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Studies on Bioactive Metabolites Produced by *Lechevalieria flava*

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Abstract: Cultural, morphological and physiological characteristics of *Lechevalieria flava* were studied. The strain exhibited sensitivity to different types of antibiotics. It could tolerate salt concentration upto 4%. The isolate had the ability to produce enzymes such as amylase, chitinase, protease and urease. Growth pattern and antimicrobial properties of the strain were studied in different media. Impact of various carbon and nitrogen sources on the production of bioactive metabolites was also investigated. The secondary metabolite from *L. flava* was found to be active against bacteria like *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and fungi such as *Candida albicans*.

Key words: *Lechevalieria flava*, Physiological characteristics, bioactive metabolites

INTRODUCTION

Actinomycetes are G+C rich gram positive bacteria that are wide spread in nature and play a significant role in the production of bioactive metabolites. The active profile of actinomycetes is very broad, which includes numerous potentially useful compounds providing the widest range and most promising array of pharmacologically and agriculturally active compounds (Berdy, 2005). After the discovery of gentamicin from *Micromonospora*, searching of bioactive compounds from rare actinomycetes has gained attention (Balagurunathan, 2004). During the screening for rare actinomycetes from laterite soils of Acharya Nagarjuna University, a strain of non-streptomycete actinomycetes related to the genus *Nocardiopsis* was isolated and identified as *Lechevalieria flava*. With the discovery of new antibiotics from strains of *Actinomadura*, *Micromonospora*, *Saccharothrix* and *Streptosporangium*, increased emphasis was placed on developing methods for the isolation and identification of non-streptomycete actinomycetes (Zitouni *et al*., 2004; Boudjella *et al*., 2006). The strain, *L. flava* has been deposited with the Microbial Type Culture Collection (MTCC) at IMTECH, Chandigarh (India) with the accession number MTCC 6470. Hence, an attempt has been made in the present study to investigate the physiological properties of the strain as well as its ability to produce the bioactive compounds from a rare actinomycete isolate, *Lechevalieria flava*.

MATERIALS AND METHODS

Isolation

Lechevalieria flava was isolated from laterite soils present surround areas of the Acharya Nagarjuna University by dilution plate technique on yeast extract-malt extract-dextrose (YMD) agar medium. The medium consisted of 0.4% yeast extract, 1% malt extract, 0.4% dextrose, 0.2% $CaCO_3$ and 2% agar. The final pH of the medium was adjusted to 7.2 before sterilization (Narayana *et al*., 2005).

Morphology and Pigmentation

L. flava MTCC 6470 was grown on YMD agar (ISP medium 2), oat meal agar (ISP medium 3) and inorganic salts-starch agar (ISP medium 4) as described by Shirling and Gottlieb

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(1966). Culture on YMD agar was examined for pigmentation, aerial mycelia and other morphological features.

Physiological Characteristics

The strain tolerance to NaCl was determined by growing the organism on YMD agar plates supplemented with 0, 4, 7, 10 and 13% (Wt./Vol.) NaCl (Tresner *et al*., 1968). Peptone-yeast extract-iron agar (ISP medium 6) and Tyrosine agar (ISP medium 7) were used to determine the melanoid pigment production (Shirling and Gottlieb, 1966). The sensitivity of the organism to various antibiotics was studied by disc diffusion technique (Schaal *et al*., 1986). Ability of the strain for selected enzyme production is also carried out (Holding and Collee, 1970).

Growth Pattern and Antibiotic Production

Growth pattern and antibiotic production of the strain was studied on six types of media viz., YMD broth (ISP-2), glycerol-asparagine broth (ISP-5), Czapek-Dox broth, inorganic salts-starch broth (ISP-4), mineral salt medium amended with corn meal and mineral salt medium containing rice bran (Suetscena and Osajima, 1990). Impact of various carbon and nitrogen sources on biomass and bioactive compound production was also investigated (Battacharyya *et al*., 1998).

