

UV/VIS, FTIR spectrum and Anticandidial activity of Streptomyces strains

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Citation

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Abstract

Two different species *Streptomyces aureofaciens* and *S. albidoflavus* were collected from Microbial Type Culture Collection Centre, Chandigarh, India. These two species were extensively studied for their in vitro anticandidial activity. The result indicated that *S. aureofaciens* was highly active against *Candida albicans*. The UV spectral analysis of the produced antifungal metabolite of *S. aureofaciens* and *S. albidoflavus* showed the absorbance peaks at 240nm. The FTIR spectral study revealed that the ethyl acetate extracts of *S. aureofaciens* and *S. albidoflavus* fermentation broth exhibited absorption from 2956 to 2360.7 cm^{-1} and from 3310 to 2358.8 cm^{-1} , respectively, which indicate hydroxyl groups and absorption at 1639 cm^{-1} indicating a double bond of non polyenic compound.

INTRODUCTION

Streptomyces is the largest antibiotic-producing genus in the microbial world discovered so far. The number of antimicrobial compounds reported from the species of this genus per year has increased almost exponentially for about two decades. Recent reports show that this group of microorganisms still remains an important source of antibiotics (Watve et al., 2001). As a result of the increasing prevalence of antibiotic-resistant pathogens and the pharmacological limitations of antibiotics, there is an exigency for new antimicrobial substances. The results of extensive screening have been the discovery of about 4000 antibiotic substances from bacteria and fungi, many of which have found applications in medicine; most of them are produced by *Streptomyces* (Korn-Wendisch and Kutzner, 1992). To prevent exponential emergence of microorganisms from becoming resistant to the clinically available antibiotics already marketed, a periodic replace of the existing antibiotics is necessary. In the present study, anticandidial activity of compound produced by *Streptomyces* species was determined and partially characterized.

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE OF ACTINOMYCETES STRAINS

Two actinomycetes strains such as *Streptomyces aureofaciens* (MTCC 325) and *S. albidoflavus* (MTCC 932) were collected from Institute of Microbial Technology,

Chandigarh, India. The collected strains were maintained in *Streptomyces* agar medium (Himedia, Mumbai).

TARGET ORGANISM

The target organism used in the present study was *Candida albicans*.

ANTAGONISM (SLAVICA ET AL., 2005)

Balanced sensitivity medium (BSM, Difco) plates were prepared and inoculated with individual *Streptomyces* strains by a single streak of inoculum in the center of the petridish. After 4 days of incubation at 28°C, the plates were seeded with test organism, *C. albicans* and again incubated the plates for 24 h at 28C. The microbial interactions were analysed by determining the inhibition zone.

FERMENTATION

Both actinomycete strains showed activity against *C. albicans*, so they were grown individually in submerged culture fermentation in 250 ml flask containing 100 ml of various liquid media such as Starch casein, glucose asparagines, glycerol asparagines, sabouraud dextrose, potato dextrose and yeast extract malt extract (Hi Media, Mumbai) to know which liquid medium stimulates maximum antifungal activity (Sahin and Ugar, 2003). The flasks were inoculated with 1ml of active *Streptomyces* strains and incubated at 37°C for 72 h with shaking at 105t/min. After incubation, the content in each flask was centrifuged at 10,000 rpm for 20 min. The culture

supernatants were used as source of anticandidial activity.

ISOLATION OF ANTICANDIDIAL COMPOUND

For the detection of anticandidial activity of Streptomyces, the agar well diffusion method was followed according to Kathiresan et al. (2005). The collected organisms were grown well on Yeast extract Malt extract medium for anticandidial activity study. The individual culture broth was centrifuged at 10,000 rpm for 15 min. and then the supernatant was filtered through membrane filter (0.45 mm pore size). Then the filtrate was extracted with ethyl acetate, chloroform and n-butanol at pH 7.0 and concentrated in a vacuum rotary evaporator. The extract was tested against target organism (*Candida albicans*) by well diffusion method.

ULTRAVIOLET (UV) AND FOURIER TRANSFORM INFRARED (FTIR) SPECTRAL ANALYSIS

The absorption spectrum of each active extract was determined in the UV region (200-400nm) by using a Shimadzu -1601 UV-VIS spectrophotometer (Sahin and Ugur, 2003). The spectra were recorded at 200-400 nm range. The FTIR spectrum of each active extract was detected by using Shimadzu IR-470 (Augustine et al., 2005). The spectra were scanned in the 400 to 4000 cm⁻¹ range. The spectra were plotted as intensity versus wave number.

