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# Evaluation of Antimicrobial Activity and Characterization of Soil Actinomycetes from Social Forest Area of Bhilai Township, Chhattisgarh

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**A total of 35 different actinomycetes strains were isolated from soil samples of social forest area of Bhilai Township. They were then evaluated for antimicrobial activity against three fungal (*Aspergillus niger, Fusarium* sp. and *Penicillium* sp.) and three bacterial strains (*E.coli, Bacillus subtilis,* and *B.cereus*). 23 isolates were found to be active against at least one of the microorganism tested by showing *in vitro* anti microbial activity. 09 isolates were selected as highly active antagonists for further characterization on the basis of their Total Inhibition properties. Tentative identification was done on the basis of growth on different agar medium, colour of aerial mycelium, morphology of spore mass, presence or absence of diffusible pigment and melanin, growth on different temperatures, utilization of different carbon and nitrogen sources and production of extracellular enzymes. Results were also compared with probabilistic identification method.**

**Key words:** Actinomycetes, Social Forest, Antimicrobial activity, Characterization.

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil. Actinomycetes are gram-positive bacteria showing a filamentous growth like fungi. They are aerobic and provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. These searches have been remarkably successful and approximately two thirds of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes. According to the World Health Organization, over-prescription and the improper use of antibiotics has led to the generation of antibiotic resistance in many bacterial pathogens. Nowadays, the drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics. Microbial screening programs have started taking into account the ecological significance of antibiotic producing microorganisms[1]. Because of this, many scientists have actively involved in isolation and screening of actinomycetes from different untouched habitats, for production of antibiotics[2-7]. Serious infections caused by pathogenic microorganisms have become resistant to commonly used antibiotics and become a major global healthcare problem in the 21st century. The resistance problem demands to discover new antimicrobial agents effective against pathogens resistant to current antibiotics. So we need to screen more and more actinomycetes from different habitats for antimicrobial activity in hope of getting

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some actinomycetes strains for production of antibiotics that have not been discovered yet and active against drug resistant pathogens[8].

The present study was undertaken to isolate actinomycetes from the soil samples of social forest area of Industrial Township Bhilai, Dist. Durg, (C.G.), to screen their anti-microbial potential and to characterize their properties.

## MATERIALS AND METHODS

### Sampling and isolation

Soil samples were collected from different social forest areas of Bhilai Township. Each collection was made from 10-15 cm depth of the soil. All the soil samples were air dried for 1 week, crushed and sieved prior isolation [9]. This helps in decreasing the population of gram negative bacteria. 1.0 g of the soil sample was taken and mixed with 100 ml of sterile distilled water. The soil suspension was shaken vigorously under room temperature (25 ± 2$^0$C) on an orbital shaker at 200 rpm for 1 hour. 200 µl of the soil suspension were inoculated over soil extract agar plates (500g fresh soil cooked with 1500 ml of distilled water, filtered and purified by adding $CaCO_3$, again filtered and used as soil extract added with glucose 1.0g, $K_2HPO_4$ 0.5g and Agar 15g makeup to 1000ml by DW). All the plates were incubated at 27±2$^0$C for 1 - 2 weeks. Isolated colonies were sub-cultured over Starch Casein Agar (Soluble Starch, 10.0 g; Casein hydrolysate, 0.3 g; $KNO_3$, 2.0 g NaCl, 2.0 g; $K_2HPO_4$, 2.0 g; $MgSO_4.7H_2O$, 0.05 g; $CaCO_3$, 0.02 g; $FeSO_4.7H_2O$, 0.01 g; Agar, 18.0 g; DW, 1000 ml; cycloheximide, 100 µg/ml) at pH 7. The pure cultures were also grown on Yeast Malt Agar (Yeast Extract, 1.2g, Malt Extract, 3.0g, Glucose, 1.0g, Agar, 6.0g and DW, 300ml) to study the cultural characteristics. Pure cultures were maintained on Starch Casein slants at 4$^0$C for further use.

### *In vitro* screening for anti-microbial activity

Morphologically distinct actinomycetes isolates were selected for anti-microbial activity screening against the pathogenic test organisms by right angle streak method on agar medium[10]. Mueller Hinton agar plates were prepared and inoculated with actinomycetes cultures by a single streak of inoculum on the petri dish and incubated at 27$^0$C for 4 days. Later, the plates were seeded with test organisms by a single streak at a 90$^0$ angle to actinomycetes strains. Antagonism was measured in terms of Total Inhibition (TI), Growth Inhibition (GI), Growth retardation (GR) and No Inhibition (NI) of test organisms.

