



***In Vitro* Antimycotic Activity against *C. Albicans* ATCC 24433 by the Soil Isolate *Streptomyces* sp. M7**

Neha Singh^{1,2*}, Khushboo Bhange³, Shikha Soni⁴, Kamlesh Jain⁴, Vibhuti Rai¹ and O. P. Sharma⁵

¹Department of Microbiology, Pt. Ravishankar Shukla University, Raipur, 492001, India

²Virology Lab, Department of Microbiology, Pandit Jawahar Lal Nehru Memorial Medical College, Raipur, Chhattisgarh. 492001, India

³Department of Biochemistry, Pandit Jawahar Lal Nehru Memorial Medical College, Raipur, Chhattisgarh. 492001, India

⁴Department of Community Medicine, Pandit Jawahar Lal Nehru Memorial Medical College, Raipur, Chhattisgarh. 492001, India

⁵Department of Agriculture and Farmers Welfare, Govt. of India, New Delhi, 110001, India

*Corresponding Author: Neha Singh, Virology Lab, Department of Microbiology, Pandit Jawahar Lal Nehru Memorial Medical College, Raipur, Chhattisgarh. 492001, India

E-mail: nehaashishsingh2015@gmail.com

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ABSTRACT

Streptomyces sp. is a promising source for secondary metabolites exhibiting diverse biological activities. The present study reports the antimycotic activity against *Candida albicans* ATCC 24433 by soil isolate *Streptomyces* sp. M7. Antimycotic activity against *Candida albicans* ATCC 24433 was tested by agar well diffusion method on sabouraud dextrose agar plate with the zone of inhibition 29 mm ± 0.5 mm. Isolate was grown in starch casein nitrate broth at 30°C on a rotary shaker at 200 rpm. The antimycotic activity was determined after 5 days of incubation. Isolate was characterized as *Streptomyces chromofuscus* based on morphological and biochemical characterization and using PIB-Win identification software with a score 1.0.

Keywords: *Streptomyces* sp., Antimycotic activity, Fungal Pathogens, Soil Isolates

INTRODUCTION

Antimycotic drug resistance has increased globally as a result of incorrect use or overuse of antimycotic medications. Antimycotic resistance is one of the major causes of illness and mortality in immunocompromised individuals [1]. Strategies for the search for and discovery of novel antibiotics are generally based on the screening of naturally existing *Streptomyces* sp. [2]. *Actinomycetes* that exist naturally are typically screened as a preliminary step for methods used in the search for and discovery of novel antibiotics [3]. *Actinomycetes* are the source of over 70% of all antibiotics that are now known and reported, of which 75% are used in medicine and 60% in the agriculture field [4]. *Streptomyces* typically make up a significant fraction of *Actinobacteria* in natural soil habitats. From the genus *Streptomyces*, almost 45% of important bioactive chemicals currently known have been identified [5,6]. Bearing the aforementioned information in mind, the current work intends to isolate *Streptomyces* from distinctive and undiscovered environments in Chhattisgarh that produce novel bioactive antimycotic compounds. As fungal diseases emerged as risks to public health. The fungal spores in the aerosols, which include species of *Aspergillus*, *Cladosporium*, *Chaetomium*, *Penicillium*, *Wallemia*, and *Stachybotrys*, among others, have been linked to numerous potentially fatal respiratory illnesses, including hypersensitivity, *pneumonia*, *Aspergillosis*, *Candidiasis*, *Mucormycosis*, and cancer [7]. Therefore, the goal of the present research was to identify *Streptomyces* sp. having antimycotic activity from the soil.

MATERIALS AND METHODS

Isolation and maintenance of Isolate M7

One gram of dried composite soil sample was dissolved in 9 ml of 0.85% normal saline solution to prepare 10-fold serial dilutions aseptically in the test tube (10^{-2} to 10^{-5}). In triplicate, aliquots of 100 μ l from each tube were transferred and evenly disseminated on the surface of starch casein nitrate agar media [8]. Plates were incubated at 30°C for 5 days -7 days in the inverted position. Isolates with distinctive morphologies, colonies, and patterns of growth were selected, transferred to the appropriate agar slants, and stored at 4°C in the refrigerator for further use.

Characterization of strain M7

The isolate was identified based on morphological, and biochemical characteristics [6,7]. Utilization of starch, guanine, esculin, gelatin, urea, and Tween 20 was noted. Reaction for the catalase, oxidase, hydrogen sulfide production, and melanin on tyrosine agar medium was observed. The isolate growth on different concentrations of NaCl (1%-5%), potassium tellurite (0.01%-0.001%), and phenol (0.1%) was observed. Utilization of different carbon and nitrogen sources such as D-glucose, dextran, L-arginine, L-rhamnose, L- cysteine, D-lactose, D-sorbitol, L-tyrosine, L-asparagine, D-maltose, L-serine, cellobiose, DL- α -amino-n-butyrac acid, L-histidine, D-mannitol, meso-inositol and growth at a different range of temperature 15°C -37°C and at pH between 4-9 was recorded. Finally, isolate M7 was identified using PIB-Win identification software.

