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Effect of Stimulatory and Inhibitory Compounds on the Growth of Antagonistic Actinomycetes from Soils of Andhra Pradesh, India.

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ABSTRACT

In the process of identification of Streptomyces species, some important additional physiological and biochemoical tests were employed. Not all authors used all these parameters for characterization of species and in differentiating from other species which are considered to be significant secondary group characters for the identification and classification of new isolates belonging to actinomycete group. The following additional tests are conducted and observed their effect on growth: i) Effect of carbon source ii) Effect of nitrogen source iii) Effect of inhibitory chemical compounds and iv) Effect of antibiotics
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INTRODUCTION

Actinomycetesare generally regarded as chemo-organotrophs that are not fastidious, needing only a suitable carbon and nitrogen source. The species may vary considerably in their carbon and nitrogen source utilization patterns which are often used as secondary group characters for identification of new isolates. The presence of various inhibitory and stimulatory compounds may profoundly influence the growth rate of micro-organisms. It is intended to study the effect of stimulatory and inhibitory compounds on the growth of indigenous actinomycetes isolated in our laboratory.

MATERIALS AND METHODS

During our continuous search for antibiotic producing actinomycetes, we isolated a total of 359 isolates from 8 different soil samples collected from a variety of terrestrial substrates of Andhra Pradesh in India by selective methods [1]. All the isolates were tested for their antimicrobial activities by conventional cross-streak method [2] and cup –plate method [3].

Finally, 10 isolates were selected with excellent antimicrobial activities for further studies. The following parameters are investigated as per the criteria described by Williams, et al [4], for the classification and identification of isolates.

Effect of carbon source

The ability of different actinomyceteisolates in utilizing various carbon compounds as sources of energy was studied on Pridham and Gottlieb's basal medium [5].

Chemically pure carbon sources certified to be free of admixture with other carbohydrates or contaminating materials were used. The following carbon sources were incorporated at 1% level into the basal agar medium as recommended by ISP: D-glucose (positive control), L-arabinose, sucrose, D-xylose, meso-inositol, D-mannitol, D-fructose, L(+) rhamnose,raffinose and cellulose. The inoculated tubeswere incubated at 28^0 C and observed on 7 th day and 14 th day. The results are presented in Table.1.

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Table.1: Effect of carbon source.

Carbon source	Isolate D21	Isolate D50	Isolate D85	Isolate E20	Isolate E90	Isolate F20	Isolate F40	Isolate F56	Isolate F80	Isolate G23
D-glucose	+++	++	++	+++	+	++	++	+++	+++	+++
L(+)-arabinose	+++	+	++	+++	+++	++	+++	++	++	++
sucrose	+++	++	++	+++	++	++	+++	++	+++	++
D-xylose	+++	++	++	++	++	+	++	_	_	+
Meso-inositol	+++	+++	+++	_	+	+++	++	++	++	+++
D-mannitol	+++	++	+++	++	+++	+++	+++	+++	+++	+++
D-fructose	++	++	++	+++	++	_	+++	_	_	++
rhamnose	+++	++	++	+++	+++	+++	+++	+	+++	+++
raffinose	+	_	++	_	±	++	+++	+++	+++	+++
cellulose	_	_	+	_	-	_	-	_	_	_

Positive: ++, +++ Negative: - Doubtful: ±

Effect of nitrogen source

The ability of isolates to use various nitrogen sources was tested and each being incorporated into the basal medium contained 0.1% w/v.

The following nitrogen sources are used: L-cysteine HCl, L-histidine, potassium nitrate, L-arginine and L-asparagine. Inoculated tubes were incubated at 28^0 C and results are recorded after 14 days (Table.2).

Table 2: Effect of nitrogen source.

Nitrogen source (0.1%W/V)	*D21*	*D50*	*D85*	*E20*	*E90*	*F20*	*F40*	*F56*	*F80*	*G23*
L-arginine	++	+++	+++	+++	+++	++	++	+++	-	**+++**
L-cyseineHCl	-	-	+++	-	++	-	-	-	-	**-**
L-histidine	-	++	+++	++	++	+	-	++	-	**+++**
Potassium nitrate	+++	+++	+++	+++	+++	++	-	+++	-	**+++**
L-valine	-	-	++	++	+	+	-	+++	-	**+++**
L-asparagine (Positive control)	++	++	++	+++	+++	+++	±	+++	-	+++

Effect of inhibitory chemical compounds

The test was carried-out on Benetts agar medium [6-8]. A range of potential chemical inhibitors at diagnostic concentrations was added to the Benetts agar medium. The Sterile molten agar medium contained inhibitory compound was mixed thoroughly and poured into sterile petri plates (6 inch diameter). The selected isolates were streaked on the solidified agar medium. The inoculated plates were incubated at 28^0C. The presence or absence of growth was recorded after 7 days. The following inhibitory compounds are used (%w/v): crystal violet (0.00001), phenol (0.1), potassium tellurite(0.001,0.01), sodium chloride (4,7,10and13). The results are shown in Table.3.

