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Screening for Novel Antibiotic Producing Actinomycetes from Western Ghats of Karnataka State, India

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ABSTRACT

perpendicular streak method for antibiotic production. 14 isolates were positive for antibiotic activity and these strains were subjected to fermentation in yeast-malt extract broth on orbital shaker for 7 days at 27° C/200 rpm and Present study aims at isolating the Actinomycetes from Western Ghats producing antibiotics effective against plant and animal pathogens. Soil samples from Dandeli forest and Shettyhalli wild life sanctuary of the Western Ghats of Karnataka were plated on starch-casein agar. Fifty Actinomycetes were isolated and preserved at 4° C. The isolates were screened by fermentation broth was extracted in 1:1 v/v ethyl acetate and evaporated on rotary evaporator and the compound wasdissolved in DMSO. The disc assay was carried out against specific pathogens. MIC was performed to the extract which showed broad spectrum activity. The isolate 60A showing a broad spectrum activity was characterized according to Bergey's manual and identified as Streptomyces sp.

Introduction:

The Actinomycetes produce about 70% of total known antibiotics, and remaining 30% are products of filamentous fungi and non-actinomycete bacteria. Antibiotics of Actinomycetes origin evidence a wide variety of chemical structures including aminoglycosides, anthracyclines, glycopeptides, β-lactams, nucleosides, peptides, polyenes, actinomycins and tetracyclins (Ceylan et al., 2008). During last 2-3 decades, focus has been centred on Actinomycetes for their ability to produce secondary metabolites mainly antibiotics (Pandey et al.,2004). The diversity of terrestrial Actinomycetes has been of extraordinary significance in several areas of science and technology. The decreasing rate of discovery of novel drugs from established terrestrial sources has motivated the evaluation of new sources of chemically diverse bioactive compounds (Magarave et al.,2004) and the increase in the multi-drug resistance pathogen are of major concern in recent years. So looking for new and safe antibiotics to tackle this problem, present study explores few new regions of Western Ghats's soil samples of Karnataka and also to understand the novel Actinomycetes strains, producing exceptional bioactive compounds as antibiotics.

Materials and methods:

Collection of soil samples

A total of 22 soil samples were collected aseptically at 15 cm depth from surface, in sterile plastic containers from 11 different places of Dandeli forest of Belgaum District and Shettyhalli wildlife sanctuary of Shivamogga District, Western Ghats region. Soil was processed at the earliest (Augustine et al., 2004).

Isolation of Actinomycetes

Soil was subjected to serial dilution and lower dilutions were plated on Starch Casein Agar (SCA) (Singh et al.,2006) supplemented with terbinafine (1mg/ml) as antifungal agent. After incubation pure colonies were isolated and stored at 4°C on Glycerol Asparagine Agar (GAA) for further use.

Primary screening

Primary screening to check antimicrobial activity was done against various known plant and animal pathogens. Isolates were streaked on yeast-malt extract agar (YMA) at the centre of Petri plate as a straight line and incubated for 7 days. After this 24hours old culture of pathogens namely, Xanthomonas compestris NCIM2691, Proteus vulgaris NCIM 2813, Staphylococcus aureus NCIM 2079, Escherichia coli NCIM 2931, P. aeruginosa NCIM 5029, Bacillus subtilis NCIM 2063 and, Xanthomonas malvacearum NCIM 2310 (obtained from NCIM, Pune, India,) were streaked perpendicular to the central Actinomycetes colony and continued incubation at 37oC for 24 hours and observed for growth inhibition of the test organism (Ceylan et al., 2008).

Fermentation

Isolates positive for antimicrobial activity in primary screening were selected for secondary screening. The isolates were subjected to fermentation in yeast-malt extract broth (YMB). Fermentation was carried out by shake flask method at 200 rpm, 27o C for 10 days. After incubation, the fermentation broth was filtered and Ethyl acetate was added to the filtrate in the ratio of 1:1 (v/v) and shaken vigorously for 1 hour for complete extraction. The solvent phase that contains antibiotic collected and it was evaporated to dryness (Debananda et al.,2009). The residue obtained was quantified by weight difference method. Thus obtained extract was dissolved and preserved in DMSO and used to determine antimicrobial activity, minimum inhibitory concentration.

Disc assay

The antimicrobial activity was determined by filter disc assay method. Lawn culture of organisms was done on solidified Muller Hinton agar plates with the 18 hour old culture of test organism the turbidity of which is adjusted equal to 0.5 Mc-Farland turbidity standards which is assumed to have a cell concentration 1.5 X 10-5cells/ml. Sterile paper discs of 8 mm diameter with Whatmann's No.1 filter paper loaded with 10 µl of crude antibiotic extract were placed on the lawn culture. Plates were incubated at 37°C for 18-24 hours and frequently observed for inhibition zone. The diameter of the zones of complete inhibition was measured to the nearest millimetre (Beer et al.,1945)

Characterization:

The isolate showing broad spectrum activity (60A) was se-

lected for characterization by morphological, Microscopical and biochemical methods(Bergeys manual). Morphological characterization was by plating 60A on various media GAA, Oat Meal Agar (OMA) and Inorganic Salts Starch Agar media (ISSA). Microscopic Characterization was done by slide culture. Starch hydrolysis, liquefaction of gelatine, casein hydrolysis, citrate utilization tests were carried out for the biochemical characterization of the organism.

