Research Article

International Journal of Pharmaceutical Biological and Chemical Sciences

e-ISSN: 2278-5191

ANTIMICROGRAM OF ACTINOMYCETES FROM SALINE SOIL OF VIDARBHA REGION

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ABSTRACT:

Total 147 actinomycetes strains were isolated from saline belt of Purna river basin which appears in Akola, Amravati and Buldhana district of Vidarbha region. In primary screening, out of 147 actinomycetes 87 isolates (59.18 %) showed an activity against 2 test bacteria such as Staphylococcus aureus and Escherichia coli by agar overlay technique. In secondary screening, out of 87 primary isolates 19 actinomycete isolates were proceeded for an antibacterial activity against Staphylococcus aureus (MTCC 7443), Bacillus subtilis (MTCC 441), Streptococcus pyogenes (MTCC 442), Escherichia coli (MTCC 443), Pseudomonas aeruginosa (MTCC 424), Proteus vulgaris (MTCC 426), Enterobacter aerogenes (MTCC 7325), Salmonella typhi (MTCC 98), Klebsiella pneumoniae (MTCC 7407) by agar well diffusion method. Nearly 78.94% isolates recorded antibacterial activity towards S. aureus followed by 68.42% isolates to Bacillus subtilis, 63.15% for Streptococcus pyogenes, 52.63% to Escherichia coli and Proteus vulgaris, 47.36% towards Klebsiella pneumoniae, 42.10% for Pseudomonas aeruginosa and Salmonella typhi and 36.84% for Enterobacter aerogenes. Promising actinomycetes isolate (N8) showed antibacterial activity against all the test bacteria and was selected for morphological, cultural, physiological and biochemical characterization. It was found that biochemically N8 was very active actinomycetes and was able to produce variety of enzymes and utilize number of sugars.

KEYWORDS: *Actinomycetes, Antimicrogram, Saline belt, Vidarbha region.*

INTRODUCTION

The actinomycetes are important in the field of pharmaceutical industries as well as in agriculture. Antibiotics are the best known products of actinomycete. For their virtual success against pathogenic microorganisms antibiotics can be truly referred as the ‘wonder drugs’ [1]. This remarkable group of compounds forms a heterogeneous assemblage of biologically active molecules with different modes of action and structures. As a result, they are effective treatments for bacterial infections. Prior to the discovery of antibiotics, people with simple wounds and infectious diseases could not be treated.

Actinomycetes were predominating in black saline soils than other type like alluvial, lateric and coastal saline [2]. Actinomycetes have more ability to bear not only at high salt concentration but also at high pH than bacteria and fungi. In uncultivated saline soil high population of actinomycetes was observed whatever may be the degree of salinity of soil [3]. In salt affected soil, the population of actinomycetes is higher at pH 7.5 to 8.0 than other pH range [4]. According to Sagare *et al.*, (2000) soil of saline belt of Vidarbha region is highly alkaline possessing pH ranging between7.9 to 9.1 [5]. Hence the present study was undertaken to isolate actinomycetes from saline belt of Vidarbha region and assess their antibacterial potential.

MATERIAL AND METHODS

Collection of soil samples: 54 soil samples were collected from 18 villages from three district of Vidarbha region, Amravati, Akola and Buldhana at different depth (10-15 cms) in sterile polythene bags with the help sterile spatula and were transported to laboratory for further processing.

Isolation of Actinomycetes from saline soil: The collected soil samples were air dried for 24-48 hours, crushed and sieved. Then soil samples were pretreated with 1% $CaCO_3$ (w/v) under humid condition to increase the number of actinomycetes propagules in the samples [6].

Actinomycetes were isolated by serial dilution and spread plate method from collected saline soil samples on Actinomycetes isolation agar (M490, Hi-media Lab. Pvt. Ltd Mumbai, India) supplemented with 5 gm glycerol/l and antifungal antibiotic Nystatin 50 µg/ml to avoid fungal contamination [7]. The isolates showed dry, tough and leathery colonies on the isolation media and purified by streak plate method on Actinomycetes isolation agar.

