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Screening of different bioactive soil *Streptomyces SP*. for broad spectrum antibacterial and antifungal activity

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ABSTRACT

The present study was aimed at screening of bioactive *streptomyces* species. Different soil samples from river beds and field were utilized for the screening of 20 different streptomyces strains on basis of morphological and biochemical identification and antibacterial and antifungal activity. Out of 20 strains 14 strains were showed varied antibacterial activity against both gram negative and gram positive human pathogenic bacteria like *Pseudomonas aeroginosa, E.coli, Klebsiella pneumonae, Clostridium, Staphylococcus aureus, Eneterobacter aerogenes*, *Streptococcus mutans* and *Scardowia wiggasae*. Six strains also showed antifungal activity against both animal and plant pathogenic fungi like *Candida albicans, Aspergillus niger. Alternaria Solani.*

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INTRODUCTION

In the present scenario *Streptomyces* is a gram positive bacterium but morphologically similar to filamentous fungi. But the most important feature is the fact that it is the largest antibiotic producing genus in the microbial world. S*treptomyces* are valuable resources for novel product like antifungals, antivirals, antitumorals, anti-hypertensives, immune-suppressants and especially antibiotics (Mitsuiki et al 2002, Watve at al 2001). Despite the discovery and development of number of antibiotics and advances in their production techniques, infectious diseases still are the main cause of worldwide deaths. Because of this, their has been overuse and self medication of antibiotic drug which has resulted in creating resistant strains of pathogenic bacteria, viruses and fungi, thus creating constant need for research and development of new antibiotics .

The end result of this phenomenon is that many strains of bacteria have become resistant, and in many cases multi-resistant to therapeutic agents, thus rendering these drugs ineffective as treatments of choice for severe infections caused by these pathogens (Alanis 2005). Rising numbers of antibiotic unresponsive infectious disease agents confront patients worldwide (Levy 2002, Livermore 2003) and consensus has emerged that it is essential that novel antibiotic classes be developed as part of the strategy to control the emerging drug-resistant pathogens (Projan 2002, Barrett and Barrett 2003). In response, there is a renewed interest in discovering novel classes of antibiotics that have different mechanisms of action (Spizek and Tichy 1995, Barsby et al. 2001). Search for new antibiotics effective against multi-drug resistant pathogenic bacteria is presently an important area of antibiotic research. Natural products having novel structures have been observed to possess useful biological activities. Soil is a natural reservoir for microorganisms and their antimicrobial products (Dancer 2004).

Filamentous soil bacteria belonging to the genus *Streptomyces* are widely recognized as industrially important microorganisms because of their ability to produce many kinds of novel secondary metabolites including antibiotics (Williams et al. 1983a, Crandall and Hamil 1986, Williams et al. 1989, Korn-Wendisch and Kutzner 1992). Of all known drugs 70% have been isolated from Actinomycetes bacteria of which 75% and 60% are used in medicine and agriculture respectively (Miyadoh 1993, Tanaka and Mura 1993).

In response to the present needs and requirement, the present study was undertaken to isolate and screen bioactive *streptomyces* strains. Here, the isolation and characterization as well as the inhibitory effects of local Streptomycete isolates were studied against various multiple antibiotic resistant bacteria and pathogenic Fungi.

MATERIAL AND METHODS

Collection of soil samples:

1. Three soil samples were collected from various locations including Garhmukteshwar, Ghaziabad and Noida.

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2. Diverse habitats in different areas were selected for the isolation of *Streptomyces sps.*
3. These habitats include the rhizospheres of plants, soil and soil of industrial area.
4. The soil was taken up after removing approximately 3cm of the soil surface.
5. The samples were collected in the sterile polythene bags, closely tighted and were taken to the laboratory.

Preparation of sample in the laboratory:

1. The collected sample were air dried and sieved to remove various contaminants
2. Then the sieved soil was pretreated with calcium carbonate (10:1) and incubated at 28 ^{0} C. for several days.
3. The air drying and mixing the sample with calcium carbonate will reduce the vegetative bacterial cell and allow many Actinomycetes to survive.
4. Then serial dilution of all the samples was done. Test tubes contaning 10 $^{-2}$ Dilution of the sample were placed in a water bath at 45^{0} C for few hrs so that the spores would separate from vegetative cells, which was then ready for inoculation.

Organisms used for antimicrobial activity- In vitro antimicrobial activities were performed against the *Bacillus subtilis, Enterobacter aerogenes ,Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus mutans, Scardowia wiggasae Aspergillus niger, Alternaria alternate, Candida albicans,* etc.

