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Screening the Antibacterial Actinomycetes from Saline Soil of Vidarbha Region

KEYWORDS: Actinomycetes, Saline soil, Vidarbha region, Antibacterial activity

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ABSTRACT *Antibacterial activity of Actinomycetes isolated from saline belt of Purna river basin which appears in Amravati, Akola and Buldhana district of vidarbha region has been studied. In primary screening, out of 147 actinomycete isolates 50 isolates (34.01%) showed an activity against 2 test bacteria such as Staphylococcus aureus and Escherichia coli by agar overlay technique. In secondary screening, out of 50 primary isolates 19 actinomycete isolates were proceeded for an antibacterial activity against Staphylococcus aureus (MTCC 7443), Bacillus subtilis (MTCC 441), Escherichia coli (MTCC 443) and Pseudomonas aeruginosa (MTCC 424) by agar well diffusion method. Nearly 78.94% isolates were found antibacterial to Staphylococcus aureus followed by 68.42% against Bacillus subtilis, 52.63% against Escherichia coli and 42.10% against Pseudomonas aeruoginosa. Similarly actinomycetes isolates KR4, N3, N8 and C6 showed antibacterial activity against all the test bacteria.*

INTRODUCTION:

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil (Mustafa Oskay et al., 2004). Actinomycetes are best known for their ability to produce antibiotics and are gram positive bacteria which comprise of a group of branching unicellular microorganisms. They produce branching mycelium which may be of two kinds' viz., substrate mycelium and aerial mycelium (P. A. Mary Helen et al., 2012). They have 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 (Okami Y. and Hotta K., 1988). Almost 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genera Streptomyces and Micromonospora (Pandey B et al., 2004)

According to geological survey of India it was reported that the saline belt of Purna river basin is a part of Payanghat plains where to its north is Melghat and Ajanta mountain ranges to the south. Due to volcanic eruption on the nearby Satpuda ranges this land becomes arid as a result of deposition of alluvial. Hydro geologically, the Purna river basin is the worst alluvial tract of India though its water holding capacity is good. Secondly Purna River remains dry throughout the year except for the rainy season. The Purna river basin lies between latitude 20-40 to 21-45 and east longitude 76-20 to 77-45. Purna River originates from Gawilgarh hills of Satpuda near Bhaisdehi in Baitul district of M.P. The Purna River travels through Akola, Amravati, and Buldana districts of Vidarbha region and finally meets Tapi River that eventually meets the Arabian Sea (Adyalkar, 1971). Purna river basin extends east-west for a stretch of 170 kms and width of 55 kms. Total area covered is 6200 sq km out of which 3000 sq km comprising 547 villages is characterized as saline belt. According to Sagare et al., (2000) soil of saline belt of Vidarbha region is highly alkaline possessing pH ranging between 7.9 to 9.1. Attempt was made to isolate actinomycetes from this saline belt to study their antibacterial potential against test bacteria.

MATERIALS AND METHODS:

Collection of soil samples: Soil samples were collected from 18 villages from three district of Vidarbha region, Amravati, Akola and Buldhana. The salinity level in these villages is highest as per the report given by Sagare et al., (2000). Soil samples were collected from different depth (25-40 cms and 50-140 cms) in sterile polythene bags. In all 36 soil samples i.e. 2 samples from each village were collected. The soil samples thus collected were subjected to pretreatment with $CaCO_3$ under humid conditions to increase the number of actinomycetes propagules in the sample. The pretreated soil samples were first air dried at temperature 25-30^0 C (Taso et al., 1960).

Isolation of Actinomycetes: Actinomycetes were isolated by serial dilution and pour plate method on Actinomycetes isolation agar plates supplemented with 5 gm of glycerol/L and antifungal antibiotic Nystatin 50µg/ml to avoid fugal contamination. Dry, tough colonies showing antagonism were selected for isolation of actinomycetes. Each such colony showing antagonism was picked up and streaked on sterilized actinomycete isolation agar plate which was then incubated at room temperature for 4 to 6 days.

