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Streptomyces rufus sp.nov., from Soils of Andhra Pradesh.

P Ellaiah* and VS Venkateswara Rao.

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

ABSTRACT

A new *Streptomycete* 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 species of *Streptomyces rufus*. 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 rufus,* Gram- positive, Gram- negative, Sporophores, Rectus flexibilis Aerial mycelium, Vegetative mycelium, Diaminopimelic acid.

Corresponding author

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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.

The present communication deals with the isolation and characterization of bioactive actinomycete from soils of Andhra Pradesh.

MATERIALS AND METHODS

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[5]and AV agar medium[6] and incubating at 28^0 C for 14 days. The media were supplemented with Benzyl penicillin (0.8mg), Nystatin(50µg/ml) to minimize the bacterial and fungal contamination. A total of 359 actinomycetes were isolated from 8 samples. Among 359 actinomycetes, isolate F_{20} with good antimicrobial activity and sporophores were straight with globose bodies were found to be interesting and it was selected for detailed taxonomic study.

Antimicrobial Activity

The isolate F_{20} was inoculated into a production medium [7] and incubated at 28^0C for 6 days on a rotary shaker. The antimicrobial activity was determined by standard cup-plate method [8]. 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.

Characterization

Characterization of isolate F_{20} was done according to ISP procedures [9]. 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_{20} are presented in Tables 2 to7.

Characterization of the selected isolate has been made by following the standard procedure [9]. For identification, the International Streptomyces Project (ISP) reports [10-12]. Bergey's Manual of Determinative Bacteriology [13] and Bergey's Manual of Systematic Bacteriology [14] have been followed.

RESULTS AND DISCUSSION

Screening of 8 different natural substrates resulted in the isolation of 359 actinomycetes. The isolate F_{20} has shown good antimicrobial activity against Gram-positive and Gram-negative bacteria and moderate activity against fungi and yeasts (Table.1). Therefore the isolate F_{20} was selected for further study.

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Table.1: Antimicrobial spectrum of F_{20} culture filtrate.

Test organism	Inhibition zone diameter (mm)
Bacillus pumilus NCIM 2327	18
Bacillus subtilis NCIM 2063	21
Staphylococcus aureus NCIM 2492	11
Sarcina lutea NCIM 2103	19
Escherichia coli NCIM 2563	slight
Pseudomonas aeruginosa NCIM 2863	13
Aspergillus niger NCIM 1222	15
Trichoderma viride NCIM 1051	slight
Candida utilis NCIM 3055	12
Candida albicans NCIM 3471	12

Table.2: Cultural characteristics of F_{20}.

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

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

The most significant characteristics of the strain F_{20} are summarized as follows:

The strain grew well on most of the media. Micro-morphological studies revealed that the strain F_{20} has shown straight sporophores and globose bodies were observed throughout the aerial mycelium. Hence it belongs to section Rectus flexibilis (RF).As shown in Table.2, the aerial mycelium developed moderately to good on most of the media at it was light brown to gray. The vegetative mycelium was brown to pale gray to gray on most of the media. The strain was chromogenic with brown to black diffusible pigment on organic media and it produced brown soluble pigment on ATCC-172 Medium.

The strain was H_2S and tyrosinase positive. It exhibited good diastatic activity. It hydrolyzed casein and gelatine. It did not coagulate and peptonise milk. It showed strong nitrate reduction. It grew well at 28^0C and it did not grow at 10^0C, 20^0C and37^0C. (Table.3). It exhibited good growth on meso-inositol, mannitol &rhamnose and poor to moderate growth on xylose, glucose, arabinose sucrose and raffinose. It did not utilize fructose and cellulose.(Table.4).

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Table.3:Physiological and biochemical properties of F_{20}.

S.no	Reaction	Response	Result
1	Melanin reaction		
	ISP-1	Browning of the medium	Positive
	ISP-6	Blackening of the medium	Positive
	ISP-7	Blackening of the medium	Positive
2	H_2S production (ISP-6)	Blackening of the medium	Positive
3	Tyrosine reaction(ISP-7)	Blackening of the medium	Positive
4	Starch hydrolysis	Growth zone : 13 mm Hydrolyzed zone : 35 mm	Positive
5	Casein hydrolysis	Growth zone : 14mm Hydrolyzed zone : 18mm	Positive
6	Gelatin hydrolysis	Growth zone : 10mm Hydrolyzed zone : 27mm	Positive
7	Milk coagulation and Peptonisation	No coagulation and no peptonization	Negative
8	Nitrate reduction	Deep pink	Strongly
9	Growth temperature range		positive
	a) 10^0 C	-	
	b) 20^0 C	-	Growth at
	c) 28^0 C	+++	28^0 C
	d) 37^0 C	-	

Table.4: Carbon source utilization pattern of F_{20}

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

Table.5: Growth of F_{20} 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)	+++

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Table.6: Resistance to antibiotics.

Antibiotic(µg/ml)	Growth response (F_{20})	Result
PenicillinG(10U/ml)	+	R
Streptomycin (100)	-	S
Tetracycline (50)	-	S
Cephalexin(100)	+	R
Gentamicin(100)	-	S
Rifampicin (50)	-	S

R: Resistance, S: Sensitive

Table.7: Effect of inhibitory chemical compounds on F_{20}:

Name of the compound(%w/v)	F_{20}
Crystal violet(0.00001)	+
Phenol (0.1)	-
Potassium tellurite	
(0.001)	-
(0.01)	-
Sodium chloride	
(4)	-
(7)	-
(10)	-
(13)	-

+: Growth, -: No growth

The Table.5 indicates that the strain F_{20} exhibited good growth on L-asparagine. It showed poor to .moderate growth on L-valine, L-histidine, potassium nitrate and L-arginine.It did not grow on L-cysteine HCl. It exhibited resistance against penicillin G & cephalexin, sensitivity to streptomycin, tetracycline, gentamicin and rifampicin(Table.6).

The analysis of cell wall hydrolyzates showed the presence of *LL*–DAP (diaminopimelic acid) and glycine without any characteristic sugars. Hence, our strain F_{20} belongs to cell wall type I and type C sugar pattern. It could grow in the presence of crystal violet and inhibited by phenol and potassium tellurite. It did not grow at 4%, 7%,10% and 13%NaCl.(Table.7).

A detailed survey of the literature indicates that our strain F_{20} is some what related to *Streptomyces globosus*[13-15] and *Streptomyces tanashiensis*[13-14,16-17] in respect of RF sporophore morphology, production of antibiotic and sensitivity to streptomycin and some biochemical reactions.

The morphological, cultural, biochemical studies advocate that the F_{20} is closer to *S. tanashiensis*.But it is differed from the reference culture in the following respects: the strain F_{20} exhibited light brown to gray aerial mycelium and the vegetative mycelium was brown to pale gray to gray(reverse colour).It showed good growth on meso-inositol, D-mannitol &rhamnose and moderate growth on sucrose and raffinose while the reference culture showed gray colour aerial mycelium and pale yellow to light brown olive vegetative mycelium. It did not grow on sucrose, inositol, mannitol, rhamnose, and raffinose. Our strain F_{20} did not grow

at 4% NaCl and exhibited antibacterial, antifungal and anti-yeast activities while the reference culture showed growth at 4% NaCl and exhibited antifungal activity.

CONCLUSIONS

In view of a number of significant differences, it led us to consider that the strain F_{20} is a new species. Hence, it is designated as *Streptomyces rufus* sp.nov. Rufus is referred to red colour of the soil from which the organism was isolated.

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