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A NTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF STREPTOMY CES ISOLATED FROM SOIL UNDER CULTIVATION OF *CURCUMA LONGA L.*

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Abstract:-The *Streptomyces* are Gram positive prolific group of actinomycetes. They are filamentous structure, strict aerobes, lack cross wall. *Streptomyces* are widely distributed in a variety of natural and manmade environments. The present research carried out to search antimicrobial activity produced by Streptomyces against different pathogens. Total three rhizospheric soil samples under cultivation *Curcuma longa* L. were collected from the villages around Barshi Dist- Solapur. Five isolates were identified as Streptomyces with the help of MICRO-IS software. Total five Streptomyces isolates were tested for their antimicrobial activity with the help of primary screening and agar overlay technique against *Bacillus megaterium, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi, Aspergillus niger, Saccharomyces cerevisae, Candida albicans and Penicillium chrysogenum. Streptomyces roseus* showed broad spectrum antimicrobial activity against all tested pathogens except *C. albicans. Streptomyces filipinensis* showed broad spectrum antimicrobial activity against tested pathogens except *Aspergillus niger*. Antimicrobial substance produced from both of these *Streptomycetes will* be used to prevent human and plant bacterial and fungal diseases caused by these tested pathogens to some extent.

Keywords:*Streptomyces, Curcuma longa* L., antifungal activity, antibacterial activity.

INTRODUCTION :-

Soil is a natural reservoir for microorganisms and their antimicrobial products (Dancer 2004). Plant rhizosphere soil is a unique biological niche with a diverse micro flora of bacteria, fungi, protozoa and algae. These communities have been played significant role in nutrition by a high input of organic materials derived from the plant roots and root exudates that are necessary for microbial growth (Lynch 1990). The plants containing medicinal substances which can be used as antibacterial, antifungal, antipyretic, anti-cancerous, etc. are termed as medicinal plants. *Curcuma longa* L. is one of the important medicinal plants. *Streptomycetes* are found in plant rhizosphere (Suzuki *et al*., 2000) and the possibility that they can protect roots by inhibiting the development of potential fungal pathogens.

The *Streptomyces* are commonly found in soil and are known antibiotic-producers. The genus *Streptomyces* was proposed by Waksman and Henrici for aerobic and spore forming Actinomycetes (Williams et al., 1989). Different *Streptomyces* species produce about 75% of commercially and medically useful antibiotics. More than half of the naturally occurring antibiotics discovered, to date and continue to be screened produced by *Streptomyces* for useful compounds (Miyadoh 1993).

Most actinomycetes in soils belong to the genus *Streptomyces* (Goodfellow and Simpson, 1987). Approximately two-thirds of naturally occurring antibiotics, including some of agricultural importance, have also been isolated from the soil microorganisms. Evidences reported that actinomycetes represent a large part of the rhizosphere microbial community and are prolific producers of diverse bioactive secondary metabolites (Strobel *et al*., 2004; Schulz *et al*., 2009), and play an important role in plant growth, protect plant roots against invasion by root pathogenic fungi and thus acts as biocontrol agents against plant pathogens (Crawford *et al*., 1993; Chamberlain and Crawford, 1999; Tokala *et al*., 2002 Doumbou *et al*., 2002). Some evidences reported antagonistic activity of actinomycetes against fungal pathogens (Ouhdouch *et al*., 2001; Getha and Vikineswary, 2002).

Very few workers studied antifungal and antimicrobial activity of *Streptomyces* from rhizosphere soil of medicinal plants. *Curcuma longa* L. is an important medicinal plant. This study revealed that antifungal and antimicrobial activity of Streptomyces from soil under cultivation of *Curcuma longa* L.

MATERIALS AND METHODS

Materials-

1. Soil samples- Three rhizosphere soil samples under cultivation of *Curcuma longa* L. were collected from the villages around Barshi, Dist. Solapur, M.S. India and used in this study for isolation of antibacterial and antifungal activity.
2. Glycerol aspargine agar
3.24 hrs. old standard culture of *Bacillus megaterium*, *Klebsiella pneumoniae*, *Staphylococus aureus*, *Salmonella typhi Aspergillus niger, Saccharomyces cerevisae, Candida albicans and Penicillium chrysogenum*. as test organism.
4. 2% Nutrient agar.
5. 2% Sabouraud agar

1. Isolation of *Streptomyces*: For the present study three soil samples collected from the villages around Barshi, Dist. Solapur M.S., India and were used for isolation of *Streptomyces*. *Streptomyces* were isolated by weighing one gram of soil sample and serially diluted up to 10^{-7} dilution. Aliquots of 0.1 ml of each dilution was streak inoculated on glycerol aspargine agar (L-aspargine- 0.1g, K_2HPO_4-0.1g, glycerol- 1g, trace salt solution- 0.1ml, agar-agar- 2.5g, distilled water-100ml pH-7.4) after incubation at an ambient temperature for 5 to7 days.

