

Original Research Article

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Antimicrobial Potential of *Streptomyces violascens* ISP 5183 (T) Isolated from Valley of Flower (VOF) India

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ABSTRACT

Keywords

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VOF Actinomycetes, VOF A167-SAct 225 (*Streptomyces violascens* ISP 5183(T)) exhibited maximum antifungal activity against *C. albicans* MTCC 183 (15.31±1.28 mm) and *C. parapsilosis* ATCC 22019 (12.25±0.35 mm) on GAA media. Comparative antimicrobial activity of culture filtrate of *S. violascens* (VOFA 167-SAct 225) grown (24 hrs, 48 hrs and 72 hrs) on GAA media, extract of culture filtrate and extract of biomass using ethyl acetate as solvent showed that extract from biomass had highest activity (25.01±0.72) against *C. albicans* (MTCC 183). HPTLC, HPLC, LC MS and mass spectrum library search indicates the probability of Amikacin (MW 485) an amino-glycoside.

Introduction

The increasing resistance strains of pathogens against antibiotics are today's key challenges for antimicrobial research. Majority of Antibiotics produced, synthetic or natural were sourced 1st from microbes. Actinomycetes are the major antibiotics producing microbes hence industrially importance since ages. Isolation of these microbes from a specific habitat and niche plays a key role in the discovery of new antibiotics. The class *Actinobacteria*, specifically bacteria belonging to the order *Actinomycetales*, are common soil inhabitants that have the unprecedented ability to produce a wide range of secondary metabolites. Many

species of actinomycetes have the capacity to inhibit pathogenic fungi (Dahiya *et al.*, 2006). However, the inhibitory effect of this isolate on the growth of human fungal pathogens and disease development is probably derived from more than one mechanism. Although the exact mechanisms by which actinomycetes isolate operated to reduce disease incidence is not much elucidated, one possibility is that this strain exerted a direct inhibitory effect on hyphal growth and structure of fungal pathogens (Zaitlin *et al.*, 2004; Zakalyukina *et al.*, 2007; Loqman *et al.*, 2009). *Actinomycetes* have provided important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances. The vast majority of

actinomycetes have originated from soil (Bisht *et al.*, 2013). The present study was undertaken to isolate and characterize antibiotics exhibiting antifungal potential from *Streptomyces violascens* ISP 5183(T) (VOF A-SAct 225) from soil of valley of flower (VOF), Uttarakhand, India.

Experimental

The actinomycetes strain VOF A167-SAct 225 (*Streptomyces violascens* ISP 5183(T)) was isolated from the soil samples collected from the valley of flower, Uttarakhand, India. Three fungal pathogens such as *Candida albicans* (MTCC 183), *Candida parapsilosis* (ATCC 22019) and *Aspergillus fumigates* (MTCC 9657) and two bacterial pathogens viz., *E. coli* (ATCC 9637) and *Staphylococcus aureus* (ATCC 25923) were tested for antimicrobial activity. The capacity of actinomycetes to inhibit growth of test pathogens was evaluated by agar cylinder method (Tortorano *et al.*, 1979). Figure 1 represents the screening of the media and Figure 2 shows the flow chart of the experiments conducted with respective detail.

Results and Discussion

Streptomyces violascens ISP 5183(T) (VOFA 167-SAct 225) prefers to grow better on media containing glycerol (AIA, GAA) as carbon source than dextrose (GSA, MYEA and SEA). There was no growth on MYEA media, whereas when dextrose was supplemented with soyabean meal (GSA) as nitrogen source the growth response was better than SEA media, where it was supplemented with yeast extract (Figure 1). Antimicrobial activity of VOFA 167-SAct 225 varies as per the culture media used. The strain was unable to inhibit the growth of *E. coli* (MTCC 443), *S. aureus* (ATCC 25923), and *A.fumigates* (MTCC 9657) on respective medium. The strain showed maximum antibacterial activity against *C. albicans* MTCC 183 (15.31±1.28 mm) and *C. parapsilosis* ATCC 22019 (12.25±0.35 mm) on GAA media. Bisht *et al.*, (2013) reported *Streptomyces violascens* IN2-10 having broad spectrum activity against *Candida* species, *Aspergillus* species, and Dermatophytes.

