ISSN 2278-7763

Streptoverticillium vepadensis sp.nov., from Soils of Andhra Pradesh

P.Ellaiah and V.S.Venkateswara Rao*
Pharmaceutical Biotechnology Division, Department of Pharmaceutical Sciences
Andhra University, Visakhapatnam-530003, Andhra Pradesh.

ABSTRACT

A new species of *Streptoverticillium* 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 *Streptoverticillium -Streptoverticillium vepadensis*. Antibiotic activity of the strain was tested against both Gram-positive and Gram-negative bacteria as well as fungi and yeasts.

Keywords: Species**,** *Streptoverticillium, Streptoverticillium vepadensis,* Gram- positive, Gram-negative, Biverticillate, Sporophores, Diaminopimelic acid

1.INTRODUCTION

Actinomycetes bearing whorls and umbels of short spore chains at regular intervals on their long aerial hyphae and originally included in the genus Streptomyces were later re-classified as a separate genus Streptoverticillium[1,2] . However, these two genera share a number of common morphological, developmental and physiological characteristics which have prevented several workers from accepting their separate generic status [3]. The similarities have also appeared to dis- courage the further study of streptoverticillia and it has been assumed that other properties are shared[4].

In the present studies, the cultural, physiological &biochemical and taxonomic characteristics of the soil actinomycete which was isolated from soils of Andhra Pradesh in India were investigated.

Corresponding author e-mail**:vaddiparthy@yahoo.com**

ISSN 2278-7763

2.MATERIALS AND METHODS

2.1 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 D_{85} with good antimicrobial activity and occurred as biverticillate sporophores was found to be interesting and it was selected for detailed taxonomic study.

2.2 Antimicrobial Activity

The isolate D_{85} 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.

2.3 Characterization

Characterization of isolate D_{85} 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 D_{85} are presented in Tables 2 to7.

ISSN 2278-7763

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, 1974[13] and Bergey's Manual of Systematic Bacteriology,1992[14] have been followed.

3. RESULTS AND DISCUSSION

Screening of 8 different natural substrates resulted in the isolation of 359 actinomycetes. The isolate D_{85} has shown good antimicrobial activity against Gram-positive and Gram-negative test organisms. No or negligible activity was observed against fungi and yeasts (Table.1). Therefore isolate D_{85} was selected for further study.

The most significant characteristics of our strain D_{85} are summarized as follows:

The strain D_{85} grew well on most of the media. Microscopic studies revealed that the isolate D_{85} occurred as biverticillate sporophores ,elements of secondary verticils were open and short spirals which belong to section 'Biverticillus-spira (BIV-S)'. The aerial mycelium developed moderately to good on most of the media and it was pale pink to pink. The vegetative mycelium was pale pink to deep pink on most of the media. The strain was chromogenic with brown diffusible pigment. It produced light brown, reddish pink soluble pigments on YEME (yeast extract- malt extract agar medium) and glycerol-asparagine agar respectively.

The strain H_2S and tyrosinase positive. It exhibited good diastatic activity. It could hydrolyze casein and gelatine. It did not coagulate and peptonise milk. It showed strong nitrate reduction. It exhibited good growth at 28^0 C, no growth at 10^0 C &20^0 C and showed poor growth at 37^0 C (Tables.2 &3).

The cell wall composition was found to contain *LL*- diaminopimelic acid (DAP), glycine, xylose and arabinose as diagnostic components. The above data suggested that the isolate D_{85} belongs to cell wall Type I and Type D sugar pattern.

ISSN 2278-7763

It exhibited good growth on meso-inositol &D-mannitol and moderate growth on glucose,L(+) arabinose, sucrose, D-xylose, D-fructose, L(+) rhamnose and raffinose(Table.4).

As indicated inTable.5, the strain D_{85} , showed good growth on L-arginine, L-cysteine HCl, L-histidine& potassium nitrate and moderate growth on L-valine & L-asparagine.

The strain D_{85} exhibited resistance against penicillin and cephalexin . It showed sensitivity to streptomycin, tetracycline, gentamicin and rifampicin(Table.6).

