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Research Article

ANTICARIES ACTIVITY OF *STREPTOMYCES* SPECIES SRDP-TK-07 ISOLATED FROM A WESTERN GHAT SOIL OF TALAKAVERI, KARNATAKA, INDIA

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Abstract

The present study aims at determining anti caries activity of a bioactive *Streptomyces* species SRDP-TK-07 isolated previously from a rhizosphere soil of Talakaveri, Karnataka, India. Efficacy of ethyl acetate extract of SRDP-TK-07 to inhibit seven cariogenic isolates of *Streptococcus mutans* was tested by employing Agar well diffusion assay. The extract was effective in inhibiting all seven isolates of *S. mutans* with zone of inhibition ranging 1.3 to 2.9 cm. The isolate SRDP-TK-07 is found promising and can be used for the development of inhibitory agents active against cariogenic bacteria.

Keywords: Talakaveri, *Streptomyces*, Dental caries, *Streptococcus mutans*, Agar well diffusion

INTRODUCTION

Dental caries is one among the common, pathological, most chronic and multi-factorial infectious diseases worldwide. In a susceptible host, the disease occurs as a result of the interaction of specific bacteria with constituents of a diet. Dietary carbohydrates are fermented into organic acids by the microbial community embedded in the bio film, the pH at tooth surface drops below 5.5, demineralization proceeds faster and results in tooth decay. Several strains of oral streptococci are capable of initiating the formation of dental plaque and play an important role in the development of dental caries and periodontal disease in humans. Two α -haemolytic streptococci viz., *Streptococcus mutans* and *S. sobrinus*, are the potent cariogenics, however, other bacteria notably lactobacilli and actinomycetes may also be involved in the development of dental caries¹⁻³. The recognition of the involvement of the microorganisms in dental caries has led to the development of prevention and control measures for eliminating them or reducing their number in the oral cavity¹. Actinomycetes are Gram positive, filamentous eubacteria possessing high G+C content. Among actinomycetes, the genus *Streptomyces* is known to encompass the largest number of species and varieties, found distributed in almost all ecological niches and differ in their morphology, physiology and biochemical characteristics. The members belonging to *Streptomyces* are dominating in soil in terms of number and the bioactive compounds they produce. These organisms are being considered as fruitful sources of bioactive compounds having pharmaceutical and agricultural importance. They have provided 2/3rd of naturally occurring antibiotics, such as tetracyclines, aminoglycosides, macrolides etc., discovered so far⁴⁻¹⁰. Talakaveri, is the place that is generally considered to be the source of the river Kaveri. It is located by Brahmagiri hill of the Western Ghats near Bhagamandala in Kodagu district, Karnataka, India 1276 m above sea level. The present study was undertaken to investigate anti caries activity of ethyl acetate extract from a

Streptomyces species SRDP-TK-07 isolated previously from a rhizosphere soil collected at Talakaveri, India. The extract of the isolate SRDP-TK-07 was reported to possess antimicrobial and antioxidant activity in our previous study¹¹.

MATERIALS AND METHODS

Streptomyces species SRDP-TK-07

The isolate SRDP-TK-07 was previously recovered from a rhizosphere soil of Talakaveri, India by Serial dilution followed by plating on Starch Casein Nitrate (SCN) agar. The isolate was characterized as a species of *Streptomyces* based on cultural and morphological studies¹¹.

Fermentation and extraction

The protocols employed for bulk cultivation of the isolate SRDP-TK-07 in SCN broth and extraction of the culture broth by solvent extraction (using ethyl acetate in separation funnel) were as described in our previous study¹¹.

Anti caries activity of ethyl acetate extract

Antibacterial activity of ethyl acetate extract was tested by Agar well diffusion assay against 7 clinical isolates of *Streptococcus mutans* (Sm-01 to Sm-07) isolated previously from dental caries subjects. The test bacteria were inoculated into sterile Brain heart infusion broth (Hi Media, Mumbai, India) tubes and incubated overnight at 37°C. The broth cultures 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 and 100 μ l of ethyl acetate extract (5 mg/ml of 25 % dimethyl sulfoxide [DMSO]), standard (Streptomycin, 1 mg/ml) and DMSO (25 %) were transferred into respectively labeled wells. The plates were incubated aerobically at 37°C for 24 hours in upright position and the zone of inhibition formed around the wells was measured using a ruler¹¹.

