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Short Communication

CHARACTERIZATION AND ANTIMICROBIAL SPECTRUM OF A POTENT *STREPTOMYCES* SP. GOS2 ISOLATED FROM WESTERN GHATS OF KARNATAKA, INDIA

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ABSTRACT

Objective: Western Ghats of Karnataka, India-a biodiversity hotspot is a rich source of microorganisms with undiscovered metabolic capabilities. The upsurge of antibiotic-resistant pathogens has created a greater demand for novel antibiotics. In context to the above a potent soil *Streptomyces* sp. GOS2 isolated from Agumbe regions of Western Ghats, and its metabolite was assessed and characterized for its antimicrobial spectrum.

Methods: The isolation was carried out by soil serial dilution plating on Starch Casein Nitrate agar media (SCN). The obtained isolate was characterized by morphological and biochemical tests. The antimicrobial activity was assessed by well in agar methods against 28 test organisms. The partial characterization of the bioactive metabolite was carried out by thin layer chromatography (TLC) and UV–Visible spectroscopy studies.

Results: The isolated GOS2 was observed as a raised powdery colony with grey colored aerial mycelium and media impregnated substrate mycelium. The spore chain was rectus with smooth spore surface. The isolate was gram positive, non-acid fast, positive for catalase, hydrogen sulphide production and starch hydrolysis, negative for casein and gelatin hydrolysis. The carbohydrate fermentation studies showed acid production in dextrose and alkali production in sucrose, lactose, maltose and starch. A prominent antibacterial activity was observed with a zone of inhibition measuring 21-27 mm. The TLC showed a purple spot and UV spectroscopy revealed λ max at 233.2 and 235 nm indicating macrolide group of antibiotics.

Conclusion: Western Ghats actinomycetes are a potent source of novel antibiotic molecules.

Keywords: *Streptomyces*, Western Ghats, Antibiotics, Macrolides, UV spectroscopy

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In the recent past the phenomenon of antibiotic resistance has acquired significance with the emergence of drug-resistant pathogens to be known antibiotics [1, 2]. *Streptomyces* sp. being the largest members of Actinomycetes has been revered for its metabolic potential and are storehouses of a diverse array of metabolites *viz*., antibiotics, antitumor agents, pharmacological agents, enzymes, immunosuppressant's, etc. [3-5].

Agumbe regions of the Western Ghats in Karnataka-a biodiversity hotspot is a surfeit source of flora and fauna [6], but has been less studied for microbial forms. The emerging need of novel antibiotic molecules and with a diversity of *Streptomyces* and Western Ghats, the present study accentuates on the isolation and partial characterization of a *Streptomyces* sp. and its bioactive metabolite with potent antimicrobial activity isolated from Agumbe regions of the Western Ghats in Karnataka, India.

All the media and chemicals used in the present study were of analytical grade procured from Himedia Laboratories, Mumbai. The UV-Visible spectrum was recorded on Systronics (117) UV-Visible spectrophotometer. The electron microscopic picture was captured using Qanta 200 electron microscope.

The soil sample for isolation of *Streptomyces* spp. was collected from Agumbe regions (13.493853, 75.085901) of Western Ghats in Karnataka. The isolation was carried out by soil serial dilution and plating on starch casein nitrate agar medium. The isolate GOS2 was as a prominent colony with a raised powdery appearance, having gray colored aerial mycelium and white-colored substrate mycelium impregnated into the medium.

The isolate was identified and confirmed as *Streptomyces* sp., as per the identification criteria in Bergey's manual of Determinative bacteriology, Systematic bacteriology and the International *Streptomyces* Project guidelines [7-9]. The microscopic studies revealed a rectus spore chain arrangement and the electron microscopic studies revealed the spore surface ornamentation to be smooth (fig. 1).

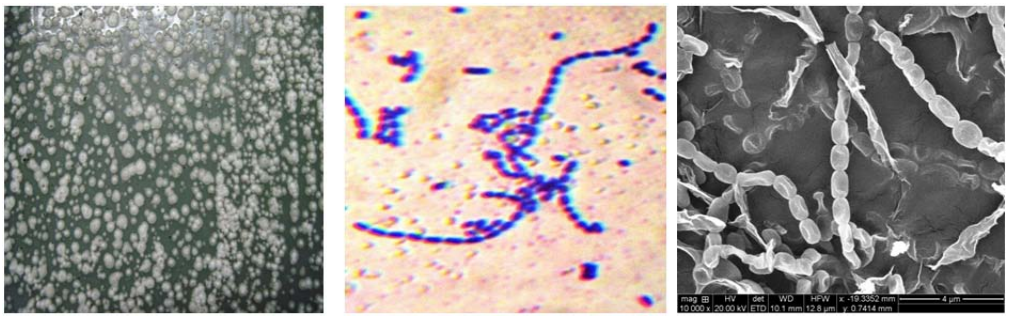

Fig. 1: Colony morphology, Spore chain and surface ornamentation of GOS2

The isolate was found to be Gram positive and nonacid-fast. Diverse biochemical test results were observed with positive results for catalase, hydrogen sulfide production and starch hydrolysis. Negative results were observed for casein and gelatin hydrolysis. The carbohydrate fermentation studies showed acid production in dextrose and alkali production in sucrose, lactose, maltose and starch. Alkali production was considerably higher in lactose and starch. No gas production was observed.

The antimicrobial activity was assessed by well in agar method [10]. The isolate GOS2 showed a broad spectrum antibiosis with a prominent zone of inhibition against both Gram positive and Gram negative bacteria. Minimal zone of inhibition was observed against yeasts and no zone of inhibition was observed against filamentous fungi. The zone of inhibition ranged from 21 to 27 mm. The zone of inhibition (ZOI) of solvent control, solvent extract, crude extract and standard antibiotics are as represented in table 1.