Antimycotic activity

To see the antimycotic activity isolate M7 grown in starch casein nitrate broth with the composition g/L: starch: 10; Casein:30; KNO₃: 2.0; MgSO₄: 0.05; K₂HPO₄: 2.0; NaCl:2; CaCO₃:0.02; FeSO₄.7H₂O: 0.01. A loopful culture of the isolate was inoculated in a 1 liter flask containing 500ml of broth in triplicates. All the flasks were incubated at 30°C at 200 rpm in a rotatory shaker for 5 days.

Agar well diffusion method

After incubation 2 ml of fermented broth was transferred to 5 ml of Eppendorf tube and centrifuged at 12,000 rpm in a cooling centrifuge. The surface of on sabouraud dextrose agar was swabbed with test organism *C. albicans* ATCC 24433 using a sterilized cotton swab to access the antimycotic activity, and 6 mm diameter wells were cut using a sterile cork borer on the same sabouraud dextrose agar plate [2,5,6]. 100 μ l of centrifuged and fermented broth was added using sterile micro tips and a pipette. Plates were refrigerated for 30 minutes to allow the metabolite to diffuse. The plates were then kept at 30°C for 48 hours-72 hours. The Zone of Inhibition (ZOI) was measured following incubation to express the antimycotic activity.

Test organism used

C. albicans ATCC 24433 were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh to assess the antimycotic activity of isolates.

RESULTS AND DISCUSSION

Isolation, morphological and biochemical characterization of strain M7

The isolate M7 was well isolated on starch casein nitrate agar media at 30°C. The optimum incubation time was 5 days and the isolate was able to degrade starch, guanine, esculin, gelatin, urea, Tween 20, and. Isolate showed a positive reaction for catalase, oxidase, and hydrogen sulfide production (Figure 1a,b). The isolate was able to produce melanin on a tyrosine agar medium. The isolate was able to grow with 1% -5% NaCl, 0.01-0.001% potassium tellurite, and 0.1% phenol. It was able to utilize D-glucose, dextran, L-arginine, L-rhamnose, L- cysteine, D-lactose, D-sorbitol, L-tyrosine, L-asparagine, D-maltose, L-serine, cellobiose, DL- α -amino-n-butyrac acid, L-histidine, D-mannitol, meso-inositol. The isolate was able to grow at a temperature of 15°C-37°C and at a pH between 4-9. M7 was identified as *Streptomyces chromofuscus* and the PIB-Win identification score was 1.0 [2].

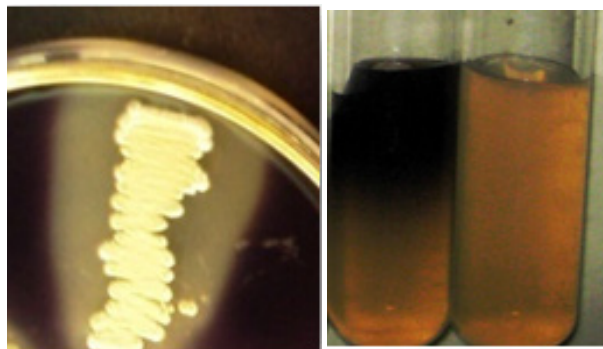


Figure 1. Biochemical properties of Isolate M7 (a) starch hydrolysis activity (b) H₂S utilization

Antimycotic activity

After incubation of 5 days of isolate M7, extracellular antimycotic activity was accessed using sabouraud dextrose agar against test organism *C. albicans* ATCC 24433. The zone of inhibition was recorded as 29 mm \pm 0.5 mm. clear area around the well indicated the antimycotic activity of the fermented broth and inhibition of the fungal strain (Figure 2). To see the extracellular activity fermented broth was centrifuged at 12000 rpm and the cell-free broth was loaded into wells on SDA plates. Most antibiotics are extracellular has been noticed in various research, and more research is needed to clarify the extraction, purification, and characterization of the antifungal metabolite [9]. Antimicrobial production is greatly affected by cultural conditions such as aeration and mixing of the nutrients in the fermentation media are impacted by agitation and so secondary metabolite production [10,11]. The yield of the antifungal metabolite is strongly impacted by temperature variations from the ideal range. Hence, the optimum production of the secondary metabolite is necessary for these isolates [12]. In our study, we investigate the isolation of novel *actinomycete* strains to produce novel compounds with antimycotic properties from largely unexplored agroecological niches in India. Also, this discovery provides the path for future characterization of these *Streptomyces sp.* isolates for optimal antifungal usage.



Figure 2. Antimycotic activity of isolate M7 against *C. albicans* ATCC 24433

CONCLUSION

Isolate M7 produced an antimycotic compound against the *C. albicans* and it can be a potential source of an antimycotic-producing strain isolated from the soil as a natural source. The author already reported various antibacterial and antimycotic compounds producing *Streptomyces sp.* From the soil of Chhattisgarh. Chhattisgarh is the central state of India and is covered 40% of the forest area. This region of unexplored and previous reports also indicated that these regions could be explored more in the search for new antimicrobial-producing *Streptomyces sp.* Secondary metabolites produced by these strains can be used to treat various bacterial and fungal diseases.

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