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RESEARCH ARTICLE

PHARMACEUTICS

ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES ISOLATED FROM THE WESTERN GHATS OF TAMILNADU

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ABSTRACT

A total of fifty two actinomycetes strains were isolated from the rhizosphere soil of medicinal plants collected from Southeastern Western ghats of Tamilnadu. They were assessed for their antibacterial activity against six human pathogenic bacteria by cross streak method. Out of 52 isolates, five strains were showed high antibacterial activity against all the tested pathogens. Highly active strain (STREP 37) was analyzed further for its morphological and biochemical characteristics for identification. Fermentation conditions were also standardized for the optimum production of antibacterial compounds.

KEY WORDS

Actinomycetes, medicinal plants, pathogens.

INTRODUCTION

Actinomycetes are the group of gram positive filamentous bacteria which are ubiquitous various natural and man-made environments. Actinomycetes are the most economically valuable prokaryotes which are well known to produce chemically diverse metabolites with wide range of biological activity (Balagurunathan and Radhakrishnan, 2007). Recent days the discovery of known metabolites and actinomycetes are increasing due to the exploitation of natural ecosystems. Exploitation of less and unexplored ecosystems for actinomycetes is highly necessary for the discovery of novel bioactive metabolites. Actinomycetes are important sources of new bioactive compounds such as antibiotics and enzymes (Vining, 1992; Edwards, 1993; Demain, 1995; Xu *et al.,* 2005) which have diverse clinical effects and are active against many organisms (Bacteria, Fungi, Parasites *etc*.). In fact more than 50% of the known natural antibiotics are produced from actinomycetes (Miyadoh, 1993). The most striking feature of the actinomycetes is their ability to produce a wide variety of secondary metabolites. These natural products have been extraordinary sources of lead structures in the development of newer drugs (Kutzner, 1986; Willams *et al.,* 1989; Weber *et al.,* 2003). In the present study we have isolated actinomycetes from the rhizosphere soil of medicinal plants obtained from the Southeastern Western ghats of India and studied for their antimicrobial activity.

MATERIALS AND METHODS

Collection of soil samples

Rhizosphere soil samples of *Phyllanthus niruri, Hibiscus rosasinensis* and *Acalypha indica* were collected from the Senbagathop forest coming under the Srivilliputtur range, Tamil Nadu. Samples were brought to the laboratory in aseptic condition and air dried in shady condition at room temperature for 7-10 days. Just before isolation, samples were kept in an incubator at 45□C for 1 hour to avoid the bacterial and fungal contaminants.

Isolation of actinomycetes

After incubation, 1g of sample was taken and transferred to an Erlenmeyer's flask containing 99 ml of sterile distilled water. The soil suspension was further diluted up to 10^{-5} levels. One ml of the diluted suspension was spread over the surface of starch casein agar medium. The pH of the media was adjusted to 7.2. To prevent the fungal and bacterial contaminants, Cycloheximide (100mg/1) and Nalidixic acid (20mg/1) were prepared separately in sterile water and mixed with the medium just before use. The petri plates were then incubated at 28 □ 2□C and the colonies were observed from third day onwards and upto one month. Strains of actinomycetes were picked out and purified by repeated streaking on yeast extract-malt extract agar (ISP 2) and were preserved in slants at 4±2□C.

Screening for antagonistic activity

Test organisms

Human pathogenic bacteria such as *Staphylococcus aureas, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumoniae* and *Escherichia coli* were obtained from Microbial Type Culture Collections (MTCC), Chandigarh were used for this study.

Primary screening

Primary screening was done by cross streak method. In this method a loop full of inoculum was streaked in the middle of the petridish containing modified nutrient agar medium. After inoculation, petri dishes were incubated at 28□ 2□C for 3 days for growing actinomycetes and then 24hrs old pathogenic bacteria were inoculated near the growth line of actinomycetes in the same petridish. The cross streaked plates were incubated at 28□2□C for 24 hrs. The inhibition zone (Cleaning zone) produced between the actinomycetes and the pathogenic bacteria were measured.

Secondary screening

Highly active isolates were further studied by shake flask culture method to conform their antimicrobial activity by paper disc method. The spore suspension of test bacteria was grown in Muller Hinton agar medium. The sterile paper disc impregnated with 0.1 ml of culture filtrate of the selected actinomycetes strains were placed in the center of the plates and incubated at 28□2□C for 48 hrs. The diameter of the inhibition zone for each strain was recorded. Among the selected strains most potent strain was selected for further analysis.

Optimization of culture conditions for antibacterial activity

Effects of carbon and nitrogen sources

To study the influence of incubation periods, the culture was maintained in the production medium for up to 7 days. The broth culture drawn at 24, 48, 96,120 and 168 hrs was tested for antibacterial activity. To study the Influence of carbon and nitrogen sources, different carbon sources, *viz*. glycerol, glucose, lactose and maltose and different nitrogen sources, namely soybean meal, Yeast extract, malt extract, peptone and sodium nitrate each at 1% of the production medium were used separately.