Screening of antibiotic producing actinomycetes: Total 147 actinomycete isolates were first primarily screened with *Staphylococcus aureus* and *Escherichia coli* by using agar overlay technique [8].

Isolates showing antibacterial activity against both bacteria were subjected to secondary screening. The spore suspension of actinomycetes isolates were prepared by scraping 7 day old slant culture of actinomycetes isolates in 5 ml sterile distilled water and this spore suspension was added into a 250 ml Erlenmeyer flask containing 50 ml of glucose soybean medium and incubated at 30°C on a rotary shaker at 220 rpm for 7 days. Then the cultures were collected and centrifuged at 4000 rpm for 20 minute and filtered through whatman's No. 1 filter paper and filtrate was used to test antibacterial activity. Antibacterial activity was assayed by using modified agar well diffusion method against *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 441), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Proteus vulgaris* (MTCC 426), *Enterobacter aerogenes* (MTCC 7325), *Salmonella typhi* (MTCC 98), *Klebsiella pneumoniae* (MTCC 7407). Results were recorded in terms of zone of inhibition (mm) produced by actinomycete isolates against these microorganisms and the experiment was performed in triplicates for each microorganism tested.

Identification of efficient antibiotic producing actinomycete isolate:

Efficient antibiotic producing actinomycetes was characterized on the basis of morphological, cultural, biochemical and physiological features. The microscopic characterization was done by cover slip culture method [9]. Mycelium structure and arrangements of conidiospores on the mycelium was observed through microscope. The observed structure was compared with Bergey's Manual of Determinative Bacteriology ninth edition and the organism was identified [10]. Cultural characteristics (growth, colouration of aerial and substrate mycelia, diffusible pigment) were tested on different media including, Tryptone Yeast Extract Agar, Starch Casein Agar, Actinomycetes Isolation Agar and Nutrient Agar with the procedure of ISP. Gram's staining was also performed. Biochemical tests including Catalase, Oxidase, Indol, Methyl Red, Voges Proskaur, Citrate utilization test, fermentation of sugars like glucose, lactose, mannitol, dextrose, galactose, sucrose, fructose, maltose and hydrolysis of starch, gelatin, urea, lipid, casein were performed by standard protocol suggested by 'International Streptomyces Project' and 'Bergey's Manual of Systematic Bacteriology'. Physiological characterization such as, effect of pH (5-8), temperature (25-40°C) and salinity (2-7%) and antibiotic sensitivity test against seven different antibiotics (Hi-media, Mumbai) [Amikacin (30 mcg/disc), Ampicillin (10 mcg/disc), Chloramphenicol (10 mcg/disc), Norfloxicin (10 mcg/disc), Streptomycin (10 mcg/disc), Tetracycline (10 mcg/disc) and Co-trimoxazole (25 mcg/disc)] was work out.

RESULTS AND DISCUSSION

In primary screening, out of 147 actinomycete isolates 87 isolates (59.18%) showed an activity against 2 test bacteria such as *Staphylococcus aureus* and *Escherichia coli* by agar overlay technique. Out of which 45 (51.72 %) isolates active against *S. aureus* while 23 (26.43%)

isolates active against *E. coli* and 19 (21.83 %) were active against both (Fig. 1).