As shown in Table.7 the strain D_{85} exhibited growth in the presence of crystal violet and potassium tellurite but it did not grow in the presence of phenol. The strain D_{85} could grow upto 7% but failed to grow at 10% and 13% NaCl.

A detailed perusal of the literature indicates that our strain D_{85} is related to *Streptoverticillium roseoverticillatum*[13-14,10,15]. and *Streptoverticillium roseoverticillatum* sub sp *albosporum*[13-14,16] in respect of verticillate sporophore, chromogenicity and some cultural characteristics and biochemical reactions .

However, some significant qualitative and quantitative differences could be noticed. Our strain D_{85} developed pink aerial mycelium and deep pink vegetative mycelium. It strongly utilized mannitol and moderately arabinose, sucrose, xylose, fructose, rhamnose and raffinose while the reference culture showed pinkish white aerial mycelium and colourless, reddish and orange yellow to brick red vegetative mycelium, it could not utilize arabinose, sucrose, xylose and mannitol. Our strain D_{85} exhibited poor growth on cellulose and produced an antibacterial antibiotic whereas reference culture did not grow on cellulose and produced streptorubin antibiotic with cytostatic activity.

ISSN 2278-7763

CONCLUSIONS

In view of a large number of differences and a few similarities, our strain D_{85} can be considered as a new species. Hence it is designated as *Streptoverticillium vepadensis* sp.nov. Vepadensis is referred to the place from which the soil sample was collected.

ACKNOWLEDGEMENT

We thank the University Grants Commission, New Delhi for providing the Financial Assistance.

REFERENCES

[1] Baldacci, E. Development in the classification of Actinomycetes. Giornale di Microbiologia . 1958; 6: 10-27

[2]Baldacci, E.,Farina, G. & Locci, R. Emmendation of the genus Streptoverticilliunz Baldacci (1958) and revision of some species. Giornale di Microbiologia. 1966; 14:153-171.

[3] Krasilnikov, N. A. The Actinomycetes (higher forms). Moscow: Nauka. Loccr, R., Baldaccj, E. & Petrolini Baldan, B. (1969).The genus Streptoverticilliun?, A taxonomic study. Giornale di Microbiologia. 1970; 17:1-60.

[4] Cross,T., Attwell, R.W & Locci,R . Fine Structure of the Spore Sheath in Streptoverticillium Species . Journal of General Microbiology. 1973;75:421-424.

[5] Williams, S.T and Cross, T, Actinomycetes , In "Methods in Microbiology", vol.4,ed.C.Booth,London,Academic press.1971.

[6] Nonomura, H. & Y.Ohara . Distribution of actinomycetes in soil. VI. A culture method effective for both preferential isolation of *Microbispora* and *Streptosporangium* in soil (Part I) .*J. Ferment.Technol.* 1969; 47: 463-469.

[7] Ellaiah,P, Rao,V.S.V, Rao,B.V.L.N, Srinivasulu,B and Ghani,T.A . A new streptomycete producing diphenyl sulfone antibiotic- *S.sulfonensis* .Hind.Antibiot. Bull. 1998;40:31-37.

[8] Grove,D.C and Randall,W.A, "Assay methods of Antibiotics-A Laboratory Manual" , (Medical Encyclopedia, NY).1955.

[9] Shirling,E.B & D. Gottlieb, D. Methods for characterization of Streptomyces species. *Int J Syst Bacteriol* .1966; 16:313-340.

ISSN 2278-7763

[10] Shirling, E.B & Gottlieb,D . Cooperative description of type cultures of Streptomyces II.Species descriptions from first study. *Int J Syst Bacteriol.* 1968*;* 18:69-189.

[11] Shirling, E.B & Gottlieb,D . Cooperative description of type cultures of Streptomyces IV.Species descriptions from the second, third and fourth studies. *Int J Syst Bacteriol.* 1969; 19:391-512.

[12] Shirling, E.B & Gottlieb,D . Cooperative description of type cultures of Streptomyces V.Additional descriptions . *Int J Syst Bacteriol.*1972*;* 22: 265-394.