Statistical analysis

Antibacterial activity was done in triplicates and the results are mentioned as Mean \pm Standard deviation.

RESULTS

Table 1 shows the result of anti caries activity of ethyl acetate extract of SRDP-TK-07 against clinical isolates of *S. mutans*. It was observed that all the test bacteria were found to be susceptible to ethyl acetate extract but to a varied extent (with zone of inhibition ranging 1.3 to 2.9 cm). Inhibition caused by reference antibiotic was higher than that of ethyl acetate extract. DMSO was not inhibitory to clinical isolates.

Table 1: Anti cariogenic activity of ethyl acetate extract of SRDP-TK-07

Test bacteria	Zone of inhibition in cm	
	SRDP-07	Streptomycin
Sm-01	2.8 \pm 0.1	3.3 \pm 0.2
Sm-02	2.8 \pm 0.3	3.3 \pm 0.1
Sm-03	2.9 \pm 0.1	3.9 \pm 0.1
Sm-04	2.2 \pm 0.1	3.1 \pm 0.2
Sm-05	1.8 \pm 0.2	3.0 \pm 0.1
Sm-06	2.6 \pm 0.1	3.9 \pm 0.1
Sm-07	1.3 \pm 0.1	3.3 \pm 0.3

DISCUSSION

Streptococcus mutans is implicated as the primary etiological agent of dental caries in humans. The acidogenic and aciduric properties of *S. mutans* along with its ability to synthesize extracellular glucans are the main factors for the development and establishment of cariogenic bio films. Glucans, synthesized from dietary carbohydrate (mainly sucrose) by glucosyltransferases (GTFs) enhance the pathogenic potential of dental plaque as it promotes the adherence and accumulation of cariogenic streptococci on the tooth surface and contribute to the bulk and structural integrity of plaque^{12,13}. It has been observed that the extracts and metabolites of *Streptomyces* species have shown to be promising against causal agents of dental caries. In a study, Yokogawa *et al.*¹⁴ isolated two lytic enzymes (N-acetylmuramidases) capable of lysing the cell walls of *Streptococcus mutans* from *S. globisporus* 1829. Similarly Nara and Morioka¹⁵ isolated a protease from *S. globisporus* which diminished the activity of glucosyltransferase produced by *Streptococcus mutans*. The crude extract from the culture filtrate of an endophytic *Streptomyces* sp. ST8 displayed inhibition of adherence of *Streptococcus mutans* on glass surfaces and saliva-coated hydroxyapatite and also decreased the activity of glucosyltransferase and glucan-binding lectin¹⁶. Raja *et al.*¹⁷ observed anti caries activity of crude extract from two *Streptomyces* species against *S. mutans* and *S. oralis*. In the present study, the ethyl acetate extract of a bioactive *Streptomyces* species SRDP-TK-07 exhibited marked anti caries activity. To the best of our knowledge, this is the first report on anti caries activity of *Streptomyces* species recovered from the soil of Western Ghats in particular from Karnataka, India. Earlier studies have shown antimicrobial, antioxidant, enzyme inhibitory, cytotoxic, anthelmintic, analgesic, anti-inflammatory, CNS depressant and antipyretic activity of soil actinomycetes from Western Ghat of Karnataka, India^{11,18-28}.

CONCLUSION

A marked inhibitory activity against cariogenic isolates of *S. mutans* by the ethyl acetate extract from *Streptomyces* species SRDP-TK-07 was observed in this study. The isolate can be a potent source for the development of agents effective in the prevention and control of dental caries. Further studies on purification of active components from the ethyl acetate extract and their bioactivity determinations are to be carried out.

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