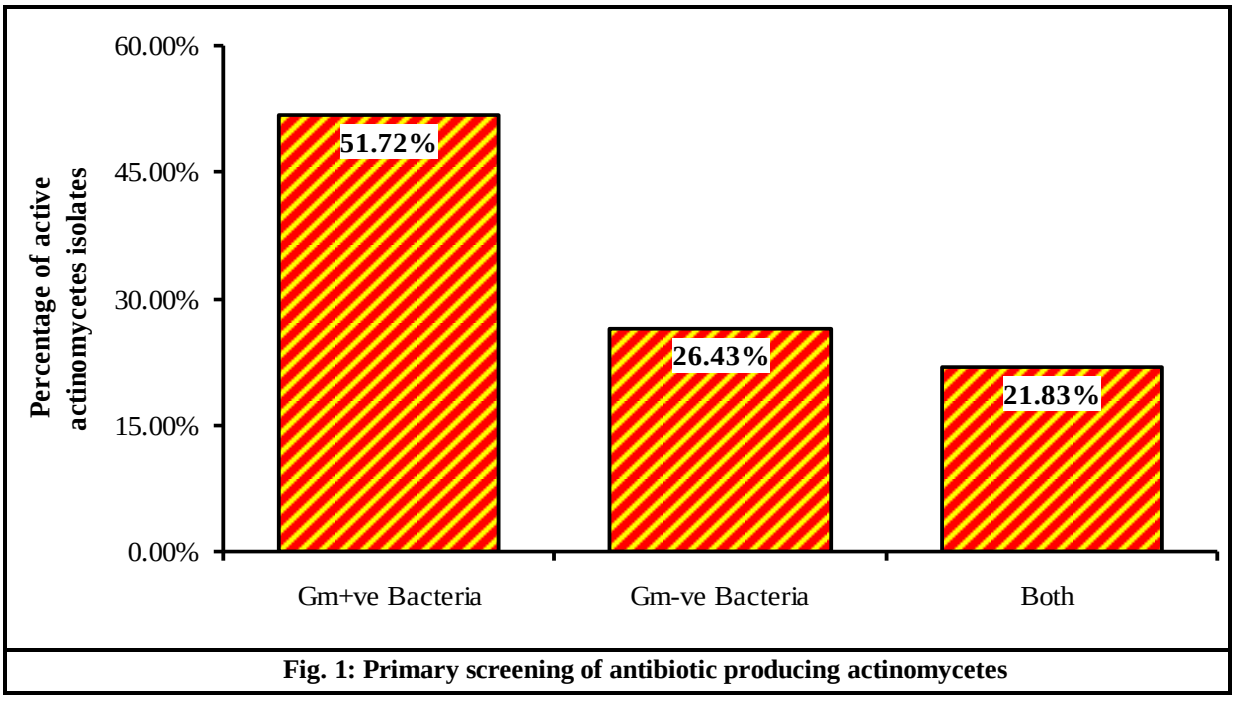

Fig. 1: Primary screening of antibiotic producing actinomycetes

From results it is obvious that the activities against Gram positive bacteria were more frequent than against Gram negative bacteria. This frequency of activities against Gram positive bacteria is similar to previous results reported by Basilio *et al.*, (2003) and Oskay *et al.*, (2004) [11,12].

In secondary screening, out of 87 actinomycete isolates only 19 isolates were selected for secondary screenings which were active against both *Staphylococcus aureus* and *Escherichia coli*. These 19 highly active isolates were subjected to secondary screening with the help of 9 test bacteria i.e. *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 441), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Proteus vulgaris* (MTCC 426), *Enterobacter aerogenes* (MTCC 7325), *Salmonella typhi* (MTCC 98), *Klebsiella pneumoniae* (MTCC 7407) by agar well diffusion method. The results of secondary screening of actinomycete isolates are depicted in Table 1.

Nearly 78.94% isolates recorded antibacterial activity towards *S. aureus* followed by 68.42% isolates to *Bacillus subtilis*, 63.15% for *Streptococcus pyogenes*, 52.63% to *Escherichia coli* and *Proteus vulgaris*, 47.36% towards *Klebsiella pneumoniae*, 42.10% for *Pseudomonas aeruginosa* and *Salmonella typhi* and 36.84% for *Enterobacter aerogenes*. Actinomycetes isolates H6, C1 and C3 showed activity against only Gram negative bacteria whereas isolates HT2, N2, N4, N5, D6, D8, C4 S6 and S9 showed activity against only Gram positive bacteria. Similarly, actinomycetes isolate N8 showed activity against all the test microorganisms.