The fermentation was carried out at 30°C for 7 days. The growth was measured in terms of dry weight of biomass (mg/100 mL). The antibiotic yield was determined in terms of diameter (mm) of inhibition zone. The secondary metabolites present in the culture broth were extracted with dichloromethane (Augustine *et al*., 2005). The solvent extract was tested for its antimicrobial activity by using the disc diffusion method (Alexander and Strete, 2001). Concentrated dichloromethane extracts (50 ppm) were placed on 5 mm sterilized filter paper discs and assayed against bacteria like *Bacillus cereus* (MTCC 430), *Escherichia coli* (MTCC 40), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 96) and yeast such as *Candida albicans* (MTCC 183).

RESULTS AND DISCUSSION

Colony Morphology

The strain grew well on ISP medium 2 and 3. On these media, the vegetative mycelium was brownish violet to brownish orange and the aerial mycelium was white. Light brown diffusible pigment was produced. Well developed vegetative mycelium with irregular branches penetrating the agar was found. Hyphae were long, moderately branched and fragmented into spores of various lengths.

Physiological Characteristics

The physiological characteristics of *Lechevalieria flava* are presented in Table 1. The strain tolerated NaCl concentration upto 4%. Melanin pigments were not produced by the strain on either peptone-yeast extract-iron agar (ISP medium 6) or tyrosine agar (ISP medium 7). The isolate had the ability to produce enzymes like amylase, chitinase, protease and urease. The strain exhibited sensitivity to many of the antibiotics like ampicillin, ciprofloaxacin, erythromycin, gentamicin, penicillin, rifampicin and tetracycline, but it was resistant to streptomycin.

Growth Pattern and Production of Bioactive Metabolites

The growth pattern of *L. flava* was studied in different media (Fig. 1). Among the various media tested, ISP medium 2, 5 and Czapek-Dox broth supported the good growth of the organism. The culture entered the stationary phase after 72 h of incubation which was extended upto 120 h. There was a gradual decline in biomass after 144 h of incubation. To determine suitable media for bioactive compound production, fermentation of the strain was carried out in six media for 7 days. For every

Table 1: Physiological characteristics of *Lechevalieria flava* MTCC 6470

Characteristics	Results
NaCl tolerance	
1-4% (w/v) NaCl	+*
7-13% (w/v) NaCl	-
Melanoid pigment production	
Tyrosine agar	-
Peptone-Iron agar	-
Enzyme production	
Amylase	+
Catalase	-
Cellulase	-
Chitinase	+
Protease	+
Nitrate reductase	-
Urease	+
Sensitivity to Antibiotics (µg/disc)**	
Ampicillin (50)	S***
Ciproflaxacin (50)	S
Erythromycin (100)	S
Penicillin-G (10 i.u)	S
Rifampicin (50)	S
Streptomycin (100)	R
Tetracycline (100)	S

*+: Positive reaction; - : Negative reaction, **: Concentration of antibiotics is in µg/disc, ***S: Sensitive, R: Resistant

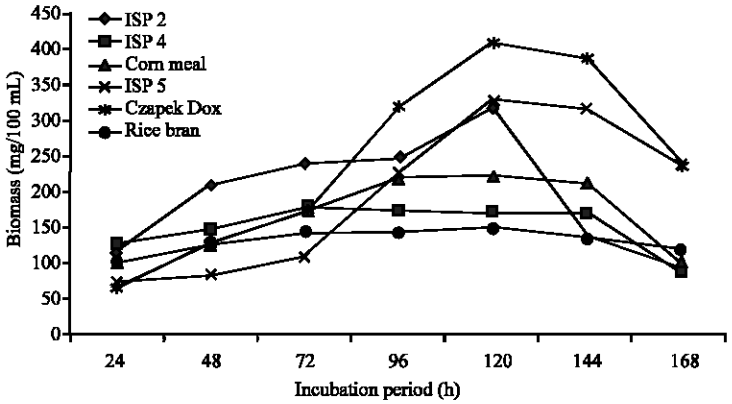

Fig. 1: Growth pattern of *Lechevalieria flava* in different media

24 h intervals, antimicrobial activity of solvent extract was examined against different microorganisms like *B. cereus*, *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans*. ISP medium 5, 2 and Czapek-Dox broth were found to be good for both biomass and antibiotic production. ISP medium 4 supported the production of antimicrobial metabolite. In the case of minimal salt media incorporated with rice bran was found to be good for antibiotic production when compared to the medium with corn meal (Table 2).

