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Research Article

A NEW VARIANT OF *STREPTOMYCES* SPECIES- *STREPTOMYCES AZUREUSVARGOSSYPII* FROM SOILS OF ANDHRA PRADESH

P. Ellaiah and VS. Venkateswara Rao*

Pharmaceutical Biotechnology Division, Department of Pharmaceutical Sciences
Andhra University, Visakhapatnam- 530003, Andhra Pradesh, India.

ABSTRACT

During our continuous search for antibiotic producing actinomycetes, a variant of Streptomyces species was isolated from soils of Andhra Pradesh in India. The morphological, cultural, physiological and biochemical characters were studied, compared to known species and identified as a new variant of *Streptomyces azureus* and designated as *streptomyces azureusvargossypii. T*he antibiotic activity of the strain was tested against both Gram-positive and Gram-negative bacteria as well as fungi and yeasts.

Keywords: Species**,** Streptomycete, *Streptomyces azureusvargossypii,* Gram- positive, Gram- negative.

INTRODUCTION

Since the isolation of actinomycin in 1940 and streptomycin in 1944 by Waksman, the actinomycetes have received tremendous attention of the scientists. Soils, composts and fodders are common sources of actinomycetes. Waksman[1] recognized a few natural substrates as ideal sources for the isolation of actinomycetes and other streptomycetes. The nature of a *Streptomyces* colony is an important property in characterizing a culture. Krainsky[2] used the structure, size, shape and texture of the colony as one of the major diagnostic criteria. According to Pridham and Lyons[3] and International Subcommittee[4], the best way to handle streptomycete classification nomenclature and identification is through application of the genus-species-subspecies concept.

The majority of antibiotic producing actinomycetes found in these species led to growing economic importance of these organisms which resulted in the isolation and description of numerous new species. It is reported that the only genus *Streptomyces*, the member of Actinomycetales accounts for approximately 93% producing secondary metabolites[5].

The present communication deals with the isolation and characterization of an antibiotic producer from soils of Andhra Pradesh.

MATERIALS AND METHODS

i) Isolation

Soil samples were collected from different locations of Andhra Pradesh, India. Actinomycetes were isolated by plating on Half-strength nutrient agar medium ,Starch – Casein agar medium[6] and AV agar medium[7] and incubating at 28^0 C for 14 days. The media were supplemented with Benzyl penicillin (0.8mg/l), Nystatin (50mg/l) to minimize the bacterial and fungal contamination. A total of 359 actinomycetes were isolated from 8 samples. Among 359 actinomycetes, isolate F_{80} with moderate to good antimicrobial activity against Gram-positive and Gram-negative bacteria and sporophores occurred as short, closed spirals was found to be interesting and it was selected for detailed taxonomic study.

ii) Antimicrobial Activity

The isolate F_{80} was inoculated into a production medium[8] with p^H 7.2 and incubated at 28^0C for 6 days on a rotary shaker. The antimicrobial activity was determined by

standard cup-plate method[9]. The potency of the isolate was measured by the degree of inhibition zone (Table.1). All the test organisms employed in the present studies were supplied by the National Chemical Laboratory, Pune.

iii) Characterization

Characterization of the isolate $F_{80}$was done according to ISP procedures[10]. The studies include morphological, cultural, physiological tests and carbon source utilization pattern. The data of cultural characteristics, physiological &biochemical properties, carbon source utilization pattern, growth in the presence of various nitrogen sources and resistance to various antibiotics, growth in the presence of various inhibitory compounds and tolerance to sodium chloride of isolate F_{80} are presented in Tables 2 to7.

Characterization of the selected isolate has been made by following the standard procedure[10] For identification, the International Streptomyces Project (ISP) reports[11-13]. Bergey's Manual of Determinative Bacteriology[14] and Bergey's Manual of Systematic Bacteriology[15] have been followed.

iv) Fermentation and Extraction of Active principles from Isolate F_{80}

The isolate F_{80} was grown on Starch-casein agar medium. After preliminary investigation, the seed culture was prepared in a medium composed of soybean meal 10g, cornsteep solids 10g, glucose 5g and calcium carbonate 5g and distilled water 1litre, adjusted to p^H 7.2 by the addition of 0.1 N NaOH and distributed into 250ml Erlenmeyer flasks and incubated for 72 hours at 28^0 C on a rotary shaker at 220 rpm of speed and allowed to develop the seed. A 10% level of seed was transferred tothe selected production medium containing soybean meal 10g, corn steep solids 5g, soluble starch 10g, dextrose 5g, calcium carbonate 7g and distilled water 1 litre with p^H 7.2. The fermentation was run at 28^0 C for 6 days on a rotary shaker at 220 rpm and antimicrobial spectrum was studied with clear centrifuged broth samples at the end of fermentation by cup-plate method[9] and the productivity of the strain was confirmed.

