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Isolation, characterization and antifungal activity of *Streptomyces sampsonii* GS 1322

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For new antifungal antibiotics from actinomycetes, a strain of *Streptomyces* GS 1322 was isolated from a sample of garden soil. The strain was found to possess antagonistic activity against four fungi i.e., *Candida albicans, Aspergillus niger, Microsporum gypseum* and *Trichophyton* sp. The strain was identified as *Streptomyces sampsonii* and the antifungal compound produced by it was found to be the heptaene group of polyene antibiotics.

Keywords: Antifungal activity, *Candida albicans*, Polyene, *Streptomyces sampsonii*

The prevalence of fungal infections has increased significantly during the past few decades, particularly in immunosuppressive and immuno-compromised patients[1-4] and are of major concern in the context of present day medicine[4-7]. Although, few antifungal agents are in use to treat fungal infections, but their clinical efficacy in some invasive fungal infections is not optimal. Thus, intense efforts in antifungal drug discovery are still needed to develop more promising and effective antifungal agents for use in the clinical arena[4,7].

Actinomycetes is most widely exploited group of microorganisms in terms of their capabilities in production of antibiotics and other compounds of biotechnological importance[8-10], while many more useful compounds including antibiotics are awaiting discovery[11-13]. The present paper reports biochemical and physiological properties of an actinomycetes strain and production of an antifungal antibiotic of polyene group.

Materials and Methods

Microorganism—The actinomycete strain GS 1322 was isolated from a sample of local garden soil (23° 50° N latitude and 78° 40° E longitude). The sample was given pretreatment by drying at 45°C for 24 h. The isolation of actinomycete was done using the method as described by Jain and Jain[10]. The isolated actinomycetes was purified using glucose yeast extract agar medium[14] and maintained at 4°C as stock cultures on yeast extract malt extract agar medium[15].

Identification—The actinomycete strain GS 1322 was got identified from IMTECH, Chandigarh based on its biochemical properties and on the basis of 16S rRNA gene sequencing by Dr. Yogesh S. Souche, National Centre for Cell Sciences (NCCS), Pune University, Pune, India.

Antifungal activity—Antifungal activity of the strain *S. sampsonii* GS 1322 was tested using 'Cross streak method' as described by Waksman and Lechevalier[16] using *Candida albicans, Aspergillus niger, Microsporum gypseum* and *Trichophyton* sp. as test organisms which were obtained from the courtesy of Dr S K Deshmukh, Nicholas Piramal India Ltd., Mumbai.

Characterization of antifungal compound

Characterization of the nature of antifungal compound produced by the test strain GS 1322 was done using following methods.

Ergosterol agar plate method—Production of polyene antifungal compounds in the .cultures of test actinomycete was assayed using Ergosterol agar plate method[17]. For this, the antifungal compound was obtained from the cultures of test actinomycete grown on beef extract broth medium for 108 h at 120 rpm on rotary shaker. The extraction of antifungal compounds from the cultures was carried out following the method as described by Jain and Jain[10] and tested for the presence of polyene compounds following the

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method as described in the Laboratory Mannual of MTCC[17]. Assay was carried out using *Candida albicans* as test organism. The results were recorded by observing the zone of inhibition developed on the assay plates. The results obtained were interpreted using criteria—(a) reduced zone size in presence of ergosterol-polyene type antibiotic present; and (b) no reduced zone in presence of ergosterol-polyene type antibiotic absent.

UV visual spectrophotometric method—For spectrophotometric analysis of the antifungal compound produced by strain GS 1322, it was grown on beef extract broth medium (beef extract, 4 g; peptone, 4 g; yeast extract, 1 g; NaCl, 2.5 g; glucose, 10 g; distilled water, 1000 ml; *p*H, 7.0) on rotary shaker (120 rpm) at 28°±1°C for 108 h. After incubation the content of the flask was centrifuged at 7000 rpm for 10 min and the mycelial pellet was obtained and used for the extraction of the polyenes using 95% methanol[10]. The presence of polyenes in methanolic extract of the mycelium was determined by spectrophotometry. The absorption spectrum was obtained at 200-500 nm using UV - visual double beam PC based spectrophotometer (ECIL model - UV 5704 SS). The presence of polyene and its type was determined on the basis of characteristic three peak spectra using standards as reported by Riviere *et al*[18].

Characterization of S. sampsonii GS 1322—To establish the identity of the test strain, it was grown on yeast malt extract agar medium and characteristics were noted following Shirling and Gottlieb[15] and Rayner[19]. For physiological and biochemical characterization its Gram's staining properties, production of pigments, growth on media having 5 different concentrations of NaCl (i.e., 2, 5, 7, 9 and 12%), seven different *p*H from 4.0 to 10.0 at an interval of 1 *p*H, effect of different temperatures (i.e., 4°, 15°, 25°, 37°, 42° and 55°C) on growth, utilization of 12 different sugars (i.e., glucose, arabinose, mannitol, xylose, meso-inositol, raffinose, rhamnose, salicin, sucrose, galactose, lactose and maltose) and nine biochemical properties including production of acid and acetoin (MR, VP), indole and H_2S production, citrate utilization; starch, casein and gelatin hydrolysis and nitrate reduction were studied following standard methods[15, 20, 21].

Results and Discussion

Identification—Actinomycete strain GS 1322 was got identified from IMTECH, Chadigarh as *Streptomyces*, based on its biochemical properties and its viable culture was deposited at its Microbial Type Culture Collection and Gene Bank with an accession number MTCC 6231. Its further identification was done on the basis of its 16S rRNA gene sequencing at National Centre of Cell Sciences (NCCS), Pune University, Pune and was identified as a strain of *Streptomyces sampsonii* GS 1322. The culture of this strain was also deposited at the Research Laboratory, Department of Applied Microbiology and Biotechnology, Dr H S Gour V V, Sagar.

