RESEARCH ARTICLE

Screening of antimicrobial Actinomycetes from saline belt of Vidarbha Region

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ABSTRACT

Total 147 Actinomycetes were isolated from saline belt of Purna river basin which appears in Akola, Amravati and Buldhana district of Vidarbha region. 147 actinomycetes isolates were recovered during primary screening and out of that 87 isolates (59.18 %) showed an antibacterial activity against two test bacteria such as *Staphylococcus aureus* and *Escherichia coli* by agar overlay method and 19 isolates were recorded antibacterial against both these test bacteria. These 19 isolates were subjected to secondary screening and found antimicrobial against *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Aspergillus niger* (MTCC 281) and *Candida albicans* (MTCC 3017) by agar well diffusion method. Nearly 78.94% isolates were active against Staphylococcus aureus followed by 68.42% against *Bacillus subtilis*, 52.63% against *Escherichia coli*, 47.36% against *Candida albicans*, 42.10% against *Pseudomonas aeruginosa* and 26.31% against *Aspergillus niger*. Similarly actinomycetes isolate N8 showed activity against all the test microorganisms.

Keywords: Actinomycetes, Saline belt, Vidarbha region, Antimicrobial.

INTRODUCTION

The emergence as well as spread of multidrug resistance in microorganism is proving to be a great threat for global public health (Rajan *et al.,* 2010). The spread of resistant strains are linked to antibiotic use as well as to the migration of people, who disperse resistant strains among people in remote communities where the use of antibiotics is very limited (Allen *et al.,* 2010). Also, improper use of antibiotic was playing a vital role for drug resistance in pathogenic microbes.

To face such kind of situations there is an interest to improve or discover novel class of antibiotics, which provide different mechanisms of action worldwide (Parungao *et al.,* 2007). For microorganisms and their antimicrobial products soil work as a natural reservoir (Dancer, 2004). Some organisms such as bacteria, fungi, plants, actinomycetes

and so forth are responsible for creating secondary metabolites (Berdy, 2005). 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' (Demain, 1999). This remarkable group of compounds forms a heterogeneous assemblage of biologically active molecules with different modes of action and structures and they are effectively used in the treatments for bacterial infections. Actinomycetes were predominating in black saline soils than other type like alluvial, lateric and coastal saline (Konde, 1978). 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 (Zaharan *et al.,* 1992). In salt affected soil, the population of actinomycetes is higher at pH 7.5 to 8.0 than other pH range (Supanekar and Patil, 1995). Hence the present study was undertaken to isolate actinomycetes from saline belt of Vidarbha region and assess their antimicrobial potential.

MATERIALS 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 (Tsao *et al.,* 1993).

Actinomycetes were isolated by serial dilution and spread plate method from collected saline soil samples on selective agar medium such as 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 (Gurung *et al.,* 2009). The isolates showed dry, tough and leathery colonies on the isolation media were selected and purified by streak plate method on Actinomycetes isolation agar.

Screening of antibiotic producing actinomycetes:

The screening method consists of two steps, Primary screening and secondary screening. Total actinomycete isolates were first primarily screened with *Staphylococcus aureus* and *Escherichia coli* by using agar overlay technique (Singh *et al*., 2006).

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 antimicrobial activity. Antimicrobial activity was assayed by using modified agar well diffusion method against *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis (*MTCC 441), *Escherichia coli (*MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Aspergillus niger* (MTCC 281) and *Candida albicans* (MTCC 3017. 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.

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).

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); Oskay *et al.,* (2004).

In secondary screening, only 19 highly active primary isolates were selected for determining their antimicrogram against 6 test microorganisms i.e. *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 443),

Pseudomonas aeruginosa (MTCC 424), *Aspergillus niger* (MTCC 281) and *Candida albicans* (MTCC 3017) by agar well diffusion method. The results of secondary screening of actinomycete isolates are depicted in Table 1.

Nearly 78.94% isolates were active against *Staphylococcus aureus* followed by 68.42% against *Bacillus subtilis,* 52.63% against *Escherichia coli,* 47.36% against *Candida albicans,* 42.10% against *Pseudomonas aeruginosa* and 26.31% isolates against *Aspergillus niger* (Fig 2). D6, D8 and S9 showed activity against only Gram positive bacteria and C1 showed activity against only Gram negative bacteria. Similarly, actinomycetes isolate N8 showed activity against all the test microorganisms. To support above findings following observations can be quoted.

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

Sr. No	Isolate code	Mean Zone of inhibition (mm)					
		Test Microorganisms					
		S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***	***A. niger***	***C. albicans***
1	H5	-	20	23	19	-	15
2	H6	-	-	21	-	-	21
3	HT2	21	23	-	-	-	17
4	KR4	19	15	24	21	-	-
5	N2	14	16	-	-	14	-
6	N3	19	21	24	23	-	-
7	N4	16	19	-	-	-	19
8	N5	19	21	-	-	-	17
9	N8	30	29	27	26	14	15
10	D1	20	-	26	19	18	14
11	D6	19	20	-	-	-	-
12	D8	21	22	-	-	-	-
13	Y3	20	15	21	-	-	-
14	C1	-	-	26	20	-	-
15	C3	-	-	21	19	20	-
16	C4	16	19	-	-	-	20
17	C6	25	24	20	26	-	13
18	S6	22	-	-	-	14	-
19	S9	26	-	-	-	-	-