Fig.1 Comparative growth of VOF A 167-SAct225 (*Streptomyces violascens* ISP 5183(T)) on five different media (AIA, GSA, GAA, SEA and MYEA) to screen the media for further study. Best growth was observed on AIA and GAA

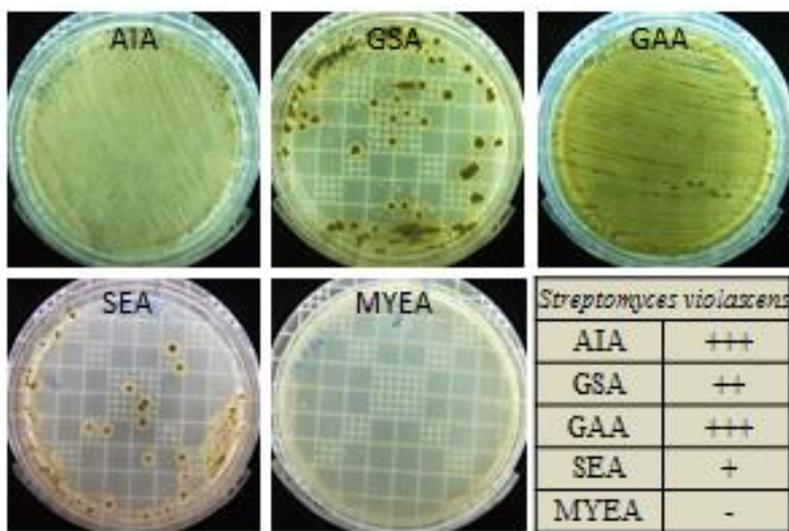
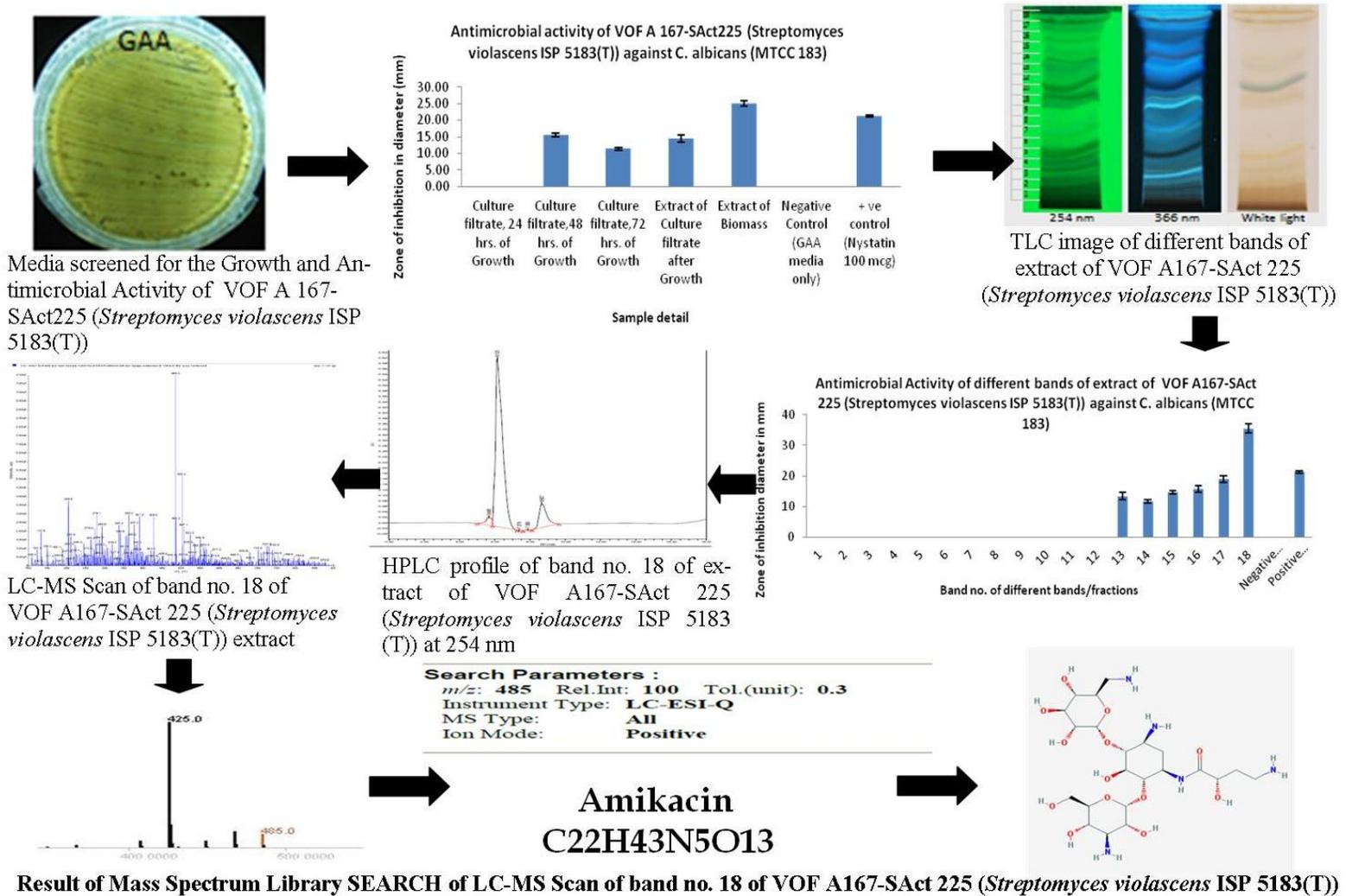