Table 3: Effect of inhibitory chemical compounds

Name of the compound(%W/V	*D21*	*D50*	*D85*	*E20*	*E90*	*F20*	*F40*	*F56*	*F80*	*G23*
Crystal violet(0.00001)	+	+	+	+	+	+	+	+	-	+
Phenol (0.1)	-	-	-	-	-	-	-	-	-	-
Potassium tellurite										
(0.001)	+	+	+	+	+	-	+	+	-	+
(0.01)	+	+	+	+	+	-	+	+	-	+
Sodium chloride										
(4))	+	+	+	+	+	-	+	+	+	+
(7)	+	+	+	-	+	-	-	-	+	+
(10)	-	-	-	-	-	-	-	-	+	-
(13)	-	-	-	-	-	-	-	-	-	-

+: Growth, -: No growth

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Resistanceto Antibiotics

The ability of the isolates to resist various types of antibiotics was one of the criteria used for the classification and identification of actinomycete strains .The test was carried-outon Benetts agar medium .The following six different antibiotics were employed (µg/ml) [9,10]: penicillin 10 IU/, streptomycin 100, tetracycline 50, cephalexin 100, gentamicin 100, rifampicin 50. The antibiotic solutions were prepared and sterilized by filtration and incorporated into the sterile molten agar medium, mixed thoroughly and poured into sterile petriplates (6 inch diameter) and inoculated with the selected isolates after solidification. The inoculated plates were incubated at 28^0 C. The presence or absence of growth was observed on 3 rd day and 7 th day. Resistance was scored as positive. The results are shown in Table.4.

Table 4: Effect of antibiotics

Antibiotic (µg/ml)	*D21*	*D50*	*D85*	*E20*	*E90*	*F20*	*F40*	*F56*	*F80*	*G23*
Penicillin (10 U/ml0	+	+	+	+	+	+	+	+	+	-
Streptomycin (100)	-	-	-	-	-	-	-	-	-	-
Tetracycline (50)	-	-	-	- + (7rd day)	-	-	-	-	-	-
Cephalexin (100)	+	+	+	+	+	+	+	+	+	+
Gentamicin (100)	-	-	-	-	-	-	-	-	-	-
Rifampicin (50)	- +(7th day)	- +(7th day)	-	-	-	-	-	-	-	-

+: Growth,-: No growth

RESULTS AND DISCUSSION

As shown in Table.1, almost all isolates exhibited moderate togood growth in the presence of arabinose, sucrose, raffinose, glucose andmeso-inositol. Poor almost no growth was observed in the presence of cellulose. Majority of isolates has shown excellent growth in the presence of D-mannitol and L(+) rhamnose.

Majority of isolates has shown good to excellent growth in the presence of L-asparagine, L-arginine and potassium nitrate. Poor to moderate growth was observed in the presence of L-cysteine HCl, L-histidine nd L-valine (Table.2). Isolate F80 did not show any growth on any of the nitrogen source employed.

As indicated in Table. 3, crystal violet (0.00001%w/v) was failed to inhibit the growth of majority of isolates whereas phenol(0.1%w/v) was inhibited the growth of all isolates. Potassium tellurite (0.001& 0.01%) was failed to inhibit the growth of majority of isolates. The growth of F20 and F80 were inhibited by potassium tellurite at both the concentrations. The growth of F20 was inhibited by Sodium chloride at all four concentrations. Majority of isolates were grown in the presence of sodium chloride at 4%whereassodium chloride at 10% and at 13% the growth was inhibited in majority of isolates.

As indicated in Table.4, almostall isolates were resistant to penicillin and cephalexin and all isolates were sensitive to streptomycin, tetracycline, gentamicin and rifampicin. The isolate D21 and D50 were developed resistance to rifampicin on 7 th day where as E20 was developed resistance to tetracycline on 7 th day.

CONCLUSIONS

The above additional physiological and biochemical characteristic features of all 10 isolates were used in our taxonomic studies for the identification and classification of our isolates along with standard protocols.

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