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Studies on Cultural, Physiological and Antimicrobial Activities of *Streptomyces rochei*

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Abstract: In the screening process of actinomycete isolates for potent antimicrobial agents, an actinomycete strain identified as *Streptomyces rochei* MTCC 8376 was found to have high antimicrobial potential. The strain produced different enzymes such as amylase, asparaginase, cellulase, chitinase, nitrate reductase, protease, tyrosinase and urease. It exhibited resistance to ampicillin, deoxycycline hydrochloride, methicillin, nalidixic acid, rifampicin and trimethoprim. Metabolites produced by the strain showed good antimicrobial activity against Gram – positive and Gram – negative bacteria. In Gram – positive bacteria, *Staphylococcus aureus* exhibited higher sensitivity followed by *Bacillus cereus* and *B. subtilis* whereas in Gram – negative bacteria ones, *Escherichia coli* was extremely sensitive to the metabolites produced by the strain followed by *Pseudomonas aeruginosa* and *Proteus vulgaris*. Among the test fungi, *Candida albicans* followed by *Aspergillus niger*, *Penicillium citrinum*, *A. flavus*, *Fusarium oxysporum* and *Alternaria alternata* were also sensitive to the metabolites elaborated by the strain.

Key words: Actinomycetes, *Streptomyces rochei*, physiological activities, antimicrobial activity

INTRODUCTION

Of all the known microbes, actinomycetes represent a rich source of biologically active metabolites such as antibiotics, agrochemicals, enzymes, immunosuppressants, antiparasitics and anticancer agents[3]. Emergence of drug resistant pathogens especially in immunodeficient patients revealed the need for new and novel antibiotics[9,10]. Majority of the antibiotics so far reported are obtained from *Streptomyces*, which are common inhabitants of soil[16]. Numerically, they cover about 80% of total antibiotic products as compared to another genera[11]. In the process of screening actinomycetes for potent antimicrobial agents[14], one promising isolate was dominant and efficient in inhibiting the growth of test organisms. The isolate was identified as *S. rochei* and the strain was deposited at Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh with accession number MTCC 8376. Basing on the broad antimicrobial potential of *S. rochei* isolated from laterite soils of Acharya Nagarjuna University, the present study was aimed to determine the cultural, physiological and antimicrobial activities of *S. rochei* MTCC 8376.

MATERIALS AND METHODS

Streptomyces rochei was isolated from laterite soils of Acharya Nagarjuna University by using soil dilution plate technique on Asparagine-glucose agar medium containing 1% D-Glucose, 0.05% L-asparagine, 0.05% dipotassium hydrogen orthophosphate, 2% agar at pH 7.2 before sterilization. The cultural characteristics of the strain was studied on different media such as Yeast extract-malt extract-Dextrose agar (ISP medium 2), Oat meal agar (ISP medium 3), Starch inorganic salts agar (ISP medium 4), Asparagine-glycerol agar (ISP medium 5), Tyrosine agar (ISP medium 7), Asparagine-glucose agar, Starch casein agar, Maltose tryptone agar, Czapek-Dox agar, Sabouraud's agar and Nutrient agar media[19]. Micromorphology of the strain was examined by slide culture method[21].

The utilization of carbon and nitrogen sources by the strain was carried out according to the method described by Gottlieb[7]. Ability of the strain to produce different enzymes was examined by using standard methods[8]. Sodium chloride tolerance of the strain was determined by the method suggested by Ellaiah *et al*.[5]. The strain was tested for its ability to produce H_2S[13], Indole[8] and acid[18]. Besides, sensitivity of the strain to different antibiotics was determined by paper disc method[22].

For the production of antimicrobial metabolites, *S. rochei* was cultivated in Asparagine-glucose broth (seed medium) for 24 h. Seed medium at the rate of 10% was transferred into the fermentation medium containing 0.4% yeast extract, 1% malt extract, 0.4% dextrose, 0.2% calcium carbonate with pH adjusted to 7.2 (YMD broth) and incubated at 28°C. The crude culture filtrate obtained from a four-day old culture was extracted with ethyl acetate and evaporated to dryness

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under vacuum at 35°C[6]. The residue dissolved in dimethyl sulphoxide (1000 µg/ml) was employed for testing antimicrobial activity against the test bacteria which included *Bacillus cereus* (MTCC 430), *B. subtilis* (MTCC 441), *Escherichia coli* (MTCC 40), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96) and fungi *Candida albicans* (NCIM 3100), *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum* and *Penicillium citrinum* by using cup-plate method[4]. The solvent (DMSO) alone was used as control. Nutrient agar and Czapek-Dox agar were the media used for culturing the test bacteria and fungi respectively. Solvent extracts (50 µL) were placed into corresponding cups and growth inhibition was measured after incubation for 18 h at 28°C. The antimicrobial activity was estimated by measuring the diameter of the zones of inhibition.

RESULTS AND DISCUSSIONS

The strain exhibited good growth on YMD agar, Oat meal agar, Starch inorganic salts agar, Asparagine-glycerol salts agar, Tyrosine agar, Starch casein agar, Maltose tryptone agar and Czapek-Dox agar media. Moderate growth was found in Asparagine-glucose agar and Nutrient agar media whereas poor growth was observed in Sabouraud's agar medium. The color of the aerial mycelium varied from grey to white while that of the vegetative mycelium was from light to dark brown. Slide culture studies showed the spore chain morphology of *S. rochei* as spiral type and may be placed in Spira group[15]. The physiological characteristics of the isolate are presented in Table 1. The strain utilized carbon sources such as D-xylose, mannitol, D-glucose, sucrose, glycerol, starch and D-fructose. Peptone, tryptone, L-asparagine, L-tyrosine, L-glycine, sodium nitrate, potassium nitrate, casein and ammonium sulphate served as good nitrogen sources for the strain.