### Study of cultural characteristics and Taxonomic grouping of active actinomycete isolates

*Actinomycete* colonies were characterized morphologically and physiologically following the directions given by the International *Streptomyces* project (ISP)[11] and Bergey's Manual of Systematic Bacteriology[12]. Cultural characteristics of pure isolates in various media were recorded after incubation for 7 to 14 days at 27$^0$C. Morphological observersations were made with a light microscope under oil immersion. Active purified isolates of actinomycetes were identified up to the species level by comparing their morphology of spore bearing hyphae with entire spore chain and structure of spore chain. This was done by using cover-slip method [13]. Individual cultures were transferred to the base of cover slips buried in medium for photomicrographs. Colors of spores were visually estimated by using a Stamp Color Key. Carbon utilization was determined on plates containing ISP basal medium 9 to which sterilized carbon sources were added to a final concentration of 1.0%. The plates were incubated at 27$^0$C and growth was recorded after 15 days. The ability to utilize nitrogen sources was determined by adding amino acid sources to the basal medium. Results were determined after 15 days. Antibiotic susceptibility was determined with susceptibility disks (diameter, 4 mm). The disks were placed on the surface of Mueller Hinton agar medium plates seeded with 14 day broth cultures. Inhibition zones observed after 2 to 5 day incubation at 27$^0$C were scored positive. Other physiological and biochemical characteristics were determined by the method described by Shirling and Gottlieb[11]. All tests were performed at 27$^0$C except growth at 45$^0$C. Two replicates were maintained throughout the study.

### Test organisms

The test organisms used in present study were indigenously isolated three fungal strains *Aspergillus niger, Fusarium sp.* and *Penicillium sp.* and three bacterial strains, gram positive *Bacillus subtilis*, *B.* cereus and Gram negative *Escherichia coli*.

## RESULTS AND DISCUSSION

Out of the total 35 different actinomycete isolates recovered from soil samples of social forest area of Bhilai Township, 23 were found to be active against at least one of the microorganism tested by showing *in vitro* anti microbial activity (Table 1). However, the intensity of inhibition was varied as Total Inhibition, Growth Inhibition and Growth Retardation. The isolates A3, A13 and A17 were found to inhibit maximum[04] microbes tested (Fig.1). Similarly, *Aspergillus niger* was maximally inhibited[17] followed by *Bacillus subtilis*[10] among all the microorganisms tested (Fig.2). Isolate A3, A4a, A5, A6c, A8, A15a, A16, A23 and A25b were selected as highly active antagonists for further characterization.

The morphological, physiological and biochemical characteristics of highly active isolates are summarised in Table 2. All isolates grew on a range of agar media showing morphology typical of Streptomycetes characteristics. The colour of the substrate mycelium and aerial spore mass was varied. Some isolates (04) produced diffusible pigments on agar media. Melanin was produced by isolate A3, A4a, A8 and A25b on Starch Casein and YMA media. The isolates A3, A4, A6c, A8, A15a and A16 produced long rectiflexible spores chains while isolate A5, A23 and A25b found to produce spiral spore chains. Verticils were not detected. Morphological examination clearly indicates that all the isolates belong to *Streptomyces* genera *Streptomycetaceae family*[12, 13].

Further comparison of physiological and biochemical characteristics among the isolates and with the help of probabilistic identification method[14]. It was proposed to designate the isolates as A3 - *Streptomyces aureofaciens*, A4a - *Streptomyces californicus* , A5 - *Streptomyces rochei*, A6c - *Streptomyces luridus*, A8 -

