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# SCREENING OF ACTINOMYCETES FOR POTENTIAL ANTIMICROBIAL ACTIVITY

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**ABSTRACT:**

This study was directed towards screening of soil samples from various crop fields of Beed District of Maharashtra as a source of actinomycetes with potent antimicrobial compounds. In all 04 soil samples 42 actinomycetes were isolated. In primary screening 18 isolates showed antibacterial activity against test organisms. To confirm antibacterial potential all isolates were subjected for secondary screening by agar well method. Out of 18 actinomycetes only 04 actinomycetal isolates showed maximum zone of inhibition against both Gram positive and Gram negative organisms. One actinomycetal isolate was selected on the basis of highest antibacterial activity and was identified as belonging to genus *Streptomyces*. This isolate was subjected to studies to find the effect of carbon and nitrogen sources on antibiotic production. The study established that maltose and potassium nitrate were the most suitable carbon and nitrogen sources for antibiotic production.

**Key Words**: Actinomycetes, Soil, Antibacterial Production, *Streptomyces*

## INTRODUCTION:

Actinomycetes are an extensive and diverse group of Gram positive, aerobic, mycelial bacteria with high G+C nucleotide content (> 55 %) playing an important ecological role in the soil cycle. The name of the group actinomycetes is derived from the first described anaerobic species *Actinomycetes bovis* that causes 'actinomycosis" the "ray-fungus disease" of cattle. They were originally considered to be intermediate group between bacteria and fungi but are now recognized as prokaryotic microorganisms. [1]

Most of actinomycetes are free living, saprophytic bacteria, widely distributed in soil and water. Actinomycets have been identified a major group of soil population. Actinomycetes have ability to form wide variety of secondary metabolites including antibiotic. Actinomycetes hold great economical and biotechnological value among prokaryotes. They are responsible for the production of majority of the discovered bioactive secondary metabolites.[2] Rare and

novel actinomycetal taxa have become a major focus in the search for pharmaceutical agents.[3]About 61% of all bioactive microbial metabolites have been isolated from actinomycetes especially from *Streptomyces*.[4]. Thousands of antibiotics are known today and most of them are produced by actinomycetes especially the genus *Streptomyces.* [5].

The actinomycetes have occupied a prominent position in the pharmaceutical industry for their seemingly unlimited capacity to produce secondary metabolites including antibiotics with divers chemical structure and biological activities. [6] *Streptomyces* is the largest antibiotic producing genus in the microbial world discovered so far. The number of antibiotic compounds reported from the species of the genus per year increased almost exponentially for about two decades. [7]

This study focuses on isolation of actinomycetes from farm soil samples of Beed district of Maharashtra, evaluation of their antibacterial activity against test microorganisms and identification of promising isolate and selection of suitable carbon and nitrogen source for antibiotic production.

## MATERIALS AND METHODS:

The farm soil samples were collected in freshly purchased polythene bags (swabbed with cotton dipped in 70% alcohol) were brought to the laboratories preventing any contamination on the way. They were stored at temperature between $6^0$C to $10^0$C until further use.

### Isolation of actinomycetes:

**Enrichment:** Dilutions of soil samples in sterile water (1/10 w/v) were made. A temperature shock $70^0$C for five minutes were given to each diluted soil samples and 5ml of soil sample were inoculated in 250 ml conical flask containing 50 ml of enrichment medium ( Starch-2.0g, Yeast extract-0.8g,Peptone-0.4g, Distilled water-1L,pH-7.2). The medium was supplemented with antifungal antibiotic griseofulvin at 50 µg/ml concentration.[8] The temperature shock depress associated gram negative bacteria and added antibiotic in medium kill fungi which create problem during isolation. The flasks were incubated at $30^o$C for 10 days. [9]

**Isolation:** The enriched cultures were isolated by streak plate method using starch nitrate agar (Starch-20.0g, KNO3-1.0g, $K_2HPO_4$-0.5g, $MgSO_4.7H_2O$-0.5g, Nacl-0.5g, $FeSO_4.7H_2O$-0.01g,Agar-20.0g,Distilled water-1L, pH 7.2). The medium was supplemented with antifungal antibiotic griseofulvin at 50µg/ml concentration. Then these plates were incubated at $30^0$C for 10 days.