The fermentation broth of the isolate F_{80} was centrifuged at 4000 rpm for 15 minutes. The clear filtrates were divided into 3 portions and each portion was adjusted to p^H 6.0,7.0 and 8.0. Each portion was extracted thrice (3x5ml) with the following solvents: chloroform, ethyl acetate and n-butanol. All extracts were concentrated to a low volume (5ml) under vacuum at 45^0 C on a rota-vapour apparatus and tested for their activity against the selected test organism (*Pseudomonas aeruginosa*). The results are given in Table.8

The mycelial cake portion of the strain was collected separately and washed 4 times with sterile distilled water and extracted with methanol (10 ml) for 1 hour. The methanolic extract was tested forits antimicrobial activity. The results are presented in Table.8.

RESULTS AND DISCUSSION

As shown in Table.1, the isolate showed moderate to good antibacterial activity against Gram-positive & Gram-negative bacteria and no or negligible activity was observed against fungi and yeasts. Therefore the isolate F_{80} was selected for further study.

The most significant characteristics of the strain $F_{80}$are summarized as follows

The strain grew well on most of the media. The micro-morphological studies revealed that the strain F_{80}has shown sporophores which occurred as short closed spirals with 2 to 4 turns, arranged in groups and formed spiral spore chains. Hence, it belongs to section 'Spira (S)'. The aerial mycelium developed well on most of the media and it was pale blue in colour. The vegetative mycelium was light brown to gray on most of the media. The strain was chromogenic with brown to blackdiffusible pigment and it produced brown soluble pigment on some media.

The strain F_{80} was H_2S and tyrosinase positive with moderate diastatic, proteolyticactivity. It could coagulate and peptonise milk. It did not exhibit nitrate reduction. It showed good growth well at 28^0 C, poor growth at 37^0 C and no growth below 20^0 C (Table.2&3).

It exhibited good growth on glucose, sucrose, mannitol, rhamnose and raffinose;moderate growth on arabinose &inositol and no growth on xylose, fructose and cellulose.(Table.4).

The strain F_{80} was failed to grow on any nitrogen source employed (Table.5). It showed resistance to penicillin G and cephalexin and sensitivity to streptomycin tetracycline, gentamicin and rifampicin (Table.6).

The cell wall composition showed the presence of *LL*—DAP (Diaminopimelic acid) & glycineand arabinose &galactosesugars. The above data suggested that the strain F_{80} belongs to cell wall type I and type A sugar pattern. It could tolerate upto10% NaCl but failed to grow at 13%.NaCl. It did not grow in the presence of crystal violet, phenol and potassium tellurite(Table.7).

A detailed survey of the literature indicates that our strain $F_{80}$is related to *Streptomyces glaucescens*[11,14,16] and *Streptomyces*

azureus[14,17] in respect of sporophore morphology, chromogenicity, production of antibioticand some biochemical reactions.However a detailed scrutiny indicates that the strain F_{80} is more closer to *S.azureus.*

However, some qualitative and quantitative differences could be noticed, the strain F_{80}differed from the reference culture in the following respects: our strain F_{80} did not grow on xylose and fructose while the reference culture could grow on both. Our strain F_{80} could grow upto 10% NaClwhere as the reference culture could tolerate upto 7% NaCl.

As shown in Table.8, the active principles from the culture filtrate of the isolate F_{80} were almost extracted with ethyl acetate at p^H 8.0 where as the other solvent extracts gave negligible activities. The methanolic extract of mycelium has also shown good antimicrobial activity (Table.8).