RESULTS AND DISCUSSION

ANTICANDIDIAL ACTIVITY

Plate 1 shows the anticandidial activity of *S. aureofaciens* and *S. albidoflavus* against *C. albicans* using active extracts. The anticandidial activity of both Streptomyces spp. against *C. albicans* in the primary selection media are presented in Table 1. Usually the organisms termed as ‘effective’ showed inhibition zone of >10 mm, and those as ‘non effective’ exhibited inhibition zone of <10 mm (Kathiresan et al., 2005). In the present study six different media were used for antifungal metabolite produced by two Streptomyces spp. Among the six media used, the highest antifungal activity (++++) was recorded by using yeast extract malt extract medium for *S. aureofaciens* against *C. albicans*. Followed by, starch casein and sabouraud dextrose media exhibited (++++) strong zone of inhibition against *C. albicans*. While using *S. albidoflavus* strain only sabouraud dextrose medium showed strong antifungal activity (+++) against *C. albicans*. But the other media showed weak or no antifungal activity. Sahin and Ugur (2003) reported that the antibacterial activity was exhibited by 45.9% of Streptomyces strains (total 74

isolates) isolated from soil sample. Denizci (1996) reported that 356 Streptomyces isolates were obtained from soils in the Aegean and East Black sea regions of Turkey, among them 36% of the isolates were found to be active against tested microorganisms. They were active against *S. aureus* (20.478%), *E. coli* (2.52%), *Micrococcus luteus* (18.25%), *Mycobacterium smegmatis* (22.47%) and *B. subtilis* (12.07%). Slavica et al. (2005) reported that 20 different Streptomyces isolates were recovered from 33 soil samples, among them only 45% exhibited antibacterial activity, while other nine isolates exhibited a very strong activity, especially against *Bortritis cinerea*, Herpes simplex, *Candida albicans* and *Staphylococcus epidermis*. Augustine et al. (2005) reported that the purified antibiotic compound was active against a number of test organisms like *A. niger*, *A. fumigatus*, *F. oxysporum*, *C. albicans* and *Cryptococcus* species. The Streptomyces, producers of more than half of the 10,000 documented bioactive compounds have offered over 50 years of interest to industry and academics (Anderson and Wellington, 2001).

Figure 1

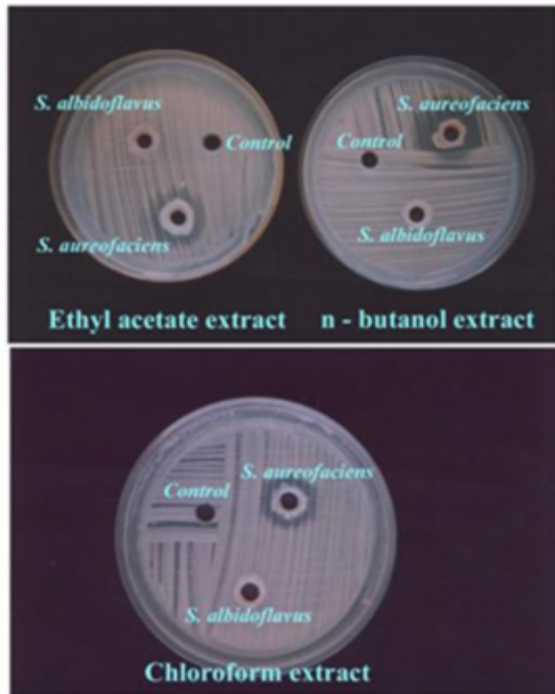
Table 1: Anticandidial activity of *S. aureofaciens* and *S. albidoflavus* on different media

S.N #.	Liquid Media used	Zone of inhibition in mm (diameter)	
		<i>S.aureofaciens</i>	<i>S.albidoflavus</i>
1.	Starch casein	+++ (18)	+ (12)
2.	Yeast extract Malt extract	++++ (24)	+ (12)
3.	Sabouraud dextrose	+++ (16)	+++ (16)
4.	Potato dextrose	+ (12)	+ (12)
5.	Glycerol asparagine	+ (11)	+ (12)
6.	Glucose asparagine	+ (12)	-

++++ very strong antifungal activity
 +++ strong antifungal activity
 ++ moderate antifungal activity
 + weak antifungal activity
 - No antifungal activity

Figure 2

Plate 1 : Antifungal activity of *S. aureofaciens* and *S. albidoflavus* against *C. albicans*



SPECTRAL ANALYSIS OF THE ANTICANDIDIAL COMPOUND

The UV spectral data for the ethyl acetate extract, n-butanol extract, and chloroform extract extracts of selected active fermented broth of both organisms are shown in Figs. 1 and 2. Maximum absorbance peaks observed at 240 nm and the characteristics of absorption peaks indicate a highly polyene nature. The spectral data are consistent with those obtained by Swaadoun et al. (1999). Slavica et al. (2005) reported that the maximum absorbance peaks of UV spectral data ranged between 215 and 270 nm of *Streptomyces* isolates from the soil samples of Southeastern Serbia.

