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Original Article

In vitro antimycotic activity of a new isolate *Streptomyces fradiae* MTCC 11051 against the multi-drug resistant pathogenic fungi

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ARTICLE INFO

Article history:

Received 28 March 2013

Accepted 24 April 2013

Available online 11 May 2013

Keywords:

Antifungal metabolites

16S rRNA sequencing

Streptomyces sp

ABSTRACT

Background: Isolation of naturally occurring *Streptomyces* sp. with an ability to produce metabolites having antimycotic property against the pathogenic fungus from unexplored area of Chhattisgarh.

Methods: Starch casein nitrate medium was used for isolation and screened for their antifungal activity by agar well diffusion method. Active isolate characterized based on the morphological, physiological and 16S rRNA sequencing. Optimization of extracellular antifungal metabolite production in terms of zone of inhibition (mm) using *Candida albicans* MTCC 183 as target organisms was performed and extracted with *n*-butanol. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of active metabolite was determined according to NCCLS.

Results and discussion: The active isolate was identified as *Streptomyces fradiae* MTCC 11051, showed the best antifungal activity against yeasts and molds including dermatophytes which showed an inimitable and stable inhibitory activity in Starch casein nitrate broth. The maximum extracellular active metabolite production was achieved in the late log phase, which remained constant during the stationary phase. The MIC and MFC values of the culture supernatant were recorded between range 50 and 100 µg/ml.

Conclusions: The metabolite from *S. fradiae* was active against pathogenic fungus with a greater potency. These results confirmed the therapeutic potential of bioactive fermented products can be used in medicinal and pharmaceutical preparations for treating the fungal infections.

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1. Introduction

Actinomycetes are diverse group of heterotrophic prokaryotes forming hyphae at some stage of their growth; hence, they are referred to as filamentous prokaryotes.¹ They are the prolific

producers of antibiotics and other industrially useful secondary metabolites.^{2,3} Approximately 70% of all antibiotics known were isolated from actinomycetes, in which 75% were employed in medicine and 60% in agriculture.^{4,5} Among the most known genus, we can find *Streptomyces*. These are used

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<http://dx.doi.org/10.1016/j.jopr.2013.04.024>

in the manufacturing fermentation of the active pharmaceutical compounds, such as the antifungal ones, antiviral, anticancer, agents of immunosuppressor, insecticides, weed killers, etc.⁶ Approaches to the search for and discovery of new antibiotics are generally based on screening of naturally occurring actinomycetes.² The objective of the present study was to isolate actinomycetes from the soil of Durg, Chhattisgarh, India, with an ability to produce metabolites having antimycotic property against the fungal pathogens. However, there is not documented information on antifungal activities of *Streptomyces* sp. isolated from the soil of Raipur, India, as a novel source for the discovery of new bioactive compounds. Such unexplored or under-exploited environments may be crucial for new strains of streptomycetes being wild types showing rich source of useful metabolites. Therefore, the study reported herein was undertaken to determine the antifungal potential of *Streptomyces* against some pathogenic fungi, the taxonomy of the antibiotic producing strain as well as detailed production optimization.

2. Methods

2.1. Isolation and screening of antifungal actinomycetes

Actinomycetes were isolated on starch casein nitrate agar medium by serial dilution method.⁷ One most promising isolate, MS02, having broad spectrum antimycotic activity, was selected for further study and grown on different agar media such as starch casein nitrate agar, glucose soybean agar, glucose asparagine, Sabouraud dextrose and yeast extract-malt extract to know which medium stimulates maximum antifungal activity. All media were obtained from Hi-Media, Mumbai. After incubation for 7 days at 28 °C, agar discs of actinomycete growth were made with a sterile cork borer (6 mm) and placed on Sabouraud dextrose agar (SDA) plates (pH 5.6) seeded with the fungal test organisms. After incubation plates were observed for bioactive property after 24 h in case of yeasts and 96 h in case of molds. The antifungal activity of the culture supernatant of the actinomycete in above mentioned liquid media was tested by agar well diffusion method.⁸ The zone of inhibition (mm) around the well was determined as antifungal activity. Values are given as mean and standard deviation (SD) of tests performed in triplicate.