2. Identification of *Streptomyces*: Isolated organisms were identified as Streptomyces on the basis of morphological and cultural characteristics. Morphological studies were carried out by cover slip culture technique and slide culture technique (Mycelium Pattern e.g. Aerial, Submerged and Surface mycelia and Structure of spore chain). The organisms were identified on the basis of cultural characteristics grown on different growth media viz. Bennet's agar and Dextrose agar and spore chain morphology.

Identification of *Streptomyces* by Probabilistic Identification of bacteria using windows, version 2.0: Based on microscopic and cultural characteristics, the actinomycete isolates were primarily identified to genus level. Biochemical and other characters were used to identify isolates to species level. Members of Streptomyces genus were identified by using PUBWin software (Bryant, 2004). The software is modification of the DOS based software MICRO-IS (Portyrata and Krichevsky, 1992). Probabilistic identification matrices used were Streptomyces species major cluster (Williams *et al*., 1983 and 1985) and

Streptomyces species minor cluster (Langham *et al*., 1989; Kämpfer and Kroppenstedt, 1991).

3. Study of antimicrobial activity of *Streptomyces*:

a. Primary screening of Isolates: *Streptomyces* obtained were grown by streaking horizontally on the nutrient agar medium plate and incubated for 7 days at room temperature. After incubation test organisms were streaked perpendicular to that growth and then incubated at 37^0 C for 24 hours. The inhibition of growth of the test organisms were observed after incubation period. Those *Streptomyces* isolates showed broad spectrum antibacterial activities were selected for secondary screening.

b. Secondary screening: Secondary screening was carried out by agar overlay method. Those *Streptomyces* isolates having maximum inhibition against test organisms were grown on glycerol aspargine agar for 7 days at room temperature. The isolates were killed by inverting colonial growth on chloroform in lid for 30 minutes. The plates were overlaid with molten cooled 2% nutrient agar (Peptone-1g, NaCl-0.5g, Yeast extract-0.3, agar-agar- 2.5g, distilled water-100ml pH- 7) and agar Sabouraud agar (Dextrose 4g, peptone 1g, agar-agar- 2.5g, distilled water-100ml pH 5.6±0.2) of 0.2 ml standard test bacterial and fungal cultures respectively. Then plates were kept at 370 C for 24 hrs. After incubation period the zone of inhibition (in mm) and growth zone diameters were measured. (Bergey's manual).

RESULTS AND DISCUSSION:

1. Identification of *Streptomyces*: By using morphological, cultural characteristics and MICRO-IS software the five isolates were identified as Streptomyces fulvissimus, *Streptomyces lydicus, Streptomyces prasinosporus, Streptomyces roseus, Streptomyces filipinensis.*

2. Antibacterial and antifungal activity of selected *streptomyces* by agar overlay method: *Streptomyces* fulvissimus showed antimicrobial activity against all tested pathogen except K. Pneumonia and *S. typhi*. *Streptomyces* lydicus showed antimicrobial activity against all tested pathogen except *S. typhi* and *S. cerevisae*. *Streptomyces* prasinosporus showed antimicrobial activity against all tested pathogen except *S.typhi* and *C. albicans*. *Streptomyces* roseus and *Streptomyces filipinensis* showed antimicrobial activity against all tested pathogen except *C. albicans* and *Aspergillus niger* respectively.

All Streptomyces isolates were sensitive to *B. megaterium, S. aureus* and *P. chrysogenum.*

Streptomyces roseus and *Streptomyces filipinensis* were sensitive to *S. typhi* and *Streptomyces fulvissimus*, *Streptomyces lydicus Streptomyces prasinosporus* were resistant to *S. typhi*. *Streptomyces prasinosporus* and *Streptomyces roseus* were resistant to *C. albicans. Streptomyces fulvissimus, Streptomyces lydicus* and *Streptomyces filipinensis* were resistant to *C. albicans*. These results were showed in Table 1.

3. The Antibacterial and antifungal activity of selected *Streptomyces* by agar overlay method is shown in Table 1.

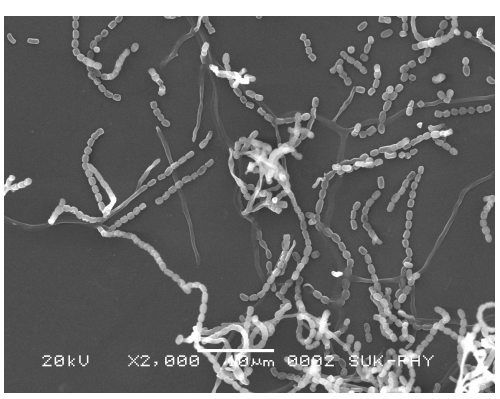
Table 1: Antibacterial and antifungal activity of selected *Streptomyces* by agar overlay method.

