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Antagonistic ability of *Streptomyces* species from composite soil against pathogenic bacteria

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ABSTRACT

In this study five *Streptomyces* species were obtained from composite soil sample. In vitro anti bacterial action of these isolates were checked for their inhibitory activities against pathogenic gram positive cocci (*Staphylococcus aureus* & *Micrococcus luteus*) and gram negative bacilli (*Escherichia coli* & *Pseudomonas aeruginosa*) by agar diffusion assay. Most of the *Streptomyces* species were highly active against gram positive cocci than gram negative bacilli tested. Inhibition zones were measured to evaluate antagonistic activity towards the tested pathogenic bacteria. One isolate was inhibitory against the tested pathogens and the four other isolates were active on gram positive cocci.

KEYWORDS

Streptomyces;
Gram positive cocci;
Gram negative bacilli;
Pathogens;
Zone of inhibition (ZOI).

INTRODUCTION

Actinomycetes occur in wide variety of natural and man-made habitats by growing on a vast range of substrates like soil, water[1]. Beijerink found the fact that actinomycetes are abundant in soil[2]. These are strict saprophytes but few exist in parasitic or symbiotic association with plants or animals that make them to inhibit or stimulate the other microbes[10]. Assessment of actinomycetes activities shows their role in decomposition of lignin, cellulose and enhancement of soil fertility (nitrogen fixation)[7]. Waksman was first to isolate streptomycin a second antibiotic after penicillin discovery that received great interest which elucidate the ability of bacteria to produce antibiotics[9]. *Streptomycetes* are aerobic, spore forming member of actinomycetes group that are potentially produce enzymes, enzyme inhibitors and several pharmacologically active substances (antibiotics) of commercial interest[6]. From the earlier studies it was found that *Streptomyces* spp. could be antagonistic against plant pathogenic fungi like *Pythium spp.* (root rot of sugar cane & corn)[11], *Fusarium* spp. (cotton wilt), *Sclerotium spp.*[8] and nematode like *Meloidogyne* spp. (tomato root gall)[3].

MATERIALS AND METHODS

Isolation and identification

Isolation and identification isolation was done by using the soil dilution technique. Ten different soil samples

were collected for isolation of *Streptomyces* with antibacterial potential. 1 gm of each soil sample was weighed and added to flask containing 50 ml of sterile water. The flasks were shaken on rotary shaker for 30 mins. The clear supernatant was used to get dilutions (10^1-10^3). 1 ml of each dilution was spreaded on the starch nitrate agar plates and incubated at 28°C for 2 weeks. The isolated colonies resembling actinomycetes growth were selected and preserved on starch nitrate agar slants. The identification of *Streptomyces* isolate was determined as per Bergey's Manual of Determinative Bacteriology[5].

Agar well diffusion assay

24 hr culture of *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli* & *Pseudomonas aeruginosa* were inoculated on the nutrient agar plates. Wells were made in the inoculated agar plate using a sterile borer (6 mm in diameter) and agar discs were cut off from the *Streptomyces* isolates cultured on starch nitrate medium that is placed in each well. The agar plates were kept in refrigerator for 1 hr to permit diffusion of antimicrobial and incubated at 30°C for 24 hr, then zone of inhibition were measured[4].

Effect of rapidly metabolized sugars on growth and antibacterial activity

Culture medium (0.3 % malt extract, 0.3 % yeast extract, 0.5 % peptone, 100 ml distill water and pH 6.8) was used to test the (1, 2, 3, 4, 5%) different concentrations of sugars (glucose, fructose, maltose) on growth and antibacterial activity of most effective *Streptomyces* isolate. After inoculation of *Streptomyces* isolate in the culture medium at 28°C with interval of 2 days incubation optical density was measured aseptically at 590 nm. After five successive readings the culture media were incubated for 10 days at 28°C.

Followed by the incubation the culture broth of different sugar concentrations were centrifuged at 10000 rpm for 20 mins. The cell free supernatants were obtained by filtering through whatman filter paper. The filtrate was passed through charcoal powder to get clear liquid. These filtrates were used to test antibacterial activity against *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli* & *Pseudomonas aeruginosa* by Agar well diffusion assay.

TABLE 1 : Effect of different sugars on the growth of *Streptomyces* isolate at various incubation periods (Generation time)

Sugars	Incubation time(days)	O.D at 590nm				
		1%	2%	3%	4%	5%
Glucose	2	0.17	0.62	0.61	0.71	0.78
	4	0.2	0.74	0.78	0.82	1.45
	6	0.72	0.87	0.98	0.96	2.56
	8	0.55	0.52	0.86	1.11	1.42
	10	0.25	0.43	0.57	0.66	0.82
Fructose	2	0.19	0.22	0.42	0.31	1.82
	4	0.25	0.37	0.52	0.62	1.43
	6	0.55	1.15	0.95	0.85	1.56
	8	0.45	1.01	0.75	0.89	2.47
	10	0.35	0.55	0.65	0.53	1.15
Maltose	2	0.24	0.62	0.61	0.71	1.82
	4	0.35	0.74	0.78	0.82	2.2
	6	0.58	0.87	0.98	0.96	2.56
	8	0.45	0.5	0.86	1.11	2.47
	10	0.39	0.54	0.5	0.66	0.82

RESULTS AND DISCUSSION

Twenty *Streptomyces* isolates were isolated and identified from the composite soil samples. Color of aerial mycelium, substrate mycelium and soluble pigments were used to characterize the isolates. Grey, blue, cream, yellow, violet and red were the main colors of aerial mycelium. The colors produced by the isolates were soluble pigments that diffuse into the agar medium. Red and grey color classes dominated in the isolates. Screening of the isolates against the test bacteria i.e *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli* & *Pseudomonas aeruginosa* were most effectively antagonized by the one isolate, four isolates were moderately effective and the fifteen isolates were least effective.