Figure 2., Flow Chart of experimental procedure followed



Jain and Jain (2007) also reported antifungal activity against *C. albicans*, *A. niger*, *M. gypseum*, and *Trichophyton* species from *Streptomyces sampsonii* GS1322. GAA and AIA media was found positive for antimicrobial activity of *S. violascens* against *Candida*. Highest activity for *C. albicans* was recorded on GAA media hence further activity study was done on this media only. Comparative antimicrobial activity of culture filtrate of *S. violascens* ISP 5183(T) (VOFA 167-SAct 225) grown (24 hrs, 48 hrs and 72 hrs of growth) on GAA media, extract of culture filtrate and extract of biomass using ethyl acetate as solvent showed that extract from biomass had highest activity (25.01 ± 0.72) against *C. albicans* (Figure 2. Flow chart).

The extract was subjected to chromatography using readymade HPTLC grade silica gel plate (60F₂₅₄) on alumina from Merck. Total 18 bands were eluted as shown in Figure 2 (Flow chart). All bands were also tested for antimicrobial activity against *C. albicans*. There was no activity in band no. 1 to 12, (R_f value 0.02 to 0.50), band no. 13 to 18 (R_f value 0.57 to 0.90) had shown activity 13.33 ± 1.23 , 11.72 ± 0.62 , 14.57 ± 0.58 , 15.73 ± 1.06 , 19.03 ± 1.09 and 35.46 ± 1.42 respectively. The qualitative activity of band no. 18 (35.46 ± 1.42) was much higher than positive control Nystatin (21.25 ± 0.35). The activity was observed in more than one band (13-18). The HPTLC chromatogram reproduced after scanning of plate and the peak display of *S. violascens* (VOFA 167-SAct 225) extract were analysed & studied.

HPLC of elute from band no. 18 of HPTLC chromatogram shows one major peak and four minor peak at different retention time. This indicates that band no 18 (R_f 0.90-0.98) may contains 4 other molecules in addition to one major as shown in Figure 2 (Flow chart). LC-MS of the eluted band no. 18 of HPTLC chromatogram shows that the mass of

prominent molecule is 485. Mass spectrum library in ESI mode had generated one hint of antibiotics related to this mass and it may be Amikacin (MW 485), an amino-glycoside. Our study supports that a new molecule for the control of candidiasis can be produced from *S. Violascens* ISP 5183(T) (VOFA 167-SAct 225). Zheng *et al.*, (2016, 2017) reported sesquiterpenoids and diterpenoids from *Streptomyces violascens*. This is the 1st report of Amikacin (MW 485) like metabolite, an amino-glycoside from *Streptomyces violascens* (VOFA 167-SAct 225).

The soil isolate from valley of flower (VOF) *Streptomyces violascens* ISP 5183(T) (VOFA 167-SAct 225) showed potent antimicrobial activity against pathogens. HPTLC, HPLC, LC MS and mass spectrum library search indicates the probability of Amikacin (MW 485) an amino-glycoside from VOF A167-SAct 225 [*Streptomyces violascens* ISP 5183(T)]. Amikacin like metabolite produced by this strain showed highest antimicrobial activity against *Candida* species, the pathogen of Candidiasis.

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