Antimicrobial Activity of Bacteria Isolated from soil against the *Pseudomonas aeruginosa, Bacillus subtilis, Escherichia.coli, Staphylococcus aureus and Shigella flexneri.*

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Abstract

The continuous use of the antibiotics makes the organisms resistive, so there is need to look for new antibiotics. Samples collected from various regions of the Jammu, Himachal Pradesh and Punjab and were screened for antimicrobial activity against the targeted bacteria to find the antimicrobial agents against these strains. 35 samples were collected out of which 6 samples showed the positive response towards various multidrug resistant strains. Isolated microorganism from Hospital dump soil (HDS), Rock soil (RS) and Himachal agriculture soil (H.S) showed a better antimicrobial activity against the targeted strains. The morphological, biochemical and molecular test were performed for the identification of microorganisms.

KEYWORDS: Antibiotic, soil sample, biochemical characterization, Molecular characterization.

INTRODUCTION

In the current scenario many bacteria are becoming resisrtant to pharmaceutical antimicrobials, it has become necessary to find an alternative to current chemical antimicrobial substances. Antibiotics are the substances that fight against the bacterial infection they either kill bacteria or keep them from reproducing (Aminov R. I., 2010). The *penicillin* was the first antibiotic used successfully to treat bacterial infections for the saving of thousands lives. Present study deals with isolation, identification and characterization of microorganism which can produce antibiotic against the targeted strains. The characterization often requires several steps for finding a microbe, the bio-characterization involve the complete biological study of the microorganisms involving the conformation of species of origin, tissue of origin and identification to which the microbe belong were done for the finding of a microbe with Novel characteristics. Antibiotic resistance microbes are resistant against particular antibiotics. However, it may possible that one microbe is resistive against many antibiotics but it is not possible that one microbe is resistive against all the antibiotics it may be killed by some antibiotics. The microbes resistive against more than one antibiotics are named as multidrug-resistant (Datta A. *et al.*, 2012).

Antimicrobial agents are the chemical substances that kill or slow the growth of microbes. Most of the antibiotics are releasing from the natural environment, the soil is the main resource for antibiotic producing microbes (Abdulkadir M. *et al.,* 2012; Popowska M. *et al.,* 2012). The Bacteria become resistant to antimicrobials through a number of mechanisms (Angela H. A. M. *et al.,* 2011). A) Permeability changes in the bacterial cell wall which restricts antimicrobial access to target sites. B) Active efflux of the antibiotic from the microbial cell. C) Enzymatic modification of the antibiotic (Lupo A. *et al.,* 2012). D) Degradation of the antimicrobial agent. E) Acquisition of alternative metabolic pathways to those inhibited by the drug. F) Modification of antibiotic targets (Davies J.2006).

In this study, soil sites have been chosen for the isolation of microorganism. Isolated microorganism were screened for their potential to produce antibiotics. Targeted microorganism were selected for the testing of antibiotic potential. Antibiotic producing microorganism were then characterized at morphological, biochemical and molecular level.

MATERIALS AND METHODS

COLLECTION OF SAMPLE (Kumar N. *et al*., 2010)

The samples of soil were collected from various places of Jammu region, Himachal and Punjab states of India. The collection of soil samples involves the random sampling and labeling was done properly. The samples were collected from Hospital dump soil (HDS), Rock soil (RS) and agriculture soil (H.S) came from multiple origins and further they were differentiated according to their sources (Chandan P. *et al*., 2010; Bahig A. E. *et al*., 2008). All the samples were collected from sterile instrument from the depth of soil and carried in sealed sterile bag to the laboratory.

ISOLATION OF MICROBES

The isolation of microbes from soil sample offered serial dilution, Serial dilution was performed due to the reason that the morphology of microorganisms in stocks solution was not clearly visible due to high concentration of cell growth. Concentration of microorganism decreases with every serial dilution step performed another advantage of this step is we can maintained our desired concentration of biomass. The dilution of sample occur 1gm in 0.9% saline, 1gm in 100ml and 1gm soil sample in 10ml of water (Oviasogie F. *et al.,* 2010).