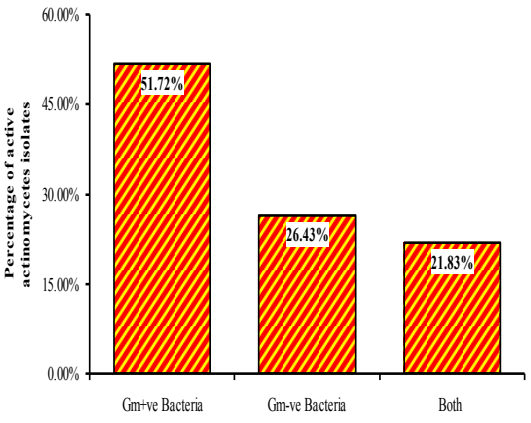

Fig. 1: Primary screening of antibiotic producing actinomycetes

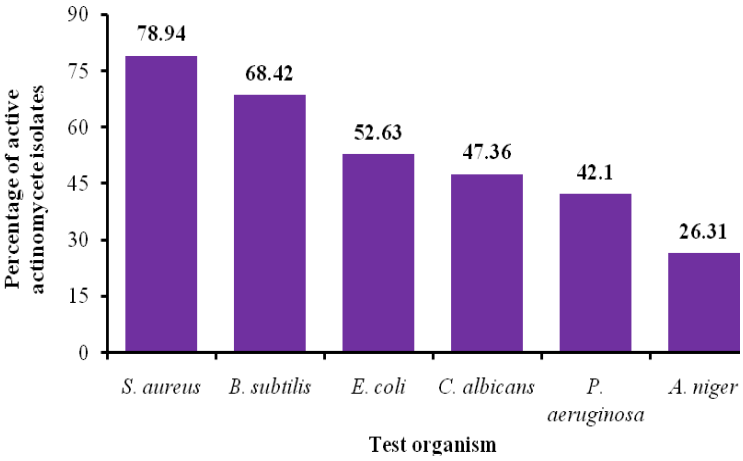

Fig. 2: Secondary screening of antibiotic producing actinomycetes

Oskay *et al*., (2004) isolated actinomycetes from dry alkaline conditions of farming soils. Chougule and Deshmukh AM (2006) also isolated actinomycetes from saline belt of Sangli district. Gurung *et al.,* (2009) studied antibacterial potential of seventy-nine actinomycetes from soil of Kalapatthar (5545 m), Mount Everest region and recorded 27 isolates (34.18%) antibacterial against at least one test-bacteria among two Gram positive and nine Gram negative bacteria in primary screening by perpendicular streak method and also 13 isolates (48.15 %) showed antibacterial activity in secondary screening. Hozzein *et al.,* (2011) studied the antimicrobial activities of desert actinomycetes as potential producers of active metabolites. Out of the 75 actinomycetes strains isolated from the Egyptian desert habitats, 32 (42.67 %) showed activity against the used test organisms.

CONCLUSION

From the present study it can be concluded that Saline belt of Vidarbha region shows great diversity regarding antimicrobial actinomycetes which provide platform for researchers to discover newer efficient antibiotic, helpful in combating many human and plant diseases

REFERENCES

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J (2010) Call of the wild: antibiotic resistance genes in natural environments. *Nat Rev Microbiol*. 8: 251–259.

Basilio A, Gonza´lez I, Vicente MF, Gorrochategui J, Cabello A, Gonza´lez A, Genilloud O (2003) Patterns of antimicrobial activities from soil actinomycetes isolated under different conditions of pH and salinity. *J Appl Microbiol.* 95: 814–823.

Berdy J (2005) Bioactive microbial metabolites. *J Antibiot*. 58(1): 1-26.

Chougule VV, Deshmukh AM (2006) Studies on actinomycetes from saline soil of Sangli district. Ph.D. Thesis, Shivaji University Kolhapur.

Dancer SJ (2004) How antibiotics can make us sick: the less obvious adverse effects of antimicrobial chemotherapy. *The Lancet Infect Dis*. 4: 611-619.

Demain AL (1999) Pharmaceutically active secondary metabolites of microorganisms. *Appl Microbiol Biotechnol.* 52: 455-463.

Gurung TD, Sherpa C, Agrawal VP, Lekhak B (2009) Isolation and characterization of antibacterial actinomycetes from soil samples of Kalapatthar, Mount Everest region. Nepal J Sci Technol. 10: 173-182.

Hozzein WN, Rabie W, Ali IA (2011) Screening the Egyptian desert actinomycetes as candidates for new antimicrobial compounds and identification of a new desert *Streptomyces* strain. *Afr J Biotechnol*. 10(12): 2295-2301.

Konde BK (1978) Studies on soil Streptomyces from Maharashtra. Ph.D. (Agri) Thesis. University of Poona.

Oskay M, Tamer A, Azeri C (2004) Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. *Afr J Biotechnol*. 3: 441–446.

Parungao MM, Maceda EBG, Villano MAF (2007) Screening of antibiotic producing actinomycetes from marine, brackish and terrestrial sediment of Samal Island, Philippines. *J Res Sci Comp Engg*. 4(3): 29-38.

Rajan BM, Kannabiran K (2010) Antimicrobial activity of *Streptomyces albofaciens vitbrk* sp. isolated from the Bay of Bengal coast of Tamil Nadu, India. *Pharmacologyonline.* 1: 124-132.

Singh LS, Baruah I and Bora TC (2006) Actinomycetes of Loktak habitat: isolation and screening for antimicrobial activities. *Biotech.* 5(2): 217-221.

Supanekar SV, Patil PL (1999) Impact of soil salinity on microflora and ground water pollution in Sangli district. Ph.D. Thesis Shivaji University, Kolhapur.

Tsao PH, Leben C, Keitt GW (1960) An enrichment method for isolating actinomycetes that produce diffusible antifungal antibiotics. *Phytopath.* 50(1): 88-95.

Zaharan HH, Moharram AN, Mohammad HA (1992) Some ecological and physiological studies on bacteria isolated from salt affected soil of Egypt. *J Basic Microbiol.* 32(6): 405-413.