Characterization of *Streptomyces sps.:*

Morphological Identification was done and colony morphology was noted with respect to color, aerial mycelium, size and nature of colony, slide color and feeling the consistency with a sterile loop.

After which Biochemical Characterization of *Streptomyces* isolates was carried out by Catalase test, nitrate reduction test, IMViC test, Starch hydrolysis test, Fermentation of citrate test, Citrate utilization test, Skim milk agar hydrolysis and different sugar utilization tests.

Streptomyces isolates were then screened for antimicrobial activity against the pathogenic test organism by well diffusion method.

RESULT AND DISCUSSION

This study was undertaken with an aim of isolation and screen *Streptomyces sps. from* soil from Noida & Ghaziabad region (Table :1) and selective media and cultivation conditions described previously a total of 14 different *Streptomyces* isolates were recovered from 20 soil samples that were collected from soil of Noida & Ghaziabad. S1 and S2 strain gives the higher number of *Streptomyces* isolates with respect to garden soils.

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Origin	Isolation temperature	Total strains isolated	No. of active Isolates against bacteria
Waste land near Sec.-63 Noida	28°C 37°C	4	3
Wasteland near sec.-2 Noida	28°C 37°C	5	5
Soil from Ghaziabad (Near River Beds)	28°C 37°C	5	3
Soil from Ghaziabad (near canal/nalla)	28°C 37°C	6	3
Total		20	14

Table 1-Total number of Actinomycetes isolates with antibacterial activity isolated at different temperature.

All isolates grew on isolation agar media showing morphology typical of *Streptomyces*. Since the colonies were slow growing, aerobic, folded and with aerial and substrate mycelia of different colors. All *Streptomyces* isolates were Gram's stain positive. The cultural characteristics (pigment production), morphological characteristics of the different *Streptomyces* isolates are presented in Table-2.

Origin	Sample Name	Color	Mycelium type	Pigment production	Gram's reaction
Waste land near Sec.-63 Noida	S1	White	Aerial	Orange	+
	S2	Green	Aerial	Yellow	+
	S3	Dark Green	Substrate	Yellow	+
Wasteland near Sec.-2 Noida	S4	White	Aerial	Orange	+
	S5	Dark green	Aerial	Black	+
	S6	White	Substrate	Yellow	+
	S7	Green	Aerial	Yellow	+
	S8	White	Aerial	Green	+
Garden soil Sec.-82	S9	Green	Aerial	Yellow	+
	S10	White	Aerial	Orange	+
	S11	Green	Substrate	Black	+
Garden soil sec-110	S12	White	Aerial	Orange	+
	S13	Green	Aerial	Green	+
	S14	Green	Aerial	Yellow	+

Table 2- Culture Characteristic of Selective isolates on Isolation agar medium.

Test	Results
Gram's staining	Positive for all strain
Methyl red	Negative for all strain
Indole test	Negative for all strain
Vogus proskauer test	Negative for all strain
Triple sugar Iron test	Negative for all strain
Fermentation of Citrate test	Negative S1,S2,S7-Positive)
Starch hydrolysis test	Positive (S1,S3,S7-negative)
Hydrogen Sulphide test	Negative (S7-positive)
Glucose hydrolysis test	Positive for all strain
Sucrose hydrolysis test	Positive for all strain

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Mannose	Positive for all (S7 negative)
Fructose	Positive for all
Rhamnose	Positive for all
Lactose hydrolysis test	Positive for all strain
Nitrate reduction test	Negative for all strain
Skim milk agar hydrolysis	Negative for all strain
Catalase test	Positive for all strain
Casien hydrolysis Test	Positive for all (S3, S1, S7 negative)
Urea	Positive for all

Table 3 – Results of Biochemical tests of isolates of *Streptomyces (according to Bergey's manual)*

Around 20 Actinomycetes were subjected for primary screening and subjected for purification methods by streak plate method. The Identification of the potent antibiotic producing strains reveals that all the strains belong to the genus *Streptomyces.* The isolated microorganism were Gram positive, having branching and were filamentous. Different isolates showed varying results in the Biochemical test as shown in Table : 3

The Streptomyces flora of 20 soil samples, collected from different locations were screened for their potential as a source of antibiotics active against antibiotic- resistant bacteria and pathogenic fungi. All of the isolates were tested for their ability to produce inhibitory substances against 10 test microorganisms

Out of 20 isolates, 14 isolates showed positive antimicrobial results. These isolates were selected for their broad spectrum of activity and zone of inhibition. Table : 4 and 5. It was observed that isolates S1, S2 and S3 gave the best inhibitory effect aginst most of the bacterial and fungal isolates.