TEST ORGANISMS

Various test organisms used for the isolation of antimicrobial agents. All strains samples were taken from IMTECH (Institute of Microbial Technology) Chandigarh. *Shigella flexneri*- MTCC 1457, *Escherichia coli*-MTCC 1680, *Bacillus subtilis*-MTCC 121, *Pseudomonas aeruginosa*- MTCC 424, *Staphylococcus aureus*-

MTCC 6908. For the revival of the pure strain, inoculate the particular strain containing nutrient broth (Sharma D. *et al.,* 2011; Kumar S. *et al.,* 2012; Eckert R. *et al.,* 2006).

PRIMARY SCREENING

The primary screening of soil sample against targeted bacteria required: Nutrient Agar plates, swabbing the strains for checking the antimicrobial activity of cultures against the strains, Gel punctured method was used, Pour 10ul of each culture in each well, keep in incubator for 24hrs at 37^{o}C. The primary screening showed the positive result against the bacterial strains. For the confirmation pick the discrete colonies from the plates and streak the colony in nutrient agar plates for secondary screening

ANTIBIOTIC SUSCEPTIBILITY TESTING (AST)

Antibiotic susceptibility test was used to find out the microbes that are sensitive and resistive against the particular antibiotic, if the bacteria was sensitive to the antibiotic, a clear ring or zone of inhibition was seen inside the petriplate if not than no change occur in the petriplate. The Antibiotic susceptibility involves the primary screening of antibiotics Isolates which showed broad spectrum activity against test organisms in primary screening were subjected to secondary screening by Kirby Bauer agar well diffusion method (Kiehlbauch J. A. *et al.,* 2000; Chang J. C. *et al*., 1997; Chait R. *et al*., 2010). The primary screening involves the crowded plate method. Antibiotic sensitivity tests were performed on targeted culture. Culture was swabbed on nutrient agar using sterile cotton swabs. Antibiotic discs (10mcg/disc) were placed on the dish and incubation of 24 hrs was given. Following antibiotic discs were used for sensitivity tests Kannamycin, Erythromycin, Gentamycin, Norfloxacin, Neomycin, Tetracycline, Streptomycin, Ciprofloxacin and Ampicillin

SECONDARY SCREENING

For the secondary screening took the discrete colonies from the petriplates and inoculated in the fresh nutrient broth for 24-48 hrs at 37^{o}C. The secondary screening showed the positive results against the various cultures. The secondary screening result showed the final isolation of antimicrobial agents against various strains means the culture showed some antimicrobial activity against the strains. Isolation of pure culture were obtained by streaking again and again on nutrient agar by picking single colony from the petriplates finally getting our desired species

MICROSCOPIC CHARACTERIZATION

The staining shows the single morphology means the culture is pure and again the culture is inoculated in broth and store the pure culture shows the antimicrobial activity against the strains. The six samples were isolated showed the antimicrobial activity against the multidrug strains. This sample is often now used for the

conformation of the species and for the conformation we have to use several biochemical tests to confirm the species of origin

RESULTS

ANTIMICROBIAL ACTIVITY OF THE PURE ISOLATES AGAINST THE MULTIDRUG RESISTANT MICROBES

The antimicrobial activity of the pure isolates is checked by the well plate method the media prepared, pouring is done then after solidification of the media make the well in the petriplates and then swabbing of pure strains is done with the help of cotton swabs (being autoclaved) after swabbing the pure strain take 20ml of our culture and pour in to the well by using micropipette (0.5-10ml or 10-100ml) and then incubate the petriplates in to the incubator for 24hr at 37^0C without inverting the petriplates (Saha S. *et al*., 2012)

S. No	Isolates	*S. aureus*	*P.aeruginosa*	*B. subtilis*	*S. flexneri*	*E. coli*
1	Isolate 1	0.6	0.8	0.6	1.5	0.0
2	Isolate 2	0.6	0.9	0.6	1.5	0.0
3	Isolate 3	0.0	0.9	0.7	1.7	0.0
4	Isolate 4	0.0	0.9	0.8	1.5	0.0
5	Isolate 5	0.0	0.0	0.6	0.8	0.0
6	Isolate 6	0.0	0.0	0.3	0.8	0.0

Fig No 1: represents the antimicrobial activity, of isolates (1, 2, 3, 4 and 5 and 6) against targeted strain in the form of zone of inhibition (in cm).