Antagonistic activity—*S. sampsonii* GS 1322 exhibited significant antifungal activity against both yeast and moulds. It caused total inhibition in growth (TIG) of *Candida albicans* and growth inhibition and retardation in the growth of *Aspergillus niger* and *Microsporum gypseum* causing 20 and 05 mm zone of inhibition, respectively, but showed only growth retardation in the growth of *Trichophyton* sp. (Table 1).

Characterization of antifungal compound

Identification of antifungal compound produced in cultures of *S. sampsonii* GS 1322 has been carried out using ergosterol agar plate assay and spectrophotometric methods. The results obtained are as follows:

(a) *Ergosterol agar plate assay*—Results of present study showed the production of polyene antifungal antibiotic by *S. sampsonii* GS 1322. The antifungal compound obtained from its culture caused a zone of inhibition of 21 mm against *C. albicans* grown on SDA medium without ergosterol, whereas the same amount of sample when tested using SDA medium containing 0.5% ergosterol, produced only 14 mm zone of inhibition against *C. albicans*. Since, ergosterol and polyene compounds possess strong affinity and presence of ergosterol in assay medium resists the diffusion of polyene compounds this reduction in zone of inhibition in the presence of

Table 1—Antagonistic activity of *Streptomyces sampsonii* GS 1322

Test organism	Zone of inhibition (mm)	Activity*
Candida albicans	No Growth	TIG
Aspergillus niger	20	GIR
Microsporum gypseum	05	GIR
Trichophyton sp.	-	GR

*TIG-Total inhibition in growth, GIR-Growth inhibition and retardation, GR-Growth retardation.

ergosterol indicated the production of polyene antifungal compound by the test strain.

(b) *Spectrophotomtric spectra of antifungal compound*—Methanolic extracts of the mycelia of test strain produced a characteristic three-peak spectrum, confirming the production of polyene group of antifungal antibiotic by this strain. The position of peaks in the spectrum was noted at 360.0, 379.5 and 402.0 nm and hence, antifungal compound produced by the present strain belongs to the heptaene group of polyene antibiotics (Fig. 1).

Cultural characteristics—*S. sampsonii* GS 1322 showed a rapid growth on yeast extract malt extract agar medium. The culture showed the presence of opaque, convex mycelial growth with irregular margins. Colour of the aerial mycelium was cream to primerose and the substrate mycelium was cream to brown colour. Production of diffusible pigments was not observed.

Biochemical and physiological characters—The strain was found to hydrolyze starch, casein and gelatin and showed weak positive results for reduction of nitrate (Table 2). The strain showed good growth at *p*H 6 to 10, but at *p*H 5 its growth was noted weak positive. The test strain was able to grow in a temperature range of 15°C to 42°C but failed to grow at 55°C. The study on sodium chloride tolerance indicated that the strain could grow up to 9% concentration of NaCl in the growth medium. It was found to tolerate 12% concentration of NaCl. It grew well in presence of potassium tellurite (0.001%) and sodium azide (0.01%), but the growth was observed weak positive in the presence of 0.1% phenol.

The strain utilized glucose, arabinose, mannitol, xylose, raffinose, salicin, galactose, lactose and maltose as sole source of carbon. The utilization of rhamnose and sucrose was found doubtful but, failed to utilize meso-inositol as carbon source.

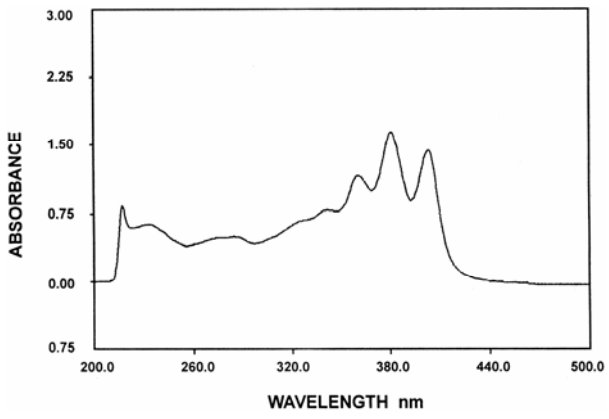

Fig. 1—Ultraviolet absorption spectrum of antifungal compound produced by *Streptomyces sampsonii* GS 1322 in methanol (95%).

Table 2—Biochemical and physiological characteristics of *S. sampsonii* GS 1322

Grams staining	+
Production of H_2S	-
IMViC	-
Hydrolysis of starch, casein and gelatin	+
Utilization of Carbon sources:	
glucose, arabinose, mannitol, xylose, galactose, maltose, raffinose, lactose.	+
rhamnose, sucrose	±
Nitrate reduction	W+
Growth in presence of NaCl:	
2%, 5%, 7%, 9%	+
12%	W+
Growth in presence of:	
sodium azide (0.01%)and potassium tellurite (0.001%)	+
phenol (0.1%)	W+
Growth at temperatures (°C)	
15, 25, 37, 42	+
4 and 55	-
Growth at *p*H 4	-
*p*H 5-10	+

+, positive; W+, weak positive; ±, doubtful; – , Negative

Ability of *Streptomyces sampsonii* GS 1322 to produce heptaene antifungal antibiotic was interesting, in view of its possible pharmaceutical application. Beside that, the present strain *Streptomyces sampsonii* GS 1322 differed from the type strain of *S. sampsonii* (ATCC 25495) in cultural and biochemical characteristics[21] that have been described in the present study.

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