CONCLUSIONS

In view of large number of similarities and a few differences, it is felt that the strain F_{80}can be considered as a new variant of *Streptomyces azureus.* Hence it is designated as *Streptomycesazureusvargossypii.*Gossypiiis referred to the cottony appearance of spore mass.

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Table 1: Antimicrobial spectrum of F_{80} culture filtrate

Test organism	Inhibition zone diameter (mm)
*Bacillus pumilus*NCIM 2327	12
*Bacillus subtilis*NCIM 2063	14
*Staphylococcus aureus*NCIM 2492	13
*Sarcinalutea*NCIM 2103	16
*Escherichia coli*NCIM 2563	14
*Pseudomonas aeruginosa*NCIM 2863	19

Table 2: Cultural characteristics of F_{80}

Medium	Cultural characteristics
Yeast extract-malt extract agar	G : good, cottony, raised AM : pale blue R : light brown SP : pale brown
Oat meal agar	G : good, cottony, raised AM : pale blue R : light brown SP : none
Inorganic salts-starch agar	G : good, cottony, raised AM : pale blue R : light brown SP : none
Glycerol-asparagine agar	G : good, cottony, raised AM : pale blue R : gray SP : none
ATCC-172 agar	G : good , cottony, raised AM : pale blue R : gray SP : brown
Starch-casein agar	G : moderate, cottony,raised AM : pale blue R : gray SP : light brown

G: Growth, AM: Aerial mycelium,
R: Reverse colour, SP: Soluble pigment

Table 3: Physiological and biochemical properties of the isolate F_{80}

S.No	Reaction	Response	Result
1	Melanin reaction on ISP-1 ISP-6 ISP-7	 Browning of the medium Browning of the medium Blackening of the medium	 Positive Positive Positive
2	H_2S production (ISP-6)	Browning of the medium	Positive
3	Tyrosine reaction(ISP-7)	Blackening of the medium	Positive
4	Starch hydrolysis	Growth zone : 11 mm Hydrolyzed zone: 27 mm	Positive
5	Casein hydrolysis	Growth zone : 13mm Hydrolyzed zone: 21mm	Positive
6	Gelatin hydrolysis	Growth zone : 11mm Hydrolyzed zone : 18mm	Positive
7	Milk coagulation and peptonisation	Coagulation followed by peptonisation	Positive
8	Nitrate reduction	No colour	Negative
9	Growth temperature range a) 10^0 C b)20^0 C c)28^0 C d) 37^0 C	 - - +++ +	Growth between 28^0 C ~37^0 C

Table 4: Carbon source utilization pattern of F_{80}

Utilization	Carbon source
Positive	D- glucose(+++), L(+) arabinose(++), sucrose(+++), meso-inositol(++),D-mannitol(+++), L(+) rhamnose(+++)&raffinose (+++)
Doubtful	Nil
Negative	D-xylose, D-fructose & cellulose

Table 5: Growth of F_{80} in the presence of various nitrogen sources

Nitrogen source (0.1%w/v)	Growth response
L-arginine	-
L-cysteine HCl	-
L-histidine	-
Potassium nitrate	-
L-valine	-
L-asparagine (positive control)	-

+: Growth, -: No growth

Table 6: Resistance to various antibiotics

Antibiotic(µg/ml)	Growth response	Result (F_{80})
Penicillin G(10 IU/ml)	+	**R**
Streptomycin (100)	-	**S**
Tetracycline (50)	-	**S**
Cephalexin(100)	+	**R**
Gentamicin(100)	-	**S**
Rifampicin (50)	-	**S**

R: Resistant, S: Sensitive

Table 7: Effect of inhibitory chemical compounds on F_{80}

Name of the compound(%w/v)	F_{80}
Crystal violet (0.00001)	-
Phenol (0.1)	-
Potassium tellurite	
(0.001)	-
(0.01)	-
Sodium chloride	
(4)	+
(7)	+
(10)	+
(13)	-

+: Growth, -: No growth

Table 8: Selection of solvent for the extraction of antibiotic principles (Intracellular& Extracellular) from F_{80}

Isolate No	Solvent for broth extract	Test organism	Broth Extract	Mycelial extract
			Inhibition zone diameter(mm)	
F_{80}	Ethyl acetate	*Pseudomonas aeruginosa*	30	22

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