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Streptomyces noursei var saccharicus: An Antibiotic Producer from Soils.

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Research Article

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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 noursei* and designated as *Streptomyces noursei var saccharicus.* The antibiotic activity of the strain was tested against both Gram-positive and Gram-negative bacteria as well as fungi and yeasts.

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.

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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[6]and AV agar medium[7] 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_{50} with moderate antibacterial activity particularly against Gram-positive bacteria and sporophores are arranged in groups and formed spiral spore chains was found to be interesting and it was selected for detailed taxonomic study.

Antimicrobial Activity

The isolate D_{50} 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.

Characterization

Characterization of the isolate D_{50} 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 $_{D50}$ are presented in Tables 2to7. 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.

RESULTS AND DISCUSSION

As shown in Table.1, the isolate showed moderate antibacterial activity predominantly active against Gram-positive and no or negligible activity was observed against Gram- negative bacteria, fungi and yeasts. Therefore the isolate D_{50} was selected for further study.

The most significant characteristics of the strain D_{50} are summarized as follows

The strain grew well on most of the media. The micro-morphological studies revealed that the strain D_{50} has shown sporophores which occurred as spiral spore chains. Hence, it belongs to section 'spira (s)'. The aerial mycelium developed moderately to good on most of the media and it was brown to pale gray to gray in colour. The vegetative mycelium was pale gray to gray on most of the media. The strain was non-chromogenic without any characteristic diffusible pigment and it did not produce any other soluble pigment.

The strain D_{50} was H_2S and tyrosinase negative . It showed good diastatic activity and it could hydrolyze the casein and gelatin. It could coagulate and peptonise milk. It exhibited weak nitrate reduction. It grew well at 28^0 C. It did not grow at 10^0 C and 20^0 C and it showed poor growth at 37^0 C (Table.2&3). It showed good growth on meso- inositol. It exhibited poor to moderate growth on arabinose, sucrose, glucose, D-xylose, D-mannitol, D-fructose and L(+)-rhamnose and no growth on raffinose and cellulose. (Table 4).

The strain D_{50} exhibited good growth on L-arginine and potassium nitrate. It showed moderate growth on L-histidine and L-asparagine but it did not show any growth on L-cysteineHCl and L-valine. (Table.5). It exhibited resistance against penicillin G and cephalexin and sensitivity to streptomycin, tetracycline & gentamicin. It showed resistance to rifampicin after 7 days. (Table 6).

The analysis of cell wall hydrolyzates demonstrated the presence of *LL*—DAP (Diaminopimelic acid) & glycine. No characteristic sugars were present. The above data suggested that the strain D_{50} belongs to cell Type I and Type C sugar pattern. It could tolerate upto 7% NaCl but failed to grow at 10%

and 13% NaCl. It did not grow in the presence of phenol but it could grow in the presence of crystal violet and potassium tellurite (Table.7).

Table 1: Antimicrobial spectrum of D_{50} culture filtrate.

Test organism	Inhibition zone diameter (mm)
Bacillus pumilus NCIM 2327	12
Bacillus subtilis NCIM 2063	15
Staphylococcus aureus NCIM 2492	14
Sarcina lutea NCIM 2103	16

Table 2: Cultural characteristics of D_{50}

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

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

Table 3:Physiological and biochemical properties of D_{50}.

Sr no	Reaction	Response	Result
1	Melanin reaction		
	ISP-1	No Color	Negative
	ISP-6	No Color	Negative
	ISP-7	No Color	Negative
2	H_2S production (ISP-6)	No Color	Negative
3	Tyrosine reaction(ISP-7)	No Color	Negative
4	Starch hydrolysis	Growth zone : 18 mm	Positive
		Hydrolyzed zone : 33 mm	
5	Casein hydrolysis	Growth zone : 17 mm	Positive
		Hydrolyzed zone : 32 mm	
6	Gelatin hydrolysis	Growth zone : 12 mm	Positive
		Hydrolyzed zone : 38 mm	
7	Milk coagulation and	Coagulation followed by	Positive
	Peptonisation	peptonization	
8	Nitrate reduction	Light orange	Weakly
9	Growth temperature		Positive
	Range		
	10^0 C	-	
	20^0 C	-	
	28^0 C	+++	Growth between 280 C ~ 370 C
	37^0 C	+	

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Table 4: Carbon source utilization pattern of D_{50}

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

Table 5: Growth of D_{50} 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 various antibiotics.

Antibiotic(µg/ml)	Growth response (D_{50})	Result
PenicillinG(10 IU/ml)	+	R
Streptomycin (100)	-	S
Tetracycline (50)	-	S
Cephalexin(100)	+	R
Gentamicin(100)	-	S
Rifampicin (50)	- (3 rd day)	S
	+ (7th day)	R

R: Resistant, S: Sensitive

Table 7: Effect of inhibitory chemical compounds on D_{50}:

Name of the compound(%w/v)	D_{50}
Crystal violet(0.00001)	+
Phenol (0.1)	-
Potassium tellurite	
(0.001)	+
(0.01)	+
Sodium chloride	
(4)	+
(7)	+
(10)	-
(13)	-

+: Growth, -: No growth

A detailed survey of the literature indicates that our strain D_{50} is related to *Streptomyces noursei* [14-17] in respect of spiral sporophore , non-chromogenicity and some biochemical reactions.

However, some qualitative and quantitative differences could be noticed, the strain D_{50} differed from the reference culture in the following respects: brown to pale gray to gray colour aerial mycelium, production of antibacterial antibiotic, utilization of arabinose, xylose and rhamnose.

CONCLUSIONS

In view of large number of similarities and a few differences, it is felt that the strain D_{50} can be considered as a new variant of *Streptomyces noursei* . Hence it is named as *Streptomyces noursei var saccharicus.* Saccharicus is referred to sugar cane field soil from which the organism was isolated.

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