Table 1: Antimicrobial activity of GOS 2 and standard antibiotics against different test organisms

S. No.	Test organisms	Zone of inhibition in mm (mean±standard deviation)			
		Solvent extract	Solvent control	Culture filtrate	Standard antibiotics
Test bacteria					Cefataxime
1	*S. aureus*	27.66±1.52	11.66±0.57	27.66±1.15	15.33±0.57
2	*S. epidermidis*	23.66±1.52	-	23.00±1.00	16.00±1.00
3	*S. lutea*	23.66±1.15	-	23.00±0.00	20.00±0.0
4	*Streptococcus* sp.	23.33±1.52	-	22.66±1.15	15.33±0.57
5	*B. subtilis*	23.66±0.57	-	21.33±0.57	13.33±0.57
6	*B. cereus*	22.66±1.15	-	22.66±1.15	0.00±0.00
7	*E. coli*	24.33±0.57	-	23.00±1.00	20.33±0.57
8	*S. typhi*	25.33±1.15	-	23.66±0.57	22.00±1.00
9	*S. flexneri*	25.33±1.15	-	24.66±1.15	23.66±0.57
10	*S. sonnei*	25.33±1.15	-	24.33±0.57	24.00±1.00
11	*V. cholera*	27.33±0.57	-	26.33±0.57	14.33±0.57
12	*P. aeruginosa*	-	10.66±0.57	-	23.66±0.57
13	*P. mirabilis*	24.33±1.52	11.33±0.57	23.66±1.15	0.00±0.00
14	*K. pneumonia*	25.66±1.52	12.66±0.57	24.33±0.57	10.66±0.57
15	*K. aerogenes*	26.66±0.57	10.66±1.15	24.66±0.57	14.33±0.57
16	*E. aerogenes*	-	-	-	24.33±1.15
Test fungi					(Clotrimazole)
17	*S. cerevesiae*	18.66±1.52	-	18.00±1.00	28.66±0.57
18	*C. albicans*	22.66±0.57	-	23.33±1.52	33.00±1.00
19	*C. neoformans*	23.33±1.15	-	23.00±1.00	28.66±0.57
20	*C. kruseii*	16.66±0.57	14.00±1.00	15.33±0.57	33.33±0.57
21	*C. lipolytica*	-	-	-	34.00±1.00
22	*A. niger*	12.66±1.15	-	11.66±0.57	33.66±0.57
23	*A. pumilis*	-	-	-	33.00±0.00
24	*A. wentii*	-	-	-	30.33±0.57
25	*Fusarium* sp.	-	10.66±0.57	-	26.66±0.57
26	*Curvularia* sp.	-	11.33±0.57	-	29.66±0.57
27	*M. canis*	-	10.66±1.15	-	28.33±0.57
28	*T. cutaneum*	-	-	-	29.66±0.57

The partial characterization of the active principle was carried out by TLC and UV absorption spectroscopy studies [11]. TLC developed on silica gel using methanol: acetic acid: water (8:2:1) solvent system showed a light purple colored spot with a Rf value of 0.7 when treated with ninhydrin. The UV absorption spectroscopy revealed two prominent peaks with maximum absorptions (λmax) at 233.2 and 235.0 nm (fig. 2).

The results of this study reveal that the isolate *Streptomyces* GOS2 isolated from Agumbe regions of the Western Ghats to be a potent isolate with a prominent antibacterial activity. The appearance of light purple color upon ninhydrin spray revealed the metabolites to possess free amino groups. The UV absorption spectral studies revealed two peaks at 233.2 and 235.0 nm.

Waksman and Henrici *et al.* (1943) [12] reported the λmax of macrolides at 232 nm. Macrolides being clinically important bactericidal antibiotics are thought to inhibit bacterial protein synthesis by binding to the 50s region of the ribosome near to the peptidyl transferase center and causing cell growth arrest [13, 14].

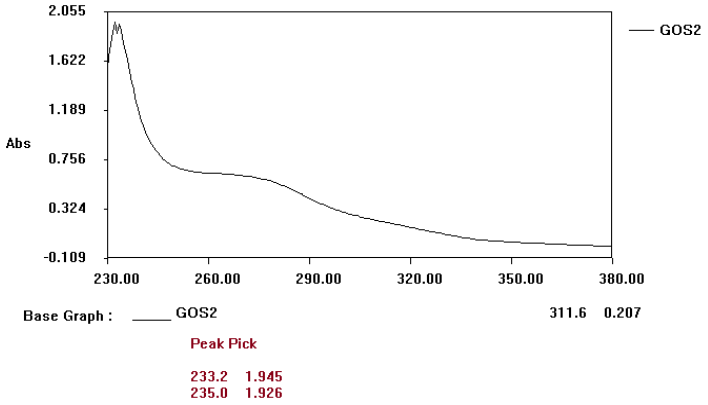

Fig. 2: UV absorption spectroscopy of isolate GOS2

In consideration with antibacterial activity and the UV absorption maxima correlating with macrolides, the metabolite can be preliminarily classified as a protein synthesis inhibitor. The prominent antibacterial activity and the insignificant antifungal activity can be attributed to the differences in the bacterial and fungal ribosomal structures. The results are also of significance because of the specificity of the molecule in curtailing the growth of bacteria only and not fungi. This specificity trait is important in drug development to delineate the adverse effects of new drug entities on eukaryotic cells.

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CONFLICTS OF INTERESTS

Declared none

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