Identification of the strain STREP 37

Identification of the actinomycete strain STREP 37 was made at genus level based on the results obtained from culture and biochemical characters including cell wall composition analysis.

RESULT

In the present study, 52 different actinomycetes strains were isolated from the three medicinal plants. Of these, 15 strains were showed antagonistic activity against six human pathogenic bacteria (Table 1). Crude extracts obtained from 5 selected strains were utilized for the secondary screening and the results obtained were presented in the Table 2.

Table 1
Antagonistic effect of actinomycetes showing inhibition zone against human pathogenic bacteria

S.No.	Strains	*Escherichia coli*	*Salmonella typhi*	*Bacillus subtilus*	*Klebsiella pneumoniae*	*Pseudomonas aeroginosa*	*Staphylococcus aureus*
1.	STREP-7	02	10	14	07	10	20
2.	STREP-9	07	04	09	08	15	16
3.	STREP-13	-	02	12	23	07	26
4.	STREP-17	11	18	07	03	13	37
5.	STREP-18	03	22	-	-	05	-
6.	STREP-21	05	12	08	14	11	28
7.	STREP-23	02	03	07	03	-	06
8.	STREP-27	06	02	03	12	11	09
9.	STREP-29	01	10	05	32	04	20
10.	STREP-35	07	20	10	12	08	22
11.	STREP-37	10	21	19	14	11	37
12.	STREP-38	06	08	06	-	07	05
13.	STREP-41	05	06	03	-	11	07
14.	STREP-43	12	11	07	22	16	09
15.	STREP-51	09	18	02	-	04	-

Table 2
Antibacterial activity of STREP 37 in the secondary screening

S.No.	Strains	*Escherichia coli*	*Salmonella typhi*	*Bacillus subtilus*	*Klebsiella pneumoniae*	*Pseudomonas aeroginosa*	*Staphylococcus aureus*
1.	STREP17	11	18	07	03	13	37
2.	STREP21	05	12	08	14	11	28
3.	STREP35	07	21	10	12	08	22
4.	STREP37	10	21	19	14	11	37
5.	STREP43	12	11	07	22	16	09

Among the five strains, only one strain (STREP37) was showed maximum activity against all the pathogenic microorganisms such as *Staphylococcus aureas* (37 mm), *Salmonella typhi* (21mm), *Pseudomonas aeruginosa* (11mm), *Bacillus subtilis* (19mm), *Klebsiella pneumoniae*(14mm) and *Escherichia coli*(10mm)(Fig 1). In the fermentation studies, we had observed maximum activity with Yeast extract *Staphylococcus aureas* 37mm, *Saccharomyces cerevisiaes* 16mm *and Aspergillus niger* 15mm as a nitrogen source and Dextrose *Staphylococcus aureas* 37mm, *Saccharomyces cerevisiaes* 16mm *and Aspergillus niger* 14mm as a carbon source (Table 4 and 5). The differences might be due to the species variation and source of isolation.