Minimum Inhibitory Concentration (MIC)

The crude extract from 60A was selected for MIC study. Disc assay was performed with discs were loaded with varying concentration of extracts prepared in DMSO ranging from 1.75ng to 300ng/10µl. The least concentration showing a zone of inhibition was considered as MIC.

Results and discussions

Western Ghats of India are the less explored regions for Actinomycetes diversity and this study has shown that Western Ghats contain diverse species producing the antibiotic. 56 different strains of Actinomycetes were isolated, among which 23 isolates showed visible inhibition of the test organisms and one isolate showed broad spectrum activity. Results are indicated in

Table 1 : primary screening results for various isolates on different pathogens

Organism	X.malvecearum	S.aureus	B.subtilis	X.compestris	E.coli	P.aerogenosa	P.vulgaris
56A	-	+	-	-	-	+	+
60A	+	+	+	-	+	+	+
38A	+	+	+	-	-	+	+
9A	-	+	+	-	+	+	+
102A	+	+	+	-	-	+	+
42A	+	+	+	-	-	+	+
100A	+	+	+	-	-	+	+
DNL-1	-	+	+	-	-	+	+
DNL-4	-	+	+	-	-	-	-
DNL-7	-	+	-	+	-	-	+
DNL-11	-	+	+	+	-	+	+
DNL-16	-	+	-	+	-	+	+
DNL-17	-	+	-	+	-	+	+
DNL-22	-	+	+	+	-	+	+
DNL-24	-	+	-	+	-	-	-
DNL-31	-	+	-	+	-	+	+
DNL-42	-	-	-	+	-	+	+
DNL-52	-	+	-	+	-	+	+

Disc assay was performed for crude extract from 6 Actinomycetes isolates based on the visible inhibition of growth of test organisms and strain 60A was found to produce broad spectrum antibiotic capable of inhibiting the growth of most of the test organism. Results are indicated in

Table 2: Disc assay results for the isolates which were positive in primary screening.

Organism	INHIBITION ZONE IN MM						
	X.malvacerum	B.subtilis	P.vulgaris	S.aureus	E.coli	X.compestris	P.aerogenosa
Streptomycin (10µg/ml)	28	31	31	21	18	18	18
60A	9	20	20	22	9	22	25
102A	10	-	10	22	11	15	12
56A	-	-	12	8	9	-	10
40A	9	8	10	-	11	10	9
42A	-	11	10	-	8	14	11
38A	12	-	-	11	9	10	9
DMSO(-)	-	-	-	-	-	-	-

Based on the result of secondary screening, the isolate 60A is selected for determination of MIC. MIC was 30ng for P.aerugenosa (10mm zone), X.compestris (9mm), B.Subtilis (9mm), S.aureus (9mm) P.vulgaris(10mm zone), X.malvacearum, E.coli showed inhibition with minimum concentration of 300 ng. Results are indicated in

Table3: Results of MIC for 60A on various pathogens

Pathogen	300ng/10µl	240ng/10µl	180ng/10µl	120ng/10µl	60ng/10µl	30ng/10µl	15ng/10µl
P.aerogenosa	25	25	23	22	20	10 MIC	No inhibition
X.compestris	22	21	20	18	16	9 MIC	No inhibition
B.subtilis	20	20	18	17	15	9 MIC	No inhibition
S.aureus	22	20	19	18	16	9 MIC	No inhibition
P.vulgaris	20	20	19	18	16	10 MIC	No inhibition
X.malvaecerum	9 MIC	No inhibition					
E.coli	9 MIC	No inhibition					

60 A produced 3 mm diameter, grey coloured powdery mass of sporulation on the colony, entire, slightly elevated colony on SCA with inhibition of neighboured colony, on Oat meal agar it produced colony with good growth, 5 mm diameter, grey coloured, watery droplets on the colony white margin, on GAA colony was 2 mm diameter, dark grey coloured entire margin, raised elevation, watery droplets on the colony, good growth, white margin, and on ISSA colony was with Irregular margin, 5 mm diameter, grey coloured, watery droplets on the colony, margin white good growth. On slide culture the organism had circular spores in conidia in the form of open spirals; spores diameter was equal to diameter of hyphae. Organism was positive for starch hydrolysis, lipase and lecithinase production, negative for casein and gelatine hydrolysis, citrate utilization. On comparing morphological, biochemical and microscopic characterization of the organism, was sharing similar results for Streptomyces sp. Padmadas and Raghunathan (2010) reports isolation of 100 actinomycetes from soil of Western Ghats of Tamilnadu, India among which 2 isolates showed very potential broad spectrum the most promising isolate in that study was a Streptomyces sp. Perusal of literature viz., Kavith and Vijayalakshmi (2007), Deepika et al., (2010), Reddy et al.,(2011) indicates that the Streptomyces spp. is potential agent for the production of novel bioactive compounds The process of purification and characterization of the compound and 16s rDNA sequencing studies of the isolate is under progress.

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