**Study of characteristics of isolates:** After incubation dry, leathery colonies of actinomycetes were studied. Color of aerial mycelium, color of vegetative mycelium was studied using ‘‘color and Streptomyces ‘and ISCC-NBS color charts. [10, 11] The cover slip cultures of actinomycetal isolates were prepared and morphological characters were studied. [12] Pure colonies were subcultured on to the respective media slants and were stored at $4^0$C for further study.

**Primary screening of actinomycetes for antimicrobial activity:** Antagonistic activity of 49 isolates was tested by using the cross streak method .[13, 14, 15, 16] The test organisms used were *Bacillus subtilis* NCIM 2195, *Staphylococcus aureus* NCIM 2602, *Proteus vulgaris* NCIM2027,

*Eschrishia coli* NCIM2685, *Pseudomonas aeruginosa* NCIM 2945.Those actinomycetes which showed prominent activity were selected for secondary screening.

**Secondary screening:** In primary screening those isolates showed prominent antibacterial activity were used for secondary screening. Sterile 5 ml of starch nitrate broth were taken in 25 ml of conical flasks. 2.5% inoculums of actinomycetes were added in the broth aseptically. The flasks were incubated on shaker $30^0$C for 10 days. After incubation supernatant was obtained by aseptic centrifugation 4000 rpm for 20 minutes, a part of which was used for testing antimicrobial activity by agar well method against test organisms.[17,18] One actinomycetal isolate showing highest antibacterial activity was selected on the basis of zone of inhibition against test organisms and was subjected to characterization following standard procedures of Shirling and Gottlieb1966 ; Holt1974.[19,20] The morphological, cultural and biochemical characters were studied . Morphology of actinomycetal isolate was further studied by scanning electron microscopy (SEM). [21,22] Cultural characters were studied on ISP4 medium. Utilization of different sugars and biochemical test were performed according to Bergey's Manual of Determinative Bacteriology.

**Effect of carbon and nitrogen source on antibiotic production:** To study the effect of carbon source on antibiotic production starch, glucose, glycerol, sucrose, maltose, xylose, lactose and arabinose sugars were used. The carbon utilization medium Shirling and Gottlieb was prepared10$gl^{-1}$ concentration of carbon source ( $(NH4)_2SO_4$ -2.64g , $KH_2PO_4$ - 2.38g $K_2HPO_4$-5.65g , $MgSO_4.7H_2O$- 1.0g ,Trace salt solution – 1ml, Carbon source -10g , Distilled water-1L, pH 7.2).100 ml of each sugar medium was taken in 250 ml capacity Erlenmeyer flask. The broth was sterilize and inoculated with 2.5% inoculums of actinomycetes. All flasks were incubated on rotary shaker $30^0$C for 10 days. After incubation supernatant was obtained by aseptic centrifugation 4000 rpm for 20 minutes, a part of which was used for testing antimicrobial activity by agar well method against test organisms (*P. vulgaris S aureus, P. aeruginosa* ). Antibiotic production was checked by measuring zone of inhibition against test organisms.

To study the effect of single nitrogen source on antibiotic production different nitrogen sources viz. Ammonium sulphates, ammonium chloride, KNO3, NaNO3, aspargine, casein, soybean meal, yeast extract were used. The above mentioned experiment was repeated except that1% maltose was used as carbon source and 2% of nitrogen source was used.

## RESULTS AND DISCUSSION:

Initially 42 actinomycetes were isolated from 6 farming soil samples. Out of these 18 isolates showed prominent antibacterial activity against test organisms in primary screening by using cross streak method. To confirm antimicrobial activity of 18 isolates giving inhibition of test organisms in primary screening were again subjected to secondary screening by agar well method. It was observed that 04 actinomycetal isolates (S1,S4,S9,S13) were showing prominent antimicrobial activity in both primary and secondary screening

methods against Gram positive and Gram negative organisms (Table-1).Finally one potent actinomycetal isolate S9 was selected for further study on the basis of maximum zone of inhibition .

