methicillin resistant and methicillin sensitive *S. aureus*. Sharma *et al.*²² found marked inhibitory activity of extracts of soil actinomycetes against multidrug resistant pathogens including methicillin resistant *S. aureus*. Kumar *et al.*²³ showed the inhibitory efficacy of EtOAc extract of marine *Streptomyces* species against multidrug resistant *S. aureus*. Bizuye *et al.*²⁴ observed inhibitory potential of EtOAc extract of Ethiopian soil actinomycetes against methicillin resistant *S. aureus*. In this study, the EtOAc extract from fermentation broth of isolates MPPO-02 showed inhibitory activity against five clinical isolates of *S. aureus* recovered from burn infection. Dental caries is one among the most important infections of the oral cavity which affect people of all age groups worldwide. Various microorganisms have been implicated in causing dental caries. Among micro flora causing dental caries, mutans streptococci in particular *Streptococcus mutans* is a primary cause. It exhibits acidogenic and aciduric properties and has the ability to adhere to tooth surfaces and forms bio film. If not treated properly, dental caries gradually leads to tooth loss along with a variety of health problems²⁵. Conventional methods employed for prevention and treatment of dental caries involve the use of antibiotics and mouth rinses. However, these agents often suffer from drawbacks such as side effects, high cost etc. The development of resistance in cariogenic flora against these agents is yet another serious problem^{25,26}. Hence,

development of new agents active against drug resistant cariogenic strains is required. The crude extracts and enzymes of *Streptomyces* species are found to possess inhibitory activity against etiological agents of dental caries. Yokogawa *et al.*²⁷ observed lytic activity of two enzymes (N-acetylmuramidases) from *S. globisporus* on cell walls of *S. mutans*. A protease, isolated from *S. globisporus* by Nara and Morioka²⁸ diminished the activity of glucosyltransferase produced by *S. mutans*. Taechowisan *et al.*²⁹ found inhibition of adherence of *S. mutans* on glass surfaces and saliva-coated hydroxyapatite and decreased activity of glucosyltransferase and glucan-binding lectin by the crude extract obtained from the culture filtrate of an endophytic *Streptomyces* sp. ST8. Raja *et al.*³⁰ showed anti caries activity of crude extract from two *Streptomyces* species against *S. mutans* and *S. oralis*. Recently, Rakesh *et al.*³¹ found inhibitory effect of EtOAc extract of *Streptomyces* species TK-07 isolated from Western Ghat soil of Talakaveri against *S. mutans* isolates. In our study, the EtOAc extract of isolate MPPO-02 displayed inhibitory activity against five isolates of *S. mutans* recovered from dental caries. Urinary Tract Infections (UTIs) are infections caused by microorganisms anywhere in the urinary tract (kidneys, ureters, bladder and urethra). UTIs are one of the common and most important infections in both community and hospital settings. UTIs are reported to affect people of all age groups in both sexes and are more common in females than in males. UTIs remain the leading cause of Gram-negative bacteraemia especially in hospitalized patients³². The aetiology of UTIs includes pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *S. saprophyticus* and *Serratia* sp. Majority of UTIs are caused by a single pathogen. Some may be poly microbial in nature. Antibiotics are commonly and routinely used to treat UTIs. However, overuse and misuse of these antibiotics resulted in the emergence of resistant bacterial strains. The prevalence of antibiotic resistance among urinary pathogens is increasing worldwide and is creating a serious threat for successful treatment of UTIs³³⁻³⁷. In the present study, the EtOAc extract of *Streptomyces* species MPPO-02 was found to possess inhibitory activity against urinary tract pathogens. Western Ghats of India, covering an area of 1, 80, 000 km² (just under 6 % of the land area of India), represent one among the biodiversity hotspots in the world and harbor > 30 % of all plant, fish, herpeto-fauna, birds, and mammal species found in India. The mountain ranges of Western Ghats are covered in five states viz., Gujarat, Maharashtra, Goa, Karnataka and Kerala, India. Biosphere reserves, national parks and wildlife sanctuaries being established protect and conserve the biodiversity of Western Ghats. A large number of globally threatened species of flora and fauna are found in the Western Ghats³⁸⁻⁴⁰. Bio activities such as antimicrobial, antioxidant, enzyme inhibitory, anthelmintic, insecticidal, cytotoxic, analgesic, antipyretic, anti-inflammatory and CNS depressant activity of soil actinomycetes from different parts of Western Ghats of Karnataka, India such as Thirthahalli, Agumbe, Mahishi, Kodachadri, Talakaveri, Dandeli and Kudremukh have been investigated^{12,13,41-50}. In the present study, bioactive *Streptomyces* species MPPO-02 isolated from rhizosphere soil of Mahishi, Karnataka, India displayed inhibitory activity against bacterial strains isolated from burn, dental caries and urinary tract infections. It was observed that Gram positive bacteria have shown higher susceptibility to

EtOAc extract when compared with Gram negative bacteria. In our previous study, the isolate MPPO-02 displayed higher inhibitory activity against Gram positive bacteria than Gram negative bacteria¹². Similar findings were observed in studies carried out by Kekuda *et al.*⁴⁵, Anansiriwattana *et al.*⁵¹, Valli *et al.*⁵², Manasa *et al.*⁴⁴, Kekuda *et al.*¹³ and Junaid *et al.*⁵⁰. The presence of an outer membrane possessing hydrophilic polysaccharides chains in Gram negative bacteria acts an additional barrier for the entry of compounds into the cells and this could be ascribed to the lower susceptibility of Gram negative bacteria^{53,54}.

CONCLUSION

The EtOAc extract of *Streptomyces* species MPPO-02 was found to possess inhibitory activity against clinical isolates of burn, dental caries and urinary tract infection. The actinomycete strain MPPO-02 can be exploited for the development of bioactive agents. The purification and characterization of bioactive components from solvent extract are to be carried out. Further studies on screening of antagonistic actinomycetes from Western Ghat soils could be fruitful.

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