Data on the impact of various carbon and nitrogen sources on biomass and antibiotic production is presented in Table 3 and 4. Among carbon sources tested, the strain utilized sucrose as good carbon source followed by arabinose and mannose, but maltose and glycerol were found to be better for antibiotic production. For biomass production, sodium nitrate served as good nitrogen source while asparagine supported the production of bioactive metabolites. Biomass as well as antibiotic production were high in medium with ammonium sulphate as nitrogen source. The strain *L. flava* exhibited good antimicrobial activity against bacteria like *B. cereus*, *P. aeruginosa*, *S. aureus* and fungi such as *C. albicans*, but *E. coli* was found to be less sensitive.

Table 2: Antimicrobial spectrum of *Lechevalieria flava* in different media

Type of medium	*Candida albicans*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
ISP 2	5	10	4	9	6
ISP4	5	10	4	7	5
Corn meal broth	4	6	-	3	3
Rice bran broth	6	7	-	3	3
Czapek-Dox	3	8	3	4	4
ISP 5	6	12	5	10	8

- : Absence of inhibition zone

Table 3: Impact of carbon sources on growth and antimicrobial spectrum of *Lechevalieria flava*

		Diameter of inhibition zone (mm)				
Carbon source	Maximum biomass (mg/100 mL)	*Candida albicans*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Arabinose	580	-	9	-	6	4
Dextrose	315	4	10	3	8	6
Glycerol	239	5	12	4	10	8
Lactose	142	-	6	-	4	3
Maltose	485	5	13	5	11	10
Mannitol	336	3	10	-	5	4
Mannose	510	-	6	-	3	3
Sucrose	1285	3	8	-	6	5
Control	90	-	-	-	-	-

- : Absence of inhibition zone, Control: Without carbon source

Table 4: Impact of nitrogen sources on growth and antimicrobial spectrum of *Lechevalieria flava*

		Diameter of inhibition zone (mm)				
Nitrogen source	Maximum biomass (mg/100 mL)	*Candida albicans*	*Bacillus cereus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Ammonium oxalate	306	4	7	-	5	-
Ammonium sulphate	508	5	11	3	7	5
Ammonium nitrate	433	4	6	-	4	-
L-Asparagine	183	6	12	3	8	6
L-Glutamine	425	3	4	-	3	-
Sodium nitrate	885	5	8	-	4	5
Tyrosine	104	-	5	-	-	-
Urea	69	-	-	-	-	-
Control	86	-	-	-	-	-

- : Absence of inhibition zone, Control: Without nitrogen source

The genus *Nocardiopsis* was described by Meyer (1976) for the species of *Actinomadura dassonvillei* on the basis of morphological characteristics and cell wall type of this organism. Only one antibiotic Madumycin was reported from *Actinomadura flava* strain INA 2171 (Gauze *et al*., 1974). Later, *A. flava* is renamed as *Nocardiopsis flava* (Gauze and Sveshnikova, 1985). *N. flava* has been reported to be transferred to the genus *Saccharothrix* on the basis of their chemotaxonomic characteristics (Grund and Kroppenstedt, 1989; Labeda and Lechevalier, 1989). Labeda *et al*. (2001) reported that the species, *Saccharothrix flava* was changed to new genus *Lechevalieria* based on polyphasic taxonomy. The strain *A. flava* INA 2171 produced maximum amounts of madumycin I and II by the 2nd, 3rd and 5th days of cultivation respectively (Kochetkova *et al*., 1976). The present study also revealed that maximum antibiotic production from *L. flava* was found after 5th day of culture fermentation. *Nocardiopsis* and *Saccharothrix* species were reported to be active against gram positive bacteria and yeast (Zitouni *et al*., 2005). *Lechevalieria flava* MTCC 6470 is a new Indian isolate and *L. flava* has not been previously reported from India. The strain not only exhibited good antimicrobial activity against gram positive bacteria such as *B. cereus*, *S. aureus* and gram negative bacteria like *P. aeruginosa* but also active against fungi such as *C. albicans*. Attempts are in progress to analyze the bioactive compounds produced by the strain.

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