The morphological, cultural, physiological and biochemical properties of the selected potent actinomycetal isolate S8 was studied. 4-8 mm diameter, circular, rough, convex, opaque, leathery, white colonies of actinomycetal isolate S8 were observed on ISP4 medium (Table-2). It was found that vegetative and aerial mycelium was present in actinomycetal isolate and long and spiral chains of spores on aerial mycelium were observed in cover slip culture and SEM images. Spores were circular and having smooth surface. Actinomycetal isolate S8 was confirmed as *Streptomyces* species on the basis of morphology by SEM analysis. Many researchers used SEM for identification of actinomycetes.

**Table -1 Antimicrobial activity of potent soil actinomycetes against test organisms**

| | | Zone of inhibition ( mm)against test organisms | | | | |
|---|---|---|---|---|---|---|
| Sr. No | Isolates | *Bs* | *Sa* | *Pv* | *Ec* | *pa* |
| 1 | S1 | 17 | 18 | 14 | 13 | 17 |
| 2 | S4 | 16 | 16 | 16 | 13 | 16 |
| 3 | S9 | 25 | 18 | 20 | 20 | 25 |
| 4 | S13 | 18 | 17 | 19 | 15 | 21 |

*Bs= Bacillus subtilis*, Sa=*Staphylococcus aureus*
*Pv= Proteus vulgaris* , Ec=*Escherichia coli*
Pa= *Pseudomonas aeruginosa*

The ability of actinomycetal isolate S8 to utilize different sugars was tested .It was able to utilize D-glucose, maltose, xylose, rhamnose, arabinose, sucrose, mannitol, lactose. Actinomycetal isolate BD8 was able to produce catalase, oxidase, gelatinase, caseinase, cellulase lecithinase and amylase enzymes .Methyl red and $H_2S$ production positive (Table- 3). Thus it was found that actinomycetal isolate S8 is biochemically versatile and it has good potential in biodegradation of varieties of organic compounds in soil. On the basis of morphological, cultural, physiological and biochemical characters the actinomycetal isolate S8 was identified as species belonging to the gnus *Streptomyces spp.*

| **Sr. No** | **Cultural characters** | ***Streptomyces* BD8** |
|---|---|---|
| 1 | Colony morphology | 4-8 mm.diameter,circular ,rough, convex, opaque, velvety, white to light brown |
| 2 | Aerial mycelium color | White |
| 3 | Vegetative mycelium color | Yellow |
| 4 | Diffusible pigment | None |
| 5 | Nature of sporulating aerial mycelium & spore | Long & spiral chains of spores on aerial mycelium. Spores are circular with smooth surface |

**Table -2. Cultural Characteristics of actinomycetes isolate *Streptomyces* BD8**

Li Hua *et al*. (1996) studied 4200 soil samples from Yunnan. He observed that the genus *Streptomyces* appears to be the most important in ecological function it represents up to 90% of all soil actinomycetes in Yunan. [22,23]. Oskay *et al*.(2004) isolated 80 different actinomycetes strain from farming soil

samples and studied antibacterial activity against phytopathogenic bacteria *Agrobacterium tumefaciens, Erwnia amylovora* and *Psedomonas viridiflora.*

Effect of carbon source on antibiotic production in a *Streptomyces* S8 was studied it was observed that when medium containing maltose as carbon source, *Streptomyces* S8 was giving maximum zone of inhibition i.e. 20, 25, 18 mm against, *P. vulgaris, S. aureus, P . aeruginosa* respectively .Thus maltose was found best carbon source by *Streptomyces* S8 as compare to starch, glucose, glycerol, sucrose, xylose, lactose and arabinose.