BIOCHEMICAL TEST FOR IDENTIFICATION OF MICROBES

The several biochemical tests is to be performed for the confirmation of the species there are different species showed different results for these tests so performing these test we were confirm the species of the origin (Oyeleke S. B. *et al*., 2008). The result of all the 6 isolates (1, 2, 3, 4, 5 and 6) were

Table 3- Morphological and Cultural Characterization of pure soil isolates

Sr No:	**Isolate**	**Gram's staining**	**Morphology**
1	**Isolates 1**	Gram negative	Rod Shaped
2	**Isolates 2**	Gram negative	Rod Shaped

3	**Isolates 3**	Gram negative	Rod Shaped
4	**Isolates 4**	Gram negative	Rod Shaped
5	**Isolates 5**	Gram negative	Rod Shaped
6	**Isolates 6**	Gram negative	Rod Shaped

Fig no 2: Morphological analysis of targeted strains

S.No	**Biochemical Test**	**1**	**2**	**3**	**4**	**5**	**6**
1.	**MR**	-	-	+	-	+	-
2.	**VP**	-	-	-	-	-	-
3.	**Indole**	-	-	-	-	-	-
4.	**Citrate**	+	+	+	+	+	+
5.	**Catalase**	+	+	+	+	+	+
6.	**Urease**	-	-	-	-	-	-
7.	**Nitrate Reduction Test**	+	+	+	+	+	+
8.	**Gelatin**	+	+	-	+	-	+
9.	**Oxidase**	+	+	-	+	-	+
10.	**Motility**	+	+	+	+	+	+
11.	**Starch Hydrolysis**	-	-	-	-	-	-

Alkaline (K), Acidic (A), Positive (+), Negative (-)

Table 1:Biochemical Characterization of pure soil isolates

Sample Code	Slant	Butt	Gas Production	H_2S
Isolates 1	K	K	-	-
Isolates 2	K	K	-	-
Isolates 3	K	A	+	+
Isolates 4	K	K	-	-
Isolates 5	K	A	+	+
Isolates 6	K	K	-	-

Table2: Results of TSI Test

Sugar fermentation test				
Sugar / Isolates	Glucose	Sucrose	Lactose	Mannitol
Isolates 1	-	+	-	+
Isolates 2	-	+	-	+
Isolates 3	+	-	-	+
Isolates 4	-	+	-	+
Isolates 5	+	-	-	+
Isolates 6	-	+	-	+

Table 3: Results of Sugar Fermentation Test

MOLECULAR CHARACTERIZATION

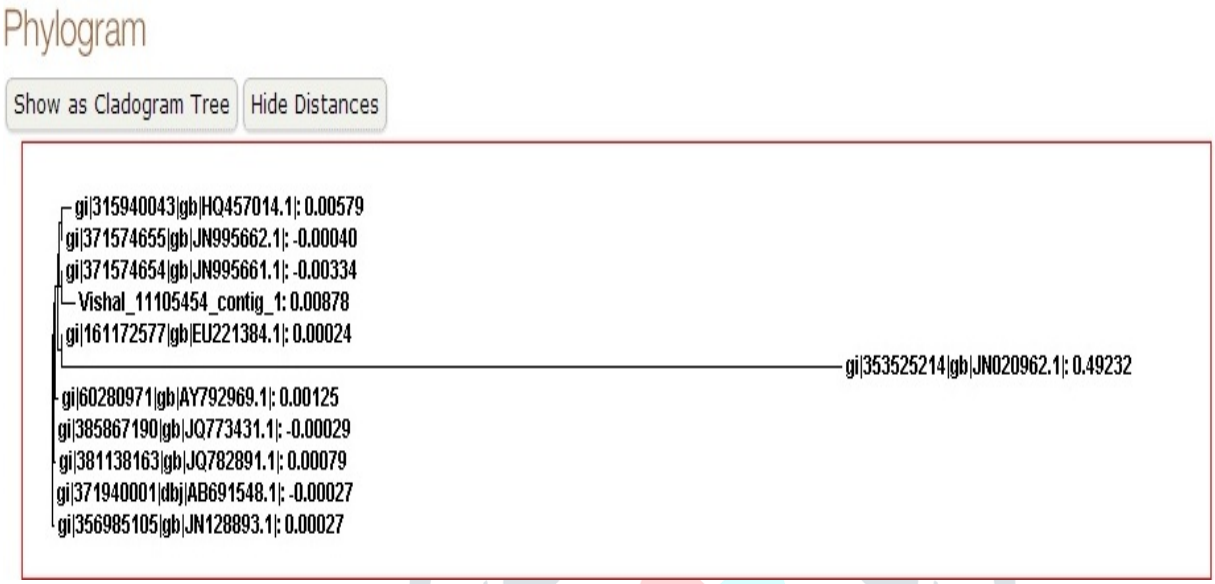
The molecular methods are used in addition to the isolation and biochemical characterization. It involves the examiner of the DNA sequence of particular bacterium either by evolutionary characteristics of organisms or by the sequencing of organisms DNA.