Screening of Actinomycetes for antibacterial activity:
The screening method consists of two steps, Primary screening and secondary screening. The 147 actinomycete isolates were first primarily screened with Staphylococcus aureus and Escherichia coli by using agar overlay technique. Isolates showing antibacterial activity against these two bacteria were subjected to secondary screening with the help of 4 test bacteria i.e. Staphylococcus aureus (MTCC 7443), Bacillus subtilis (MTCC 441), Escherichia coli (MTCC 443) and Pseudomonas aeruginosa (MTCC 424) by agar well diffusion method. Results were recorded in terms of zone of inhibition (mm) produced by actinomycete isolates against these bacteria.

RESULTS AND DISCUSSION:

In primary screening, out of 147 actinomycete isolates 50 isolates (34.01%) showed an activity against 2 test bacteria such as Staphylococcus aureus and Escherichia coli by agar overlay technique. Of the 50 isolates 22 isolates (44%) active against Gm+ve bacteria, 09 isolates (18%) active against Gm-ve bacteria and 19 isolates (38%) active against both Gm+ve and Gm-ve bacteria. In secondary screening, out of 50 primary actinomycete isolates only 19 isolates (38%) were selected for secondary screening which was active against both Staphylococcus aureus and Escherichia coli. These 19 highly active isolates were subjected to secondary screening with the help of 4 test bacteria i.e. Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa by agar well diffusion method.

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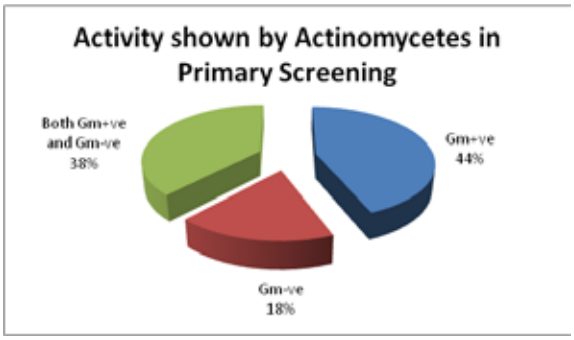
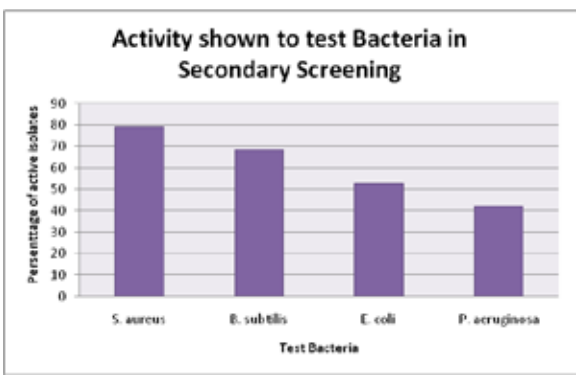


The results of antibacterial activity of actinomycete isolates are depicted in table 1. The results indicate that nearly 78.94% isolates were found antibacterial to Staphylococcus aureus followed by 68.42% against Bacillus subtilis, 52.63% against Escherichia coli 42.10% against Pseudomonas aeruoginosa. Similarly actinomycetes isolates KR4, N3, N8 and C6 showed antibacterial activity against all the test bacteria.



There are many references to supports occurrence of actinomycetes in alkaline condition. Supanekar and Patil (1995) reported that actinomycetal population was higher at pH range 7.5 to 8.0 in salt affected soils. Mustafa Oskay et al., (2004) isolated actinomycetes from dry alkaline conditions of farming soils. Chougule and A.M. Deshmukh (2006) also isolated actinomycetes from saline belt of Sangli district. Mohamoud et al., (2007) isolated 188 Streptomyces species from Egyptian soils and were screened for their antagonistic activity against 19 fungal and bacterial species.

It is obvious from the results 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), Anansiriwattana et al. (2006) and Charoensopharat et al. (2008). The reason for different sensitivity between gram positive and gram negative bacteria could be ascribed to the morphological differences between these microorganisms, gram negative bacteria having an outer polysaccharide membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, The gram positive should more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Williams, S. T. and Cross, T., 1971).

CONCLUSION:

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

Table 1: The antibacterial activity of selected actinomycetes isolates.

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

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