The promising actinomycetes strain N8 was Gram positive, aerobic and it form circular, tough, leathery colonies that adhere to the starch casein agar surface. Cover slip culture studies indicate the spore chain morphology of N8 strain as spiral type and may placed in spira group. On actinomycetes isolation agar growth was excellent with white-gray aerial mycelium and bright yellow substrate mycelium with no diffusible pigment. Again growth was excellent on starch casein agar with gray aerial mycelium and yellow substrate mycelium. On tryptone yeast extract agar isolate showed good growth with whitish-yellow aerial mycelium and yellow substrate mycelium. Similarly on nutrient agar good growth with creamish-white aerial mycelium and

cream colour substrate mycelium was obtained. In any case diffusible pigment was not found.

Table 1: Secondary screening of actinomycete isolates for antibacterial activity by agar well diffusion method.

Sr. No	Isolate code	Zone of inhibition (mm)*								
		Gram positive bacteria			Gram negative bacteria					
		S. aureus (MTCC 7443)	*B. subtilis* (MTCC 441)	*S. pyogenes* (MTCC 442)	*E. coli* (MTCC 443)	*P. aeruginosa* (MTCC 424)	*P. vulgaris* (MTCC 426)	*E. aerogenes* (MTCC 7325)	*S. typhi* (MTCC 98)	*K. pneumoniae* (MTCC 7407)
1	H5	-	20±1.00	21±0.58	23±0.00	19±1.00	26±1.00	21±1.00	17±1.00	-
2	H6	-	-	-	21±1.00	-	23±0.58	-	22±1.00	19±1.00
3	HT2	21±0.00	23±1.00	-	-	-	-	-	-	-
4	KR4	19±1.00	15±1.00	19±1.00	24±0.00	21±1.00	18±1.00	17±0.58	-	22±1.00
5	N2	14±1.00	16±1.00	21±1.00	-	-	-	-	-	-
6	N3	19±1.00	21±0.00	-	24±1.00	23±0.00	25±1.00	20±1.00	18±0.58	17±1.00
7	N4	16±1.00	19±1.00	20±0.00	-	-	-	-	-	-
8	N5	19±1.00	21±1.00	22±1.00	-	-	-	-	-	-
9	N8	30±0.00	29±1.00	32±0.58	27±0.00	26±0.00	31±1.00	22±1.00	27±0.58	26±1.00
10	D1	20±1.00	-	25±1.00	26±1.00	19±1.00	16±1.00	17±0.00	24±0.00	24±0.58
11	D6	19±1.00	20±1.00	20±0.58	-	-	-	-	-	-
12	D8	21±1.00	22±1.00	-	-	-	-	-	-	-
13	Y3	20±0.58	15±1.00	23±1.00	21±1.00	-	19±1.00	17±0.00	25±1.00	18±1.00
14	C1	-	-	-	26±1.00	20±1.00	17±0.58	-	16±1.00	22±1.00
15	C3	-	-	-	21±1.00	19±1.00	24±1.00	-	22±0.58	17±1.00
16	C4	16±1.00	19±1.00	-	-	-	-	-	-	-
17	C6	25±1.00	24±0.00	17±1.00	20±0.00	26±1.00	27±0.00	19±1.00	-	21±0.58
18	S6	22±1.00	-	21±1.00	-	-	-	-	-	-
19	S9	26±1.00	-	23±1.00	-	-	-	-	-	-

***Values are mean of three replicates ± Standard Deviation (SD), (-): no zone of inhibition**

The biochemical tests like Indol, Methyl Red, Voges Proskauer were negative except Citrate utilization test which was positive. Similarly, sugar fermentation was also studied and result indicates that it has capability to ferment all tested sugars such as glucose, mannitol, dextrose, galactose, sucrose, fructose with acid production except lactose which was not fermented. Also, the strain N8 produced amylase, gelatinase, lipase, protease, urease except oxidase and catalase.

The strain N8 was grown at different incubation temperatures and most favorable for growth was found to be 28 to 30°C. The prominent growth was obtained at pH range 6-8. Similarly, the organism showed excellent growth at 2-5% (w/v) NaCl Isolate N8 was also subjected to the antibiotic sensitivity test by disk diffusion method. The results showed that the isolate exhibits high sensitive to Streptomycin (ZOI 27 mm) followed by Amikacin (ZOI 23 mm), Tetracycline (ZOI 21 mm), Norfloxacin (ZOI 18mm), Chloramphenicol (ZOI 17 mm). However it was found resistant to Ampicillin and Co-trimoxazole. To support above findings following observations can be quoted.