[13] Buchanan, R. E. & Gibbons, N. E., eds. Bergey's Manual of Determinative Bacteriology. 8th ed. Williams & Wilkins Co., Baltimore.1974.

[14] Williams,S.T, Sharp,M.E and Holt, J.G . " Bergey's Manual of Systematic Bacteriology", Vol.4, The Williams and Wilkins Co., Tokyo. 1992-93.

[15] Shinobu, R. \"Three new species of Streptomyces forming whirls.\" Mem. Osaka Univ. Lib. Arts Educ.1956; 5B:84-93.

[16] Locci ,R , Baldacci ,E & Petrolini Baldan, B. The genus *streptoverticillium*. A taxonomic study. G*iornale di* M*icrobiologia*, 1969; 17: 1-60.

Table.1

ANTIMICROBIAL SPECTRUM OF D_{85} CULTURE FILTRATE.

Test organism	Inhibition zone diameter (mm)
Bacillus pumilus NCIM 2327	18
Bacillus subtilis NCIM 2063	19
Staphylococcus aureus NCIM 2492	17
Sarcina lutea NCIM 2103	31
Escherichia coli NCIM 2563	15
Pseudomonas aeruginosa NCIM 2863	18

ISSN 2278-7763

Table.2

CULTURAL CHARACTERISTICS OF D_{85}.

Medium	Cultural characteristics
Yeast extract-malt extract agar	G : good, wrinkled, raised AM : pale pink R : pale pink to pink with brown tinge SP : light brown
Oat meal agar	G : good, wrinkled, raised AM : pink R : pale pink SP : none
Inorganic salts-starch agar	G : moderate, wrinkled AM : pink R : pale pink SP : none
Glycerol-asparagine agar	G : good, wrinkled AM : pink R : deep pink SP : reddish pink
ATCC-172 agar	G : good, wrinkled, spreading AM : pale pink R : pink SP : none
Starch-casein agar	G : moderate to good, wrinkled, spreading AM : pink R : pale pink to pink SP : none

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

ISSN 2278-7763

Table.3

PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF D_{85}.

S.no	Reaction	Response	Result
1	Melanin reaction		
	ISP-1	Browning of the medium	Positive
	ISP-6	Browning of the medium	Positive
	ISP-7	Browning of the medium	Positive
2	H_2S production (ISP-6)	Browning of the medium	Positive
3	Tyrosine reaction(ISP-7)	Browning of the medium	Positive
4	Starch hydrolysis	Growth zone :15mm Hydrolyzed zone :32mm	Positive
5	Casein hydrolysis	Growth zone :16mm Hydrolyzed zone: 25mm	Positive
6	Gelatin hydrolysis	Growth zone :11mm Hydrolyzed zone :41mm	Strongly positive
7	Milk coagulation and Peptonisation	No coagulation and No peptonization	Negative
8	Nitrate reduction	Deep red colour	Strongly positive
9	Growth temperature range		
	a) 10^0 C	-	
	b) 20^0 C	-	Growth between
	c) 28^0 C	+++	28^0 C~37^0 C
	d) 37^0 C	+	

Table.4

CARBON SOURCE UTILIZATION PATTERN OF D_{85}

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

ISSN 2278-7763

Table.5

GROWTH OF D_{85} 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)	++

Table.6

RESISTANCE TO ANTIBIOTICS.

Antibiotic(µg/ml)	Growth response (D_{85})	Result
PenicillinG(10U/ml)	+	R
Streptomycin (100)	-	S
Tetracycline (50)	-	S
Cephalexin(100)	+	R
Gentamicin(100)	-	S
Rifampicin (50)	-	S

R: Resistant, S: Sensitive

ISSN 2278-7763

Table.7

EFFECT OF INHIBITORY CHEMICAL COMPOUNDS ON D_{85}.

Name of the compound(%w/v)	***D_{85}***
Crystal violet(0.00001)	+
Phenol (0.1)	-
Potassium tellurite	
(0.001)	+
(0.01)	+
Sodium chloride	
(4)	+
(7)	+
(10)	-
(13)	-

+: Growth, -: No growth