**Table 1.** Anti-microbial activity of active isolates by cross streak method

| S. No. | Isolates | Anti-micobial Activity | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Aspergillus niger* | *Fusarium* sp. | *Penicillium* sp. | *Bacillus subtilis* | *Bacillus cereus* | *E. coli* |
| 1 | A2 | + | - | - | + | - | - |
| 2 | A3 | ++ | ++ | +++ | - | ++ | - |
| 3 | A4a | +++ | - | - | + | - | - |
| 4 | A5 | + | - | +++ | - | - | - |
| 5 | A6c | + | - | +++ | - | - | - |
| 6 | A7 | - | - | - | - | - | ++ |
| 7 | A8 | + | - | - | +++ | + | - |
| 8 | A9b | - | + | + | ++ | - | - |
| 9 | A10 | + | - | - | ++ | + | - |
| 10 | A11 | - | - | - | - | - | + |
| 11 | A13 | ++ | ++ | ++ | - | ++ | - |
| 12 | A14 | ++ | - | + | - | - | - |
| 13 | A15a | - | - | - | +++ | - | - |
| 14 | A16 | +++ | - | - | ++ | - | - |
| 15 | A17 | + | - | ++ | + | - | + |
| 16 | A18 | ++ | - | - | - | ++ | - |
| 17 | A19a | ++ | - | - | - | - | - |
| 18 | A20b | ++ | ++ | - | - | - | - |
| 19 | A21 | + | - | + | + | - | - |
| 20 | A22a | - | - | - | - | - | + |
| 21 | A23 | +++ | ++ | + | - | - | - |
| 22 | A24b | - | - | - | - | - | ++ |
| 23 | A25b | +++ | - | - | ++ | - | - |

+++ Total inhibition*; ++ Growth inhibition; + Growth retardation; - No inhibition * Highly active isolates

**Table 2.** Morphological, Physiological and biochemical characteristics of highly active isolates

| Characteristics | Isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A3 | A4a | A5 | A6c | A8 | A15a | A16 | A23 | A25b |
| Spore chain | | | | | | | | | |
| Rectiflexible | + | + | - | + | + | + | + | - | - |
| Spiral | - | - | + | - | - | - | - | + | + |
| Verticillat | - | - | - | - | - | - | - | - | - |
| Spore mass colour | Grey | Peach | Grey | Grey | Peach | White | Grey | Grey | Grey |
| Diffusable pigment | - | + Brown | - | - | - | - | + Pink | + Brown | + Yellow |
| Melanin production | | | | | | | | | |
| Starch casein agar | + | + | - | - | + | - | - | - | + |
| Yeast malt agar | + | + | - | - | + | - | - | - | + |
| Biochemical tests | | | | | | | | | |
| Amylase production | + | + | - | - | + | - | - | - | + |
| Lipase production | + | - | - | + | - | + | + | - | + |
| Nitrate reduction | + | + | - | + | + | - | + | + | - |
| Casein hydrolysis | + | - | + | - | - | - | - | - | + |
| $H_2S$ production | - | - | + | - | + | - | - | + | + |
| Urease production | - | - | - | - | - | - | - | - | - |
| Resistance to | | | | | | | | | |
| Kanamycin | - | + | + | + | - | - | + | - | + |
| Streptomycin | - | + | + | - | + | + | - | + | + |
| Ampicillin | + | + | + | + | + | + | - | + | + |
| Bacitracin | - | - | - | - | - | - | + | - | - |
| Chloramphenicol | - | + | - | + | - | + | - | + | + |
| Tetracyclin | - | + | + | + | + | - | - | - | + |
| Penicillin | + | + | + | + | + | - | - | - | + |
| Growth at 45°C | - | - | - | - | - | - | - | - | - |
| Carbon source | | | | | | | | | |
| Glucose | + | + | + | + | + | + | + | + | + |
| Sucrose | + | - | - | + | - | - | - | - | + |
| Lactose | - | + | + | - | + | - | - | + | - |
| Xylose | + | + | - | + | - | + | - | + | + |
| Maltose | - | + | - | - | - | - | - | + | - |
| Nitrogen source | | | | | | | | | |
| D-Phenylalanin | + | + | - | + | - | - | + | - | + |
| D-Methionin | + | + | + | + | + | + | + | - | + |
| L-Cystine | + | - | + | + | + | + | + | + | + |
| L-Lysine | + | - | - | - | + | - | + | + | - |
| L-Valine | + | + | - | + | - | - | + | + | - |

**Table 3.** Probable Identification of Active isolates by PIB Win software

| Code | Probable Identification | ID Score |
|---|---|---|
| A3 | *Streptomyces aureofaciens* | 0.90 |
| A4a | *Streptomyces californicus* | 0.83 |
| A5 | *Streptomyces rochei* | 0.99 |
| A6c | *Streptomyces luridus* | 0.69 |
| A8 | *Streptomyces xanthochromogenes* | 0.89 |
| A15a | *Streptomyces rimosus* | 0.98 |
| A16 | *Streptomyces exfoliates* | 0.99 |
| A23 | *Streptomyces graminofaciens* | 0.54 |
| A25b | *Streptomyces cyaneus* | 0.99 |

