Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 2 (12): 992-994, 2007
ISSN 1816-4935

Screening of *Streptomyces purpeofuscus* for Antimicrobial Metabolites

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Abstract: While screening the actinomycetes for bioactive metabolites, *Streptomyces purpeofuscus* was isolated from laterite soil. An attempt was made to screen *S. purpeofuscus* for the production of antimicrobial metabolites. The cell growth as well as antimicrobial metabolite production was studied on different culture media. Secondary metabolites from the strain were active against bacteria like *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* as well as fungi including *Aspergillus flavus*, *A. niger* and *Penicillium citrinum*.

Key words: *Streptomyces purpeofuscus*, antimicrobial metabolites

INTRODUCTION

Actinomycetes have been isolated from different soils, plant materials, water and marine sediments (Mincer *et al*., 2002). At least 90% of the population among actinomycetes isolated from soils have been reported to be *Streptomyces* spp. Among microorganisms, actinomycetes are the important source for bioactive metabolites especially antibiotics (Berdy, 2005). *Streptomyces* and *Micromonospora* are reported to be most prolific producers of pharmacologically and agriculturally active agents (Magarney *et al*., 2004). During the isolation and enumeration of actinomycetes from laterite soil, a widely distributed strain of *Streptomyces* sp. was isolated and identified as *Streptomyces purpeofuscus*. The strain has been deposited with the Microbial Type Culture Collection (MTCC) at IMTECH, Chandigarh (India) with accession number MTCC 6473. The main objective of the present study is to screen *S. purpeofuscus* for the production of antimicrobial metabolites.

MATERIALS AND METHODS

The strain, *S. purpeofuscus* MTCC 6473 was isolated from laterite soil samples in 2005 by dilution plate technique on yeast extract-malt extract-dextrose (YMD) agar medium (Narayana and Vijayalakshmi, 2005). Micromorphology of the strain was observed by slide culture method (Williams and Cross, 1971).

Growth pattern of the strain was studied on YMD broth. Pure culture of the isolate was inoculated and incubated at 35°C for one week. At every 24 h interval, the culture was harvested and its biomass was separated from culture filtrate and dry weight of the biomass was recorded (Narayana *et al*., 2004). The culture filtrate collected from five-day old fermented YMD broth was extracted with dichloromethane. Concentrated solvent extracts (50 ppm) were used for antimicrobial assay by employing cup-plate method (Cappuccino and Sherman, 2004). The test organisms include *Bacillus cereus* (MTCC 430), *B. subtilis* (MTCC 441), *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96) and fungi like *Aspergillus niger*, *A. flavus*, *Fusarium udum* and *Penicillium citrinum*. The zone of growth inhibition was measured after incubation for 18 h at 28°C.

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The strain was grown on different media including YMD broth, starch-casein broth, glycerol-asparagine broth, oatmeal broth and nutrient broth to understand its growth pattern as well as antimicrobial metabolite production (Pridham and Lyons, 1980). Biomass was articulated as cell dry weight per 100 mL of culture medium. Production of antimicrobial metabolites by the strain was expressed in terms of inhibition zone against most sensitive bacterium and fungus exerted by 50 ppm of solvent extract obtained from different media respectively.

RESULTS AND DISCUSSION

The culture had straight to flexuous sporophores arising from the aerial mycelium and may be placed in rectus-flexibilis group of *Streptomyces* (Pridham *et al*., 1958). Kawamura *et al*. (1976) described the species, *S. purpeofuscus* strain ATCC 23952 and strain S15-1 with rectus-flexibilis type of spore chains. The growth pattern of *S. purpeofuscus* was studied on YMD broth. The culture entered the log phase after 24 h incubation. The length of stationary phase ranged from 96-120 h of incubation (Fig. 1). The 5 day old strain exhibited good antimicrobial activity against *B. cereus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus* and fungi such as *A. flavus*, *A. niger* and *P. citrinum* (Table 1). The secondary metabolites produced by *S. purpeofuscus* were found to inhibit *S. aureus* (gram-positive) and *P. aeruginosa* (gram-negative). Among fungi, *A. niger* and *A. flavus* were highly sensitive to the metabolites of the strain, however the growth of *F. udum* was not affected.

Among different media tested, YMD broth supported good growth of the strain as well as antimicrobial metabolite production (Table 2). Nutrient broth and starch-casein broth were also found suitable for bioactive metabolite production. The strain exhibited maximum growth when

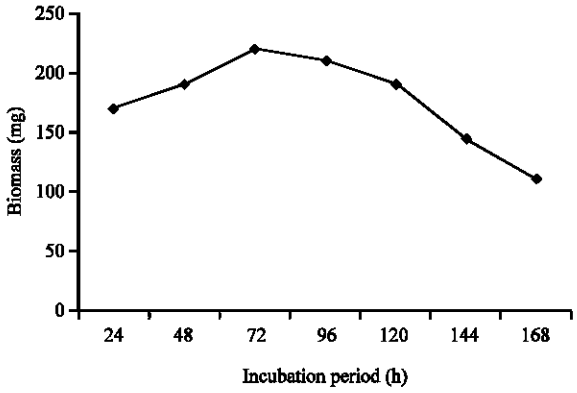

Fig. 1: Growth pattern of *Streptomyces purpeofuscus* in YMD broth

Table 1: Antimicrobial activity of *Streptomyces purpeofuscus*

Test organisms	Area of inhibition zone (mm^2)
Bacteria	
Bacillus cereus	113.07
B. subtilis	123.47
Escherichia coli	134.27
Pseudomonas aeruginosa	157.15
Staphylococcus aureus	157.07
Fungi	
Aspergillus flavus	141.49
A. niger	112.97
Fusarium udum	0.00
Penicillium citrinum	102.97

Table 2: Effect of different nutrient media on biomass and antimicrobial metabolite production by *Streptomyces purpeofuscus*

Medium	Biomass (mg/100 mL)	Area of inhibition zone (mm^2)	
		Pseudomonas aeruginosa	*Aspergillus flavus*
YMD	185	157.15	141.49
Starch-casein	167	68.87	100.27
Glycerol-asparagine	92	66.92	93.41
Oatmeal	214	58.87	73.35
Nutrient	89	93.41	105.37

cultivated on oatmeal broth, but metabolite production was not up to the mark. *S. purpeofuscus* strain ATCC 83952 was reported to produce the antibiotic Negamycin, while *S. purpeofuscus* strain S15-1 produced antiviral antibiotic belonging to the streptothricin group of antibiotics (Kawamura *et al*., 1976). A haptene antifungal antibiotic was reported from *S. purpeofuscus* strain CM 1261 (Jain and Jain, 2004) indicating the variation in metabolite production by different strains. The secondary metabolites from the strain 6473 isolated from laterite soil of Nagarjunanagar exhibited strong antimicrobial activity against gram-positive, gram-negative bacteria and fungi.

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