Most effective isolate incubated in glucose from 1-5% concentration showed O.D at 590nm in the range of 0.17-0.78 on second day of incubation. Optical density was high at sixth day incubation at 5% glucose (TABLE 1). Fructose and maltose sugars were supporting the maximum growth at 5% concentration on eighth and sixth day of incubation.

Culture filtrate obtained from different sugars like glucose, fructose and maltose at various concentrations

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TABLE 2 : Effect of glucose on Antagonistic activity by *Streptomyces* isolate

% of Glucose	Test Organisms			
	S. aureus	*M. luteus*	*E.coli*	*P.aeruginosa*
1	12	11	10	14
2	13	11	13	18
3	22	26	20	25
4	13	10	11	13
5	11	10	11	14

TABLE 3 : Effect of fructose on antagonistic activity by *Streptomyces* isolate

% of Fructose	Test Organisms			
	S. aureus	*M. luteus*	*E.coli*	*P.aeruginosa*
1	15	13	15	18
2	12	17	18	15
3	13	14	19	18
4	19	15	13	17
5	11	10	11	13

TABLE 4 : Effect of maltose on antagonistic activity by *Streptomyces* isolate

% of Maltose	Test organisms			
	S. aureus	*M. luteus*	*E.coli*	*P.aeruginosa*
1	13	13	10	10
2	12	17	18	12
3	15	14	10	18
4	13	12	11	15
5	11	10	10	12

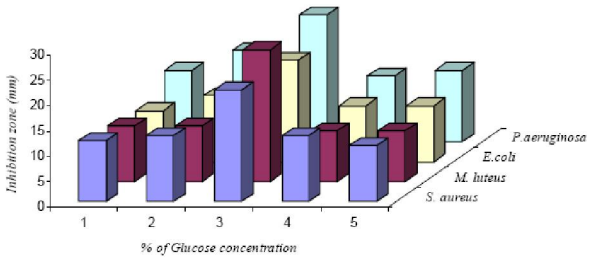

Figure 1 : Effect of glucose on antagonistic activity by most effective *Streptomyces* isolate

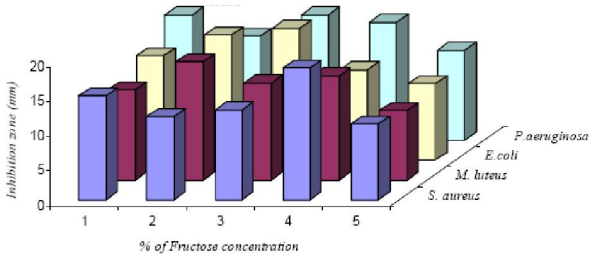

Figure 2 : Effect of fructose on antagonistic activity by most effective *Streptomyces* isolate

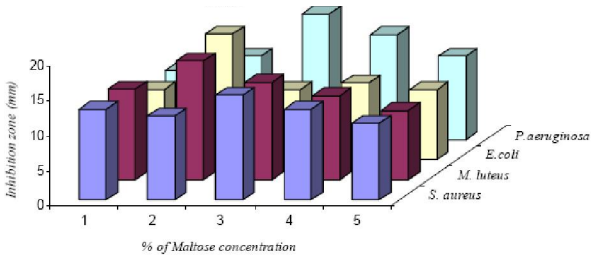

Figure 3 : Effect of maltose on antagonistic activity by most effective *Streptomyces* isolate

incubated with most effective isolate when tested against the bacterial strains like *Staphylococcus aureus, Micrococcus luteus, Escherichia coli* & *Pseudomonas aeruginosa* was found to be inhibitory (TABLE 2,3,4). 3% glucose was strongly inhibitory against the tested bacterial strains indicating the sugar concentration effective to enhance the antagonistic action of the isolate (TABLE 2, Figure 1). Fructose was varied in the concentration to increase the antibiotic activity against the tested bacteria. 4% fructose produced highly inhibitory activity against *Staph. aureus*, 2% was strongly effective towards *M. luteus*, 3% was effective against *E. coli* & *P. aeruginosa* (TABLE 3, Figure 2). 2% concentration of maltose was equally inhibitory for *M. luteus*, *E. coli* and 3% sugar inhibited *Staph. aureus* & *P. aeruginosa* (TABLE 4, Figure 3).

From this study it can be concluded that reducing sugars effects the growth and antibiotic production by *Streptomyces*. Reducing sugars possess unique property that favor in accepting hydrogen ions from metals. Increase and decrease in variety degrees of sugars affected the antibiotic production that was checked by zone of inhibition. 2, 3% sugar concentrations were found to stimulate the antibiotic production and growth of *Streptomyces*.

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