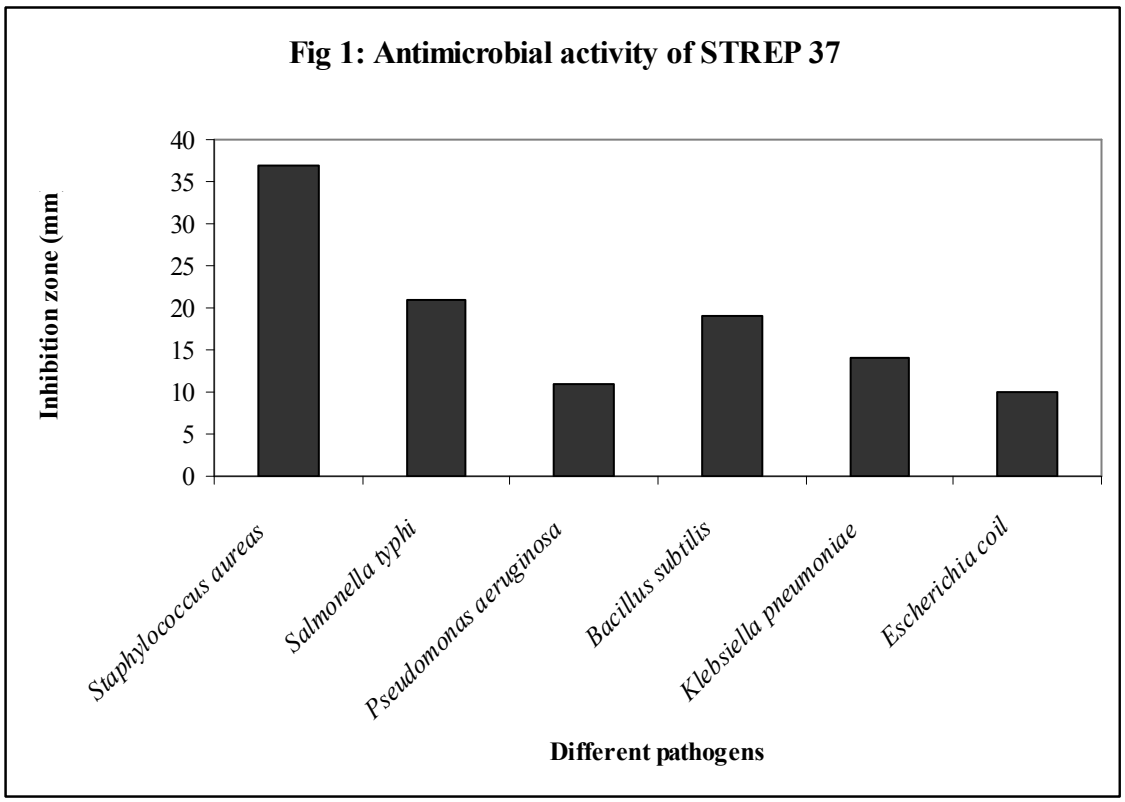

Fig. 1
Antibacterial activity of STREP 37

Table 4
Influence of various carbon sources on the antimicrobial activity of actinomycete isolate (STREP37) against various pathogenic organisms

S.No.	Various Carbon Sources	Inhibition Zone [mm] against		
		Saccharomyces cerevisiaes	*Staphyllococcus aureas*	*Aspergillus niger*
1.	Glucose	7	20	14
2	Sucrose	15	28	15
3.	Lactose	9	24	12
4.	Maltose	14	26	8
5.	Dextrose	16	37	15

Table 5
Influence of various nitrogen sources on the antimicrobial activity of actinomycetes isolate (STREP37) against various pathogenic organisms

S.No.	Nitrogen Source	Inhibition Zone [mm]		
		Saccharomyces cerevisiaes	*Staphyllococcusaureas*	*Aspergillus niger*
1.	Yeast Extract	16	37	14
2.	Malt Extract	16	32	13
3.	Peptone	11	27	9
4.	Sodium Nitrate	14	28	4

In general, the maximum growth of actinomycetes was reported between 96 and 120 hrs of fermentation. In the present study, 120hrs old fermented broth of actinomycetes was showed maximum antimicrobial activity against *Saccharomyces cerevisiaes, Staphylococcus aureas* and *Aspergillus niger* (Table 3).

Table 3
Antagonistic effect of actinomycetes in different time periods

S.No.	Inhibition Time [hrs]	Inhibition Zone [mm]		
		Saccharomyces cerevisiaes	*Staphylococcus aureas*	*Aspergillus niger*
1.	12	0	0	0
2.	24	0	4	0
3.	36	2	6	0
4.	48	7	18	5
5.	60	9	22	8
6.	72	14	27	10
7.	84	15	30	11
8.	120	16	37	12

The antagonistic strain STREP 37 was found to possess LL-DAP and contained glycine in their cell wall, which indicates the cell wall chemo type 1 i.e. the wall property of the genus *Streptomyces*. All the five antagonistic strains were found to possess LL-DAP and all of them contained glycine in their cell wall.

DISCUSSION

The World Health Organization (WHO) have observed that up to 80% of the rural population in the developing countries depend on herbal or alternative medicine and requested member countries to explore safe indigenous medicines for their natural health care (Sofawara, 1984). Medicinal plant research offers a good chance of discovering new drugs (Malone, 1983). Natural products and their derivatives represent more than 50%

of the drugs in clinical use in the world (Sofawara, 1984; Cowan, 1999).

This is the attempt to isolate novel and newer antibiotic producer from the medicinal plants of Western Ghats, India. In the present study, actinomycetes isolated from the rhizosphere of medicinal plants were found to be antagonistic to both bacteria and fungi. Approximately 60% of antibiotics developed for agricultural use were isolated from *Streptomyces* sp. (Okami and Holta, 1988).

The antibacterial activity of the test isolates was varied.17 of 50 actinomycetes isolates were shown to have very potent *in vitro* antibacterial activity against both phytopathogenic and other G (+) and G (-) bacteria. Mustafa *et al.,* (2004) reported that the 50 isolates of actinomycetes were obtained. Approximately 34% (17) of the isolates produced antibiotics, included among these were broad and narrow spectrum. 16% (8) isolates produced antibacterial substances against only on Gr (+) bacteria, 6% (3) isolates only against Gr (-) bacteria and 12% (6) isolates against both Gr (-) and Gr (+) bacteria. The most antibacterial activity on phytopathogen bacteria selected as test organisms were showed by isolate 3Ba3 (18 mm against on *Agrobacterium tumefaciens*, 15 mm against on *Erwinia amylovora* and 13 mm against on *Pseudomonas viridiflova*) and isolate 5C12 (26 mm against on *E. amylovora*, 18 mm against on *Clavibacter michiganensis subsp. michiganensis* and 11 mm against on *P. viridiflova*). Kathiresan *et al.,* (2005) observed that Soya bean was found to be the best nitrogen source and glucose was found to be the best carbon source. Actinomycetes appeared to produce high antifungal compound at 120 hrs of incubation period. LL-DAP and contained glycine in their cell wall, which indicates the cell wall chemo type 1 i.e. the wall property of the genus *Streptomyces*. *(*Muthurayar e*t al*., 2006).

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