2.2. Fungal test organisms used

Candida albicans MTCC 183, *C. albicans* MTCC 1346, *C. albicans* ATCC 10231, *C. albicans* ATCC 2091, *C. albicans* MTCC 2512, *Penicillium citrinum* MTCC 1751, *Candida tropicalis* ATCC 750, *Cryptococcus terreus* ATCC 11799, *Trichophyton rubrum* MTCC 296, *Alternaria alternata* MTCC 1362, *Rhizoctonia oryzae* MTCC 2162, *Aspergillus terreus* DSM 826, *Aspergillus niger* DSM 63263, *A. niger* DSM 2182, *Aspergillus fumigatus* ITCCF 1628, *Aspergillus versicolor* DSM 1943, *Aureobasidium pullulans* DSM 2404.

2.3. Characterization of the selected actinomycete isolate

Morphological features of the isolate were studied by cover slip method.⁹ the cover slips were observed under light

microscope (1000×) after incubation for one week at 28 °C. The morphology of the spore bearing hyphae with entire spore chain along with the substrate and aerial mycelium was also observed under scanning electron microscope (SEM) (JEOL, Tokyo, Japan). Cultural characterization was done on ISP (International Streptomyces Project) media; yeast extract - malt extract agar (ISP-2), oatmeal agar (ISP-3), glycerol asparagine agar (ISP-5), peptone yeast extract iron agar (ISP-6), inorganic salts starch agar (ISP-4), tyrosine agar (ISP-7) and nutrient agar at 28 °C. All media were obtained from Hi-Media, Mumbai. The growth of the organism was studied at different temperatures and salt concentrations such as 22, 28, 37, 42 °C and 2, 4, 6, 8, 10% respectively. Utilization of different carbon and nitrogen sources such as D-glucose, D-galactose, D-fructose, D-mannitol, D-xylose, L-arabinose, L-rhamnose, L-raffinose, L-cysteine, L-histidine, L-tyrosine, D-alanine, L-leucine, L-phenylalanine and L-valine was studied. Chemotaxonomic studies were done by analyzing the cells for 2,6-diaminopimelic acid.⁹

2.4. 16S rRNA gene sequencing

16S rRNA studies were conducted and isolate MS02, was submitted in Microbial Type Culture Collection, IMTECH, Chandigarh, India. The preparation of total genomic DNA was conducted in accordance with the methods described by Sambrook et al⁷ PCR amplification of the 16S rRNA gene of the local *Streptomyces* strain MS02 was conducted in accordance with the method described by Edwards et al¹⁰ The sequence data were deposited in the GenBank database, under the accession number JF915304. The BLAST program (www.ncbi.nlm.nih.gov/blst) was employed in order to assess the degree of DNA similarity. Multiple sequence alignment and molecular phylogeny were evaluated using BioEdit software and the phylogenetic tree was displayed using the TREE VIEW program.¹¹

2.5. Production of antifungal metabolite

Spore suspension of *Streptomyces* isolate MS02, was prepared from the freshly grown culture on starch casein nitrate agar slant and inoculated into 100 ml starch casein nitrate broth (10⁷ spores/ml of the medium) in 500 ml Erlenmeyer flask. The flask was incubated on rotary shaker (180 rpm) for 5 days at 28 °C. The culture was centrifuged at 8000 rpm for 20 min. The culture supernatant was used as a source of antifungal metabolite against *C. albicans* MTCC 183, as a target organism.

2.6. Optimization of antifungal metabolite production

Antifungal metabolite production was carried out in 100 ml starch casein nitrate broth (soluble starch – 10 g, Potassium phosphate dibasic – 2 g, Potassium nitrate – 2 g, Sodium chloride – 2 g, Casein – 0.3 g, MgSO₄ · 7H₂O – 0.05 g, CaCO₃ – 0.02 g, FeSO₄ · 7H₂O – 0.01 g, Distilled water – 1000 ml, pH – 7) in 500 ml Erlenmeyer flasks.

2.7. pH

The initial pH of the starch casein nitrate broth was adjusted to 4, 5, 6, 7, 8 and 9 separately with 0.1N NaOH/0.1N HCl. The

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