Streptomyces sp. Strains isolated	I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*IX*
S1	2.2	4.4	1.8	1.6	3.2	1.2	1.1	2.1	2.3
S2	2.8	2.0	2.0	2.4	2.6	2.1	1.6	1.4	1.9
S3	3.2	1.8	2.6	2.2	3.3	3.6	2.8	2.3	3.1
S4	1.3	0.5	1.4	1.1	1.2	1.3	1.6	1.5	1.9
S5	1.5	0.6	1.6	1.3	1.3	0.6	1.7	0.8	1.2
S6	0.9	0.7	0.8	0.9	0.8	0.9	0	0.8	0
S7	0.8	0.5	0.3	0.5	0.4	0.4	0	0.3	0.2
S8	0.75	0.4	0.6	0.5	0.4	1	0.9	0.1	0.6
S9	0.50	0	0.4	0.8	0.7	0	0.5	1.7	0.8
S10	0.45	0	0.5	0.9	0.5	0	0.4	1.9	0.2
S11	0.9	0	1	0	1.5	0	0.9	2.4	1.1
S12	0.6	0	0	0	0.5	0	0.5	0	0.1
S13	1.15	0	0	0.3	0.45	0	0.4	0	0.5
S14	0.5	0	0.3	0.9	0.4	0	0.5	0	0

Table 4- Antibacterial activity of Isolates (Agar well diffusion methods).

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I- E.coli., II- Bacillus subtilis, III- Staphylococcus aureus, IV- P. auroginosa, V- E.aerogenes, VI- K. pneumonae, VII- Clostridium sps., VIII- Streptococcus mutans, IX- Scardowia wiggasae

Isolates	*Aspergillus Niger*	*Candida albicans*	*Alternaria soloni*
S1	3.9	2.1	0
S2	2.8	0.5	0
S3	4.8	2.4	3.8
S4	1.1	0.9	0.9
S5	0.9	0.6	0.5
S6	1.0	0.3	0.4
S7	1.1	0.2	0.4
S8	0.3	0.5	0.9
S9	0.1	0	0.2
S10	1.2	0.9	0.9
S11	1.5	0.9	1
S12	1.1	0.6	0.5
S13	1.15	0	0
S14	0.5	0.1	0.6

Table 5- Antifungal activity of Isolates (Agar well diffusion methods).

	Rf Values
S1	0.83
S2	0.68
S3	0.67
Standard	0.68

Table 6 – Comparison of Rf values (TLC) of Standard Streptomycin antibiotic and crude antibiotic produced by different isolates.

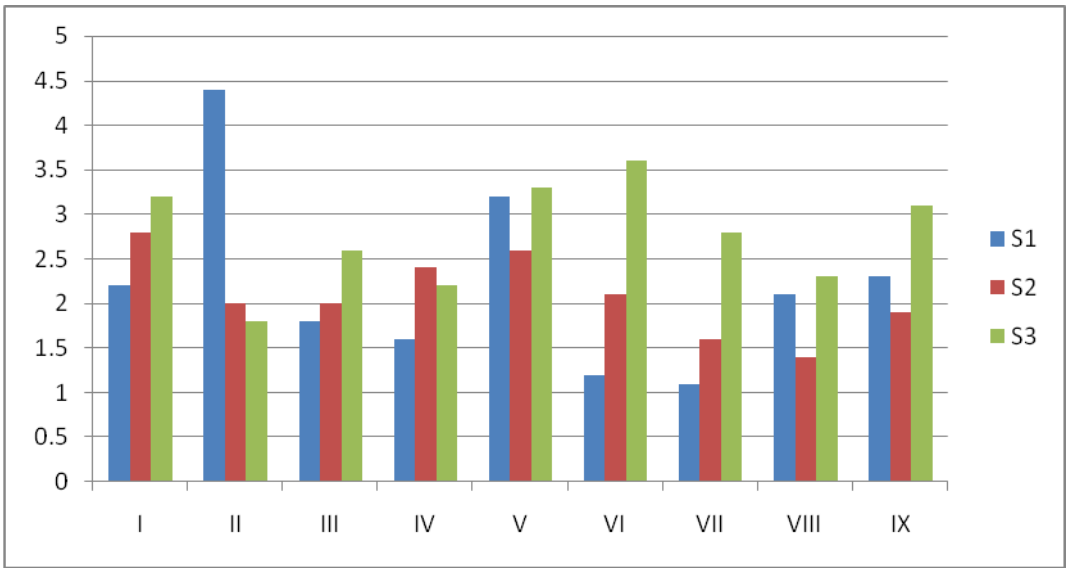

Figure 1 - Comparative Zone of inhibition of S1, S2, S3 *Streptomyes* strain against different pathogenic bacteria