Streptomyces.?	Zone of inhibition Against Pathogens(mm)							
	B. megaterium	*K. pneumoniae*	*S.aureus*	*S.typhi*	*Aspergillus niger*	*S. cerevisae*	*C. albicans*	*P. chrysogenum*
Streptomyces fulvissimus	38 (10)*	R	37 (11)*	R	30 (11)*	18 (10)*	17 (12)*	31(11)*
Streptomyces lydicus	45 (10)*	20(10)*	12(10)*	R	20 (10)*	R	20(10)*	12(10)*
Streptomyces prasinosporus	30 (10)*	34 (11.5)*	50 (12)*	R	14 (13)*	29 (10)*	R	21(10)*
Streptomyces roseus	30 (10)*	30 (10)*	50 (10)*	40(10)*	25 (10)*	35 (11)*	R	12(12)*
Streptomyces filipinensis	28 (10)*	50 (10)*	25(10)*	50(11)*	R	20 (10)*	16(13)*	11(10)*

( )* Parenthesis indicates Zone of growth of actinomycetes isolates in mm.
R= Resistant to tested the pathogen
Values out of bracket indicate Zone of inhibition in mm.

Fardos M. Bokhari (2009) studied antifungal activity of some medicinal plants in Saudi Arabia viz. lemon grass (*Cymbopogon citrates* DC.) Stapf.), lantana (*Lantana camara* L.), nerium (*Nerium oleander* L.), basil (*Ocimum basilicum* L.) and olive leave (*Olea europaea* L.). These medicinal plants were extracted with either water or different organic solvent to investigate their antifungal activities *in vitro*. During the study he reported that the methanol extract of lemon grass, lanta and nerium followed by their ethyl acetate extracts showed the highest activities against *Trichophyton rubrum*. The activity of the methanolic extracts of the 5 selected plants determined against different pathogenic fungi including *Microsporum canis*, *M. gypseum*, and *T. mentagrophytes*. Extracts of lemon grass showed the most effective followed by lantana. The study supports the traditional medicine use of different plant extracts in treating different infections caused by pathogenic fungi either by using a single or combined extracts

Figure 1: Scanning Electronic photograph **Plate 1: Primary screening of *Streptomyces. of Streptomyces* spores**



Secondary screening of *Streptomyces*:

Plate 2: Antifungal activity of *Streptomyces* **Plate 3: Antibacterial activity of *Streptomyces*.**

Santhanam, and Masilamani Selvam (2011) studied antimicrobial activity of

actinomycetes isolated from the rhizosphere soil of medicinal plants collected from Southeastern Western Ghats of Tamilnadu. They reported that antagonistic strain STREP 37 was found to possess LL-DAP and glycine in their cell wall, indicates the cell wall chemo type 1 i.e. the wall property of the genus *Streptomyces* and 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).

Kamble and Kulkarni (2012) studied antibiotics susceptibility pattern of actinomycetes isolated from soil under cultivation of *Curcuma longa* L. During the study they reported that actinomycetes isolated from soil under cultivation of Curcuma longa L. were resistant to penicillin, chloramphenicol and tetracycline may have potential to produce antibiotics of this category. These actinomycetes will be used for production of this type of antibiotics which will be used to prevent human and plant bacterial and fungal diseases. These actinomycetes may have ability to degrade chemical or antibiotics and so used to control soil pollution.

Madhurama Gangwar et al. (2014) studied diversity and biopotential of endophytic actinomycetes from three medicinal plants viz. *Aloe vera*, *Mentha arvensis* and *Ocimum sanctum* in India. They reported *Streptomyces* ranked first (60% of all isolates) followed by *Micromonospora* (25%), *Actinopolyspora* (7.5%), and *Saccharopolyspora* (7.5%) spp. The highest numbers of endophytic actinomycetes were isolated from *Ocimum sanctum* (45%). Out of the nine, eight endophytic actinomycetes (88.9%) showed a significant antagonistic activity against one or more phytopathogenic fungi which showed possible role as plant biocontrol agents.

Comparative to these studies we have found that *Streptomyces roseus* and *Streptomyces filipinensis* showed broad spectrum antimicrobial activity against tested pathogens. *Streptomyces roseus* showed antimicrobial activity against all tested pathogens except *C. albicans*. *Streptomyces filipinensis* showed antimicrobial activity against all tested pathogens except Aspergillus niger. They were further studied for production of antifungal and antibacterial substance to cure human and plant bacterial, fungal diseases.

CONCLUSION:

From the result it is concluded that *Streptomyces* sensitive to tested bacteria and fungus showed antibacterial and antifungal activity and they will be studied further for the production of this type of antimicrobial substance because they showed potential for the antimicrobial activity. These antimicrobial substances will be used to prevent human and plant bacterial and fungal diseases.

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