**Table 3a Biochemical Characters of actinomycetal isolate *Streptomysces- BD8***

| Sr .No. | | Results |
|---|---|---|
| 1 | Indol | -ve |
| 2 | Methyl Red | +ve |
| 3 | V P | --ve |
| 4 | Citrate | --ve |
| 5 | $NO_2$ Reduction | --ve |
| 6 | $H_2S$ Production | +ve |
| 7 | Catalase | +ve |
| 8 | Oxidase | +ve |
| 9 | Gelatinase | +ve |
| 10 | Caseinase | +ve |
| 11 | Cellulase | +ve |
| 12 | Lcithinase | +v |
| 13 | Amylase | +ve |

**+++ - Very good, ++ Moderate , + Poor , - No growth**
**+ = Positive Test , - = Negative Test**

Narayana *et al*. (2001) stated that *Streptomyces albiaqflavus* was giving maximum antibiotic production in presence of maltose as carbon source. [24]

When inorganic carbon source $KNO_3$ was used as nitrogen source in medium the maximum zone of inhibition developed by antibiotic compound produced by *Streptomyces* S9 i.e. 18, 19, 16 mm again *P. vulgaris, S. aureus, p. aeruginosa* respectively. Thus inorganic $KNO_3$ was found the best nitrogen source for antibiotic production in *Streptomyces* S9.

**Table 3b. Physiological Characters of actinomycetal isolate *Streptomysces- BD8***

| Sr. No. | Sugar | Result |
|---|---|---|
| 1 | Maltose | +++ |
| 2 | Xylose | ++ |
| 3 | Glucose | +++ |
| 4 | Raffinose | ---- |
| 5 | Rhamnose | + |
| 6 | Arabinose | ++ |
| 7 | Sucrose | ++ |
| 8 | Mannitol | ++ |
| 9 | Lactose | ++ |

**+++ - Very good, ++ Moderate , + Poor , - No growth**
**+ = Positive Test , - = Negative Test**

Bulchandani and Parvateesam (2007) have reported that ammonium nitrate was most suitable for antibiotic production by *Streptomyces*. [25] Aruna *et al*. studied optimum condition required for antibiotic production in *Streptomyces spp.* They reported that yeast extract and $KNO_3$ was best for antibiotic production. [26] Vorar laid Rabah *et al.* (2007) found a novel actinomycetes strain designated RAF 10 isolated form Egyptian soil. It was active against Gram positive and Gram negative bacteria, yeast and filamentous fungi. [27]

## CONCLUSION

Based on the screening results, it has been shows that farming soil samples of Beed, Maharashtra possess antibiotic producing actinomycetes and may be tapped as one of the potential source of novel antibiotics.

**REFRENCES**:

**[1]** Kuster HJ,(1968) Ubr die Bulding Von Huminstoffeen durch Streptomyceten. *Land Wirtsch Forsch* 21: 48-61.

**[2]** Berdy ,J (1974) Resent development of antibiotic research and classification of antibiotic according to chemical structure. *Adv,App.Microbiol*.14: 390-406.

**[3]** Tracy,j,Mincer, Paul,R.Jencen Christopher,A. Kauffman,W. Fenical (2002)Wide spread and persistant population of a major new marine actinomycetes. *Apl. Environ Microbiol* 68:505-511.

**[4]** Goodfellow M. and S. T. Williams . 1983. Ecology of actinomycetes . *Annu Rev. Microbiol*. 37: 189- 216.

**[5]** Edward,D.I(1980) History of chemotherapy.In: Antimicrobial drug action . Mac Millan press Ltd . 1-9

**[6 ]** Waksman S. A.and Lechevalier (1961). The actinomycetes Vol II Classification ,identification and description of genera and species. The Williams and Wilkins Company ,Baltimore USA.331

**[ 7 ]** Watve M.G., Tickoo R., Jog M.N.and Bhole B.D. (2001). How many antibiotics are produced by the genus *Streptomyces ? Arch Microbial* 176. (5): 386-390.

**[8]** Navine B. Ghanem, Soraya A., Sobry, Zeinab M.El.Sherif , and Gehan A., Abu El-El (2000) Isolation and enumeration of marine actinomycetes from sea water and sediments in Alexandria *J. Gen.Appl. Microbiy*. 46 : 105-111.

**[ 9]** Ratnakala R. and Chandrika, V. (1993.) Effect of different media for isolation , growth and maintenance of actinomycetes from mangrove sediments. *Indian J. Mar Sci*. 22;297-299

**[10]** Pridham Thomas (1965). Color of *Streptomycetes*. Report of an international workshop on determination of color of *Streptormycetes Appl Microbiol*.13 (1) : 43-61.