Figure 3

Fig. 1 : UV spectrum of the antifungal metabolite from *S. aureofaciens*

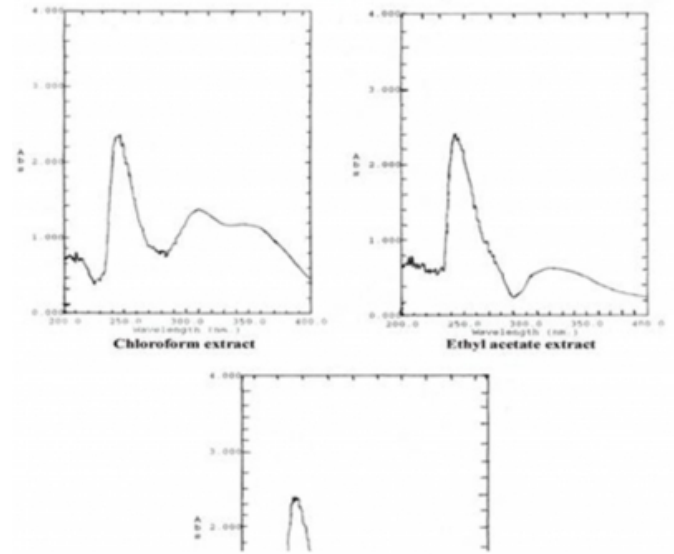
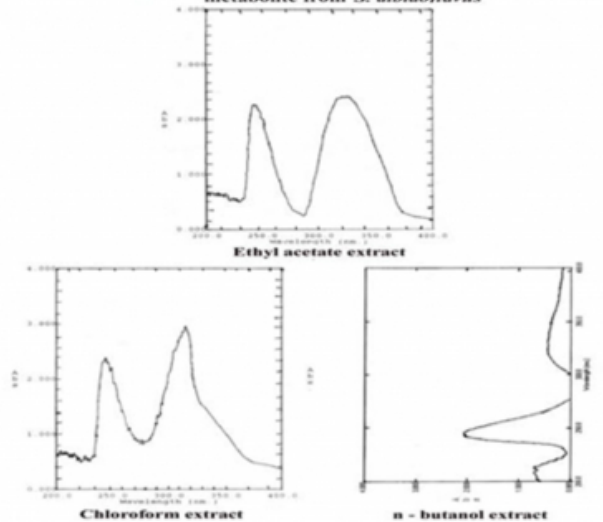


Fig. 2 : UV spectrum of the antifungal metabolite from *S. albidoflavus*



Figs. 3 and 4 show the FTIR spectrum of the anticandidial compound from *S. aureofaciens* and *S. albidoflavus*. The FTIR spectrum of ethyl acetate extracts of *S. aureofaciens* fermentation broth exhibited absorption at 2956 and 2360.7 cm^{-1} , which indicate hydroxyl groups, and the absorption at 1639 cm^{-1} indicating a double bond of polyenic compound. The FTIR spectrum of ethyl acetate extracts of *S. albidoflavus* fermentation broth exhibited absorption at 3310 and 2358.8 cm^{-1} , which indicate hydroxyl groups, and the absorption at 1683 cm^{-1} indicating a double bond. More or less similar trend was observed by Augustine et al. (2005), when they tested the FTIR spectrum of ethylacetate extract

of *S.albidoflavus* PU23 exhibited absorption bands at 3296 and 1031.8 cm^{-1} , which indicate hydroxyl groups, and absorption at 1639 cm^{-1} indicate double bonding.

The need for new, safe and more effective antifungals is a major challenge to the pharmaceutical industry today, especially with the increase in opportunistic infections in the immuno compromised host. The biotechnological potential of *S. aureofaciens* and *S. albidoflavus* in terms of production of antibiotic inhibiting pathogenic fungi is noteworthy. The result obtained in the present investigation indicated that *S. aureofaciens* produced a non proteinic, stable antifungal antibiotic, which seems to be novel. We also further propose that the actinomycetes, even today, are a source for new antifungal antibiotics.

Figure 4

Fig. 3 : FTIR Spectrum of the antifungal metabolite from *S. aureofaciens*

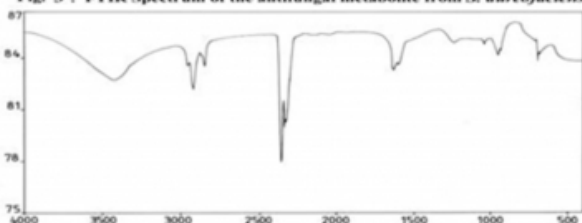
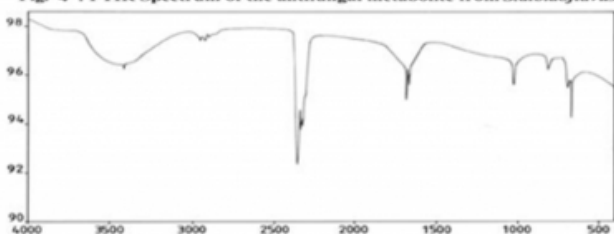


Fig. 4 : FTIR Spectrum of the antifungal metabolite from *S.albidoflavus*



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