*Streptomyces xanthochromogenes,* A15a - *Streptomyces rimosus*, A16 - *Streptomyces exfoliates*, A23 - *Streptomyces graminofaciens* and A25 - *Streptomyces cyaneus* (Table 3). Sivakumar *et al*.[15], reported that the morphological and biochemical characters can be used as marker by which an individual strain can be recognized. Particularly, chemotaxonomy plays an important role in identification of actinomycetes to generic level. Baskaran *et al.*[16], also concluded that the physiological characteristics of actinomycetes varied depending on the available nutrients in the medium and the physical conditions.

Studies on diversity of actinomycetes desires regular visits to the sampling stations, isolation from different substrates collected from the habitat and the usage of different culture media. Such attempts need to be continued both in the same area as well as from the adjoining places during various climatic conditions as to screen more isolates for novel therapeutics. The search for novel metabolites especially from actinomycetes requires a large number of isolates of pharmaceutical interest. The search will be more promising if diverse actinomycetes are sampled and screened. This is based on the hypothesis that actinomycetes diversity may be influenced by the diversity of cultivated plant species as these bacteria grow profusely in the humus and leaf litter layer. Different plants produce different type of secondary metabolites and some of these chemical compounds are toxic to soil microorganisms [17]. Effective antimicrobial properties of these isolated can also be exploited. Kavitha *et al*.[18], reported

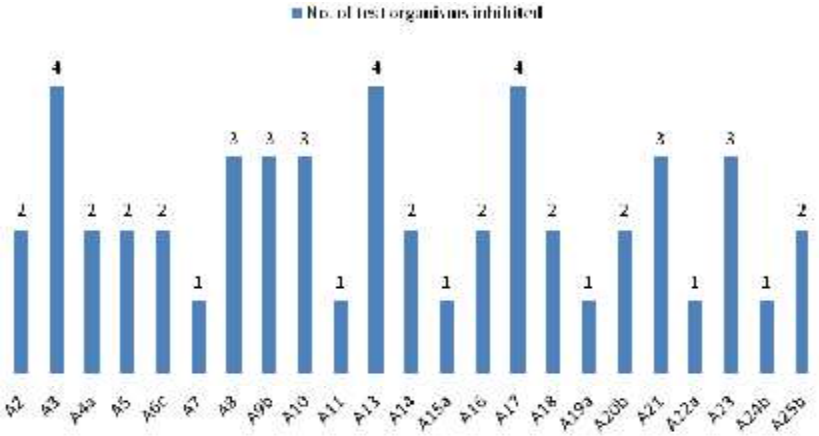


**Fig. 1.** Screening of Actinomycetes isolates for antimicrobial activity

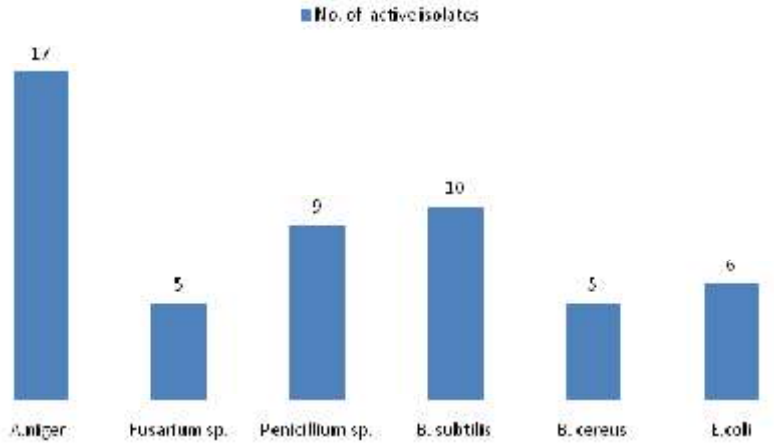


**Fig. 2.** Screening of Test organisms for inhibition by Actinomycetes isolates

their 4 strains as the most promising collection of pharmacologically new antifungal compound producers. Researchers suggested the use of their isolates for decontamination of mold-infested samples in soil isolation studies.

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