**[11]** Kenneth ,L. K. and B. J. Deane .(1955) Color universal language and dictionary of names.United states department of commerce. National Bureau of standards. Washington, D. C. 20234.

**[12]** Kawato, N. and R. Shinobu (1959). On *Streptomyces herbaricolor, nov SP* supplement : a simple technique for the microscopic observation. Mem. Saka Univ. Lib. Arts Educ. *J.Nat. Sci* 8 : 114-119.

**[13]** Williams and Cross, (1971). Actinomycetes In : J.R. Norris; D.W. Robbins (eds) Methods in Microbiology, Vol4. London, 295-334, Academic press,, New York.

**[14]** Lakshmanaperumalsamy, P.(1978) Studies on actinomycetes with special reterance to antagonistic *Streptomyces* from sediments of Porto Novo Coastal Zone, Ph.D. Thesis, Annamalai University India. 192 pp.

**[15]** Pande, B., Ghimire , P. andV.P. Agrwal. (2003) Studies on the antibacterial activity of the actinomycetes isolated from the Khumbu region of Nepal. *The Journal of Pharmaceutical Sciences.* 1 – 6.

**[16]** Oskay, M., Tamer A.U.and Azeri , C.( 2004) Antibacterial activity of some actinomycetes isolate from farming soils of Turkey. *Afr. J. Biotech* 3 (9): 441-446.

**[17]** Williams and Cross (1971) Actinomycetes In : J.R. Norris; D.W. Robbins (eds) Methods in Microbiology, Vol4. London, 295-334, Academic press New York.

**[18]** Mohammed A. I. & Gupta S.G. (2011) "Studies on Enzymatic Characteristics of select Cultures for Preparation of Beneficial

Microbial Consortium for Composting of Municipal Solid Waste" Adv. In Bioresearch 2 (1):166-173.

**[19]** Shirling E.B. and D. Gottlieb (1966). Methods for characterization of *Streptomyces species. Int. J Syst. Bacteriol.* 16 : 313- 340.

**[20]** Holt J. G( 1994) Bergey's Mannual of Determinative Bacteriology 9th edition (Willian & Wilkin Baltimore). PP 667-669.

**[21]** Chi Nam Seong , Ji Heok Choi and Keunshik Baik .2001. An improved selective isolation of rare actinomycetes from forest soil *J.microbiol* 39. (1): 17-23.

**[22]** Rong-yangwu, Ming-shi Shiao and How-ming Lee,1983. Studies on the *Streptomyces* Sc4*:* chemical formulation of antibiotic Sc4-x. *Bot.Bull.Aca.Sin* 24:71-87

**[23]** Li-Huo xu, Qi-Ren Li, and cheng-Lin, Jiang (1996) Diversity of soil actinomycetes in Yunan.China.*Appl.and Environmental Microbi.* 62(1) :244-248.

**[24]** Narayana K.J.P.and M.Vijayalakshimi. (2008) Optimization of antimicrobial metabolites production by *Streptomyces albidoflavus. Res. J. Pharmacol.* 2 (1) : 4-7.

**[25]** Bulchadani B. and Parvateesam (2007) Antibiotic producing by *Streptomyces Spp.* and influence of various C and N Sources on its production . *Asian Jr.* of *Microbiol. Biotech. Enc. Sc.* 9 (1): 123-128.

**[26]** Aruna, S. K. Vijayalakshmi, M. Shashikanth, N.D. Prasanna S.K. Shasheen and K.P. Jyotsna (2009) Optimization of antibiotic compound produced *Streptomyces* Sp isolated from earthworm gut ( Eisenia foetida ). *Asian Jr. of Microbiol. Biotech Env. Sc.* 11 (1): 77 – 82.

**[27]** Vorar Laid Rabah , Ali Elshafei , Mahamoud Sqker , Bengra Cheikh and Macerie Hocine (2007) Screening isolation and characterization of novel antimicrobial producing actinomycetes, strain RAF 10. *Biotechnology* 6 (4):489-496.