The strain exhibited salt tolerance (up to 7%) and may be placed in the intermediate salt tolerance group. The strain also showed various biochemical activities such as H_2S, Indole and acid production. The strain produced melanoid pigments on tyrosine agar medium (ISP medium 7). It had the capability to produce different enzymes such as amylase, asparaginase, cellulase, chitinase, nitrate reductase, protease, tyrosinase and urease. It exhibited sensitivity to a number of antibiotics such as amikacin, bacitracin, ciprofloxacin, clindamycin, erythromycin, furoxone, gentamicin, kanamycin, metronidazole, neomycin, oxytetracycline, penicillin-G, polymyxin-B, roxithromycin, streptomycin, tetracycline and vancomycin while it was resistant to ampicillin, deoxycycline hydrochloride, methicillin, nalidixic acid, rifampicin and trimethoprim (Table 2). Basing on these morphological and physiological characteristics, the strain has been identified as *Streptomyces rochei* and deposited at Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh with the accession number MTCC 8376.

Table 1: Morphological and physiological characteristics of *Streptomyces rochei*

Characteristics	Results
Gram staining	Gram positive
Spore chain morphology	Spira
NaCl tolerance	
1-7% (W/V) NaCl	+*
10-13% (W/V) NaCl	-
H_2S production	0
IAA production	0
Acid production from	
Carbon free medium	-
Arabinose	-
Fructose	0
Glucose	0
Glycerol	0
Lactose	0
Maltose	0
Mannitol	0
Starch	0
Sucrose	0
Xylose	0
Enzyme production	
Amylases	0
Asparaginase	0
Cellulase	0
Chitinase	0
Citrate utilization	0
DNase	-
Nitrate reductase	0
Protease	0
Tyrosinase	0
Urease	0

+*: Positive reaction; -: Negative reaction

Antimicrobial spectrum of *S. rochei* is shown in Table 3. The metabolites produced by the strain showed good antimicrobial activity against the test organisms and the zone of inhibition varied from 12 to 25 mm. Among the test Gram – positive bacteria, *S. aureus* followed by *B. cereus* and *B. subtilis* were extremely sensitive to the metabolites elaborated by *S. rochei* whereas in Gram – negative ones, *E. coli* exhibited higher sensitivity followed by *P. aeruginosa* and *P. vulgaris*. Metabolites produced by the strain also showed strong antifungal activity against *Candida albicans* followed by *Aspergillus niger*, *Penicillium citrinum*, *A. flavus*, *Fusarium oxysporum* and *Alternaria alternata*.

Kotake *et al.*[12] extracted two antifungal substances, butyrolactols A and B from the broth culture of *S. rochei* S785-16. Butyrolactols A showed good antifungal activity against *A. fumigatus* and *Trichophyton mentagrophytes* whereas moderate activity on yeasts was reported. Ugur and Sachin[20] reported that the Poly ß-hydroxy butryric acid producing *S. rochei* (MU119) had no activity against the test

Table 2: Sensitivity of *Streptomyces rochei* to antibiotics

Antibiotic (µg/disc)**	Sensitivity (R/S)
Amikacin (30)	S
Ampicillin (10)	R
Bacitracin (10)	S
Ciprofloxacin (5)	S
Clindamycin (2)	S
Deoxycycline hydrochloride (30)	R
Erythromycin (15)	S
Furoxone (100)	S
Gentamicin (10)	S
Kanamycin (30)	S
Methicillin (5)	R
Metronidazole (5)	S
Nalidixic acid (30)	R
Neomycin (30)	S
Oxytetracycline (30)	S
Penicillin-G (10)	S
Polymyxin-B (300)	S
Rifampicin (5)	R
Roxithromycin (30)	S
Streptomycin (10)	S
Tetracycline (30)	S
Trimethoprim (5)	R
Vancomycin (30)	S

**: Concentration of antibiotics in µg/disc, S: Sensitive, R: Resistant

Table 3: Antimicrobial spectrum of *Streptomyces rochei*

Test organisms	Zone of inhibition (mm)
Bacteria	
Bacillus cereus	24
B. subtilis	23
Escherichia coli	25
Proteus vulgaris	12
Pseudomonas aeruginosa	15
Staphylococcus aureus	25
Fungi	
Alternaria alternata	13
Aspergillus flavus	14
A. niger	18
Candida albicans	20
Fusarium oxysporum	14
Penicillium citrinum	15

organisms *B. subtilis*, *E. coli*, *S. aureus* and *C. albicans*. They also found that the production of melanin and other diffusible pigments by the strain were negative. *Streptomyces rochei* F20 was reported to produce the antibiotic, Streptothricin[1]. Metabolites of *Streptomyces* species including *S. rochei* showed antimicrobial activity against the mushroom blotch disease pathogen, *Ps. tolaasii* and the diameter of the zone of the inhibition was greater than 20 mm[17]. Augustine *et al.*[2] informed that the metabolites of *S. rochei* AK 39 showed antifungal activity against *Epidermophyton floccosum*, *T. rubrum* and *Microsporum gypseum* while these metabolites had no effect on *C. albicans, A. niger* and *Fusarium oxysporum*. In the present study, *S. rochei* MTCC 8376 inhibited a wide variety of Gram - positive (*B. subtilis*, *S. aureus*), Gram - negative bacteria (*E. coli*) and fungi (*A. niger*, *F. oxysporum* and *C. albicans*). These results revealed that the metabolites elaborated by *S. rochei* MTCC 8376 may be quite different from that of *S. rochei* reported earlier which needs further indepth studies. In view of its broad antimicrobial spectrum, attempts are in progress to characterize the antimicrobial compounds produced by *S. rochei* MTCC 8376.

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