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I- E.coli., II- Bacillus subtilis, III- Staphylococcus aureus, IV- P. auroginosa, V- E.aerogenes, VI- K. pneumonae, VII- Clostridium sps., VIII- Streptococcus mutans, IX- Scardowia wiggasae

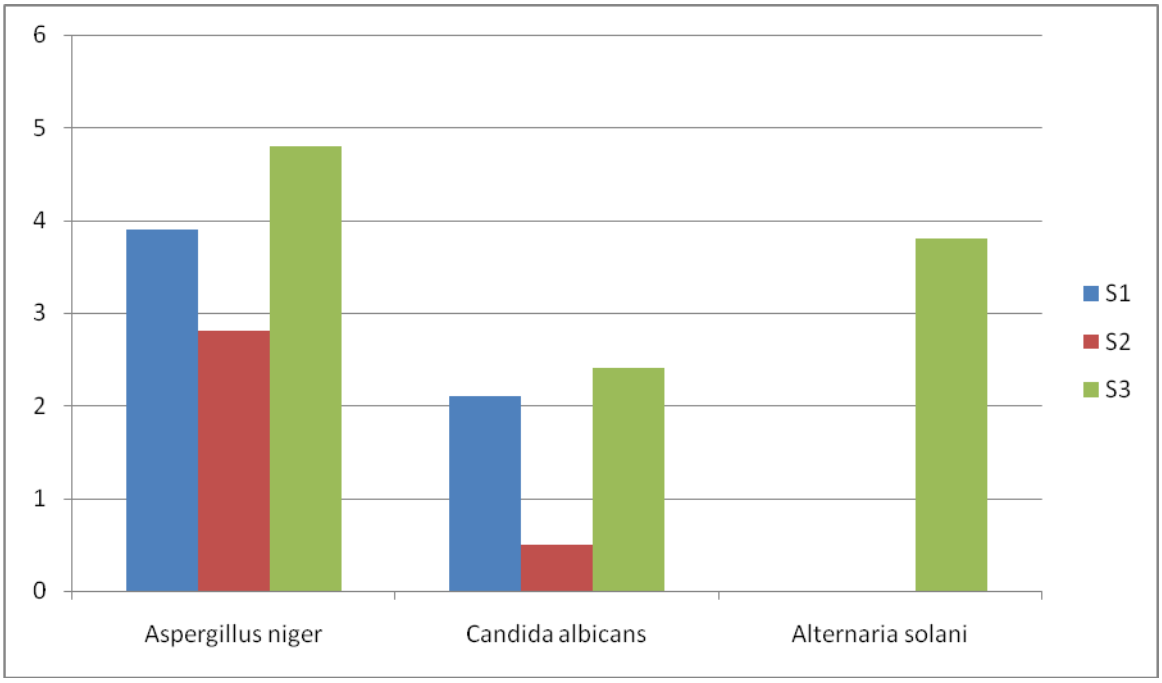

Figure 2 - Comapritive Zone of inhibition of S1, S2, S3 *Streptomyes* strain against fungal pathogens

The results of physiological, biochemical characteristics of actinomycetes isolate,such as degradation of gelatin and starch , sucrose maniose, D - xylose, D -xylose, fructose, arabinose, calactose, rabinose clucose considered to classification of isolates strain as recommended by different authors (Williams et al. 1983).The results shown in table-3 indicated that the *Streptomyces sp* .was distinguished by its inability to hydrolyzed starch, H_2S production. (Bergey's manual 2000) to a certain extent.On other hand, they have ability to utilize different carbon sources. Broadly most of the isolates show similar results, thus this clarifies that all the isolates which had be isolated from soil were from the *Streptomyces* group.

CONCLUSION

14 isolates showed activity against bacteria in which most of them from NCR (Noida) soil. These isolates showed very high antimicrobial activity against plant and animal pathogen. Our studies will establish the rich actinomycetes diversity of the region, especially the various niche habitats of NCR and also help conserve and utilize them in bioindustry.

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