The isolated sample (1) was sent for the molecular characterization to the BR BIOCHEM LIFE SCIENCES. 16S rRNA sequences were examined for the bacteria for the identification (Cheneby D. *et al.*, 2000). Out of total 6 samples, One Sample (isolate 1) was sent for the molecular characterization in BR BIOCHEM LIFE SCIENCES. The 16S rRNA sequences were obtained from the company. The sequences were searched for similarity by using NCBI- BLASTn. Several sequences providing similar alignments were found but the top ten results of BLASTn were selected containing sequences (HQ457014.1, EU221384, JN995661, AY792969, JQ773431, JQ782891, JN995662, AB691548, JN128893 and JN020962) and used for the phylogenetic tree construction using ClustalW2. In ClustalW2 all the sequences were added and make the multiple sequences alignment the results shows the evolutionary relationship of our desired sequence against the existing bacteria

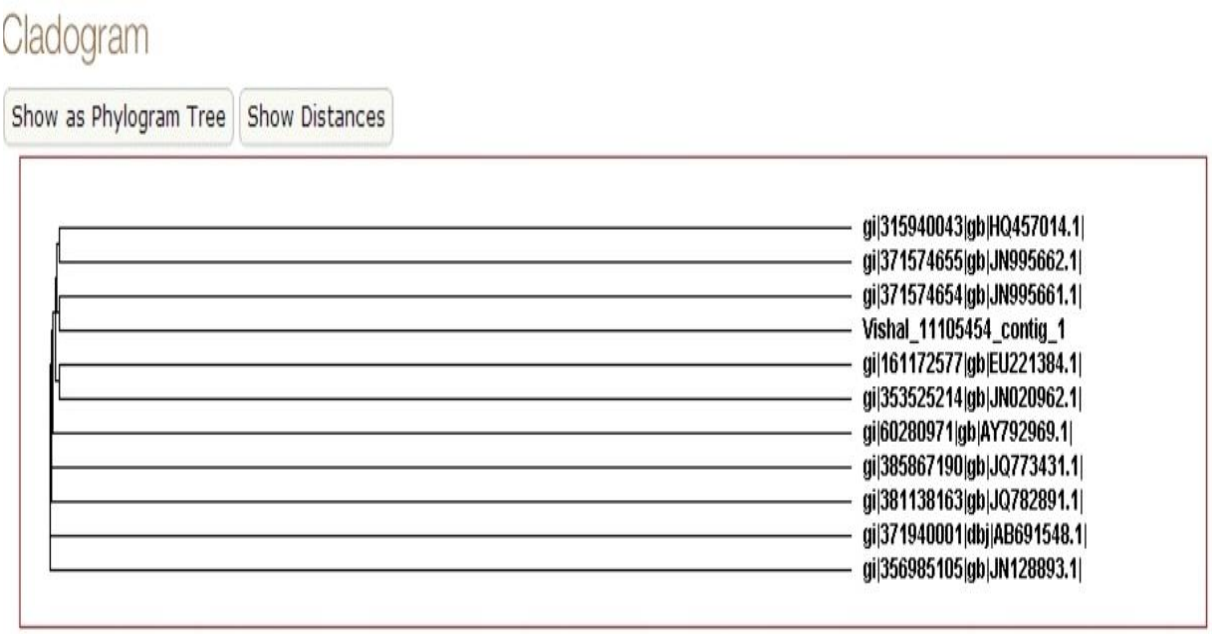
present. The results of microscopic, biochemical and Molecular characterization implemented that the organism was most related to *Pseudomonas aeruginosa* Strain HSD1.

6.9.1 PHYLOGENIC TREE CONSTRUCTION

The phylogenic trees were constructed using ClustalW2.



Phylogram tree



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