In a study, from the soil samples of Kalapatthar (5545 m), Mount Everest region, about seventy-nine actinomycetes were isolated and screened for their antibacterial activity [7]. In their screening work, they found that twenty seven (34.18%) of the isolates showed an antibacterial activity against at least one test-bacteria among two Gram positive and nine Gram negative bacteria in primary screening by perpendicular streak method. Thirteen (48.15 %) showed antibacterial activity in secondary screening. In another study, 75 actinomycetes strains isolated from the Egyptian desert habitats, and 32 (42.67 %) of the isolates were found to

be active against the used test organisms [13]. In a recent study performed in 2011 by Gautham *et al.*, *Streptomyces* species were isolated from Western Ghats soil of Agumbe, Karnataka which were characterized on the basis of cultural, staining and biochemical tests [14]. Actinomycetes strain showed good growth in medium containing 10 to15% (w/v) NaCl and with 30 to 36°C temperature [15].

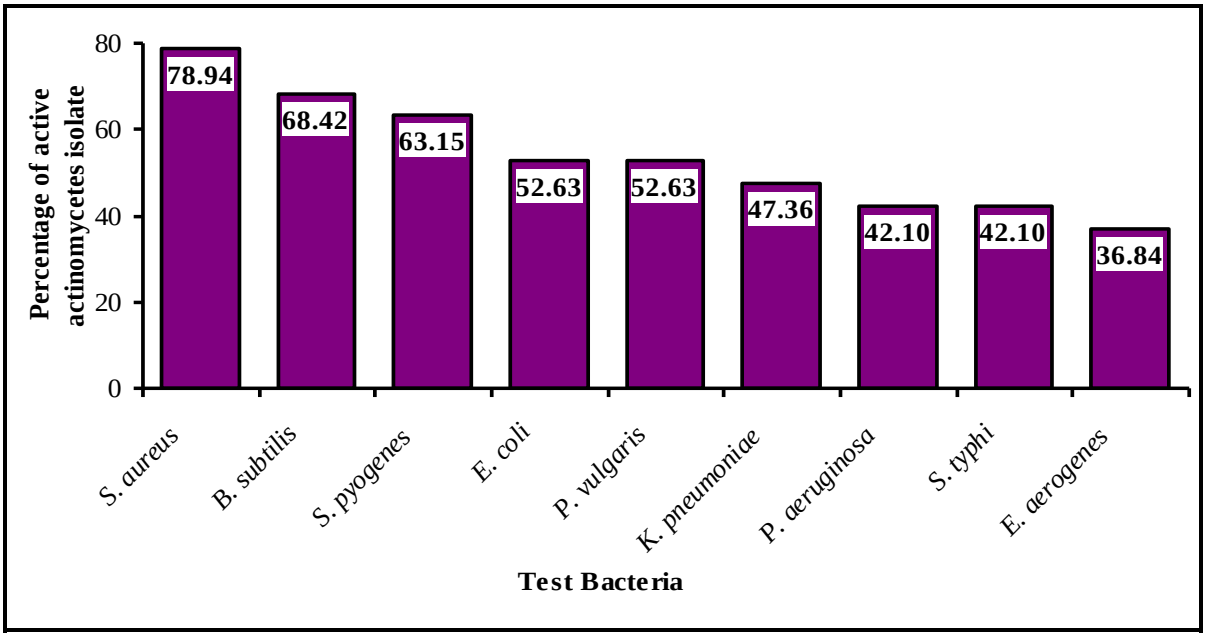

Fig. 2: Secondary screening of antibiotic producing actinomycetes

CONCLUSION

Novel antibiotic producing actinomycetes may be found in this belt which will be helpful in combating many human and plant diseases. Saline belt of Vidarbha region is a high potential source of antibiotic producing actinomycetes useful in various fields such as Pharmaceutical industries and Agricultural industries. These isolates also open a new avenue for researchers to discover newer efficient antibiotic.

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