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Research Article

Isolation and Characterization of Antibiotic Producing Microorganisms from Soil Samples of Certain Area of Punjab Region of India

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ABSTRACT

Soil samples containing antibiotic producing organism are commonly employed for the production of suitable antibiotics. Isolated antibiotic may be bactericidal or bacteriostatic in nature. In the present investigation, antibiotics producing microorganisms were isolated from soil and tested against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Mycobacterium smegtitis, Proteus vulgris and Bacillus subtilis. Disc diffusion method, agar well method, streak agar method and biochemical methods have been employed to investigate the antibiotics producing microorganisms. Out of twelve isolates, two isolates; No. 5 and No. 6 were found suitable in inhibition of the growth of S. aureus and P. vulgris and zone of inhibition were found to be in ranging between 11 and 12mm respectively. The present work suggests that soil isolates, having antibiotic producing properties can be utilized commercially after proper standardization.

Keywords: Soil sample, Antibiotic producing microorganisms, Zone of inhibition, Streak agar method, Disc diffusion method, Agar well method.

INTRODUCTION

In the beginning of 20th century, the idea of growth inhibition of one microorganism present in the vicinity of other one came into existence. Later, it was demonstrated that growth inhibition of microorganism was mediated by secretion of certain toxic metabolites which was termed as antibiotic. In nature, there is universal distribution of antibiosis among the microorganisms owing to which they are involved in antagonism (Euanorasetr et al., 2010).

Those microorganisms which have capacity to produce more antibiotics can survive for longer time than the other producing antibiotics in fewer amounts. However, antibiotics produced by microorganisms have been very useful for the cure of certain human diseases caused by bacteria, fungi and protozoa. Due to continuous endeavor made in this field, the antibiotics discovered at present are about 5,500. Total world production of antibiotics is more than one million tons per annum. This success has been possible only due to continuous researches made during the last 4 decades (Walsh, 2003).

As more antibiotics were discovered, designed and studied and found that they had different properties. Some of these properties include their source, range of activity and their kinds. These were used to classify them into different categories such as ß lactam, tetracycline, rifamycins, aminoglycoside-aminocyclitol antibiotics, macrolide, polypeptide antibiotics, glycopeptide antibiotics etc. (Russell, 2004).

Most of the antibiotic producers used today are the soil microbes. Fungal strains and streptomyces members are extensively used in industrial antibiotic production. Bacteria are also reported for their antibiotic production. Bacillus species being the predominant soil bacteria because of their resistant endospore formation and production of vital antibiotics like bacitracin etc. have always been found inhibiting the growth of the other organisms. It is advisable to screen antibiotic producing bacteria as they are easy to isolate, culture, maintain and to improve their strains. Keeping in view the above-mentioned points and antimicrobial activity of soil isolates, the present study was aimed to isolate antibiotics producing strains and screening of isolates against test organisms.

MATERIAL AND METHOD

Soil collection: Soil samples were collected from different gardens, playgrounds and from nearby the laboratory of Lovely Professional University, Punjab, India.

Isolation: Serial dilution method was employed for isolation of strains producing antibiotics. Nutrient Agar (NA) was used for screening of bacteria while potato dextrose agar (PDA) was used for fungi. Media and water blanks were prepared and sterilized. Different dilutions (10-3, 10-4 and10-5) and (10-5 and 10-6) were used for fungi and bacteria respectively. Pour plating method was used during the present study. The plates were incubated at 37^0C for 24 hours for bacterial growth and 27^0 C for 72 hours for fungal growth.

Preservation of isolates: Total twelve soil isolates (No. 01-12) were isolated and these isolates were preserved in the slants of PDA and NA by keeping them into the refrigerator at 4^0C and maintained for longer period by serial subculturing.

In vitro screening of isolates for anti-bacterial activity: Twelve soil isolates were selected for anti-bacterial activity screening against the pathogenic test organisms by

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Table 1: Zone of inhibition in mm (millimeter)

S. No.	Isolates	*S. aureus*	*P. vulgris*
1.	I-5	12	11
2.	I-6	11	12

Table 2. Sensitivity of test organisms against various soil isolates

Soil isolates	*E.coli*	*P. aeruginosa*	*S.aureus*	*B.subtilis*	*P.vulgris*	*M. smegtitis*
I-1	-	-	-	-	-	-
I-2	-	-	-	-	-	-
I-3	-	-	-	-	-	-
I-4	-	-	-	-	-	-
I-5	-	-	++	-	++	-
I-6	-	-	++	-	++	-
I-7	-	-	-	-	-	-
I-8	-	-	-	-	-	-
I-9	-	-	-	-	-	-
I-10	-	-	-	-	-	-
I-11	-	-	-	-	-	-
I-12	-	-	-	-	-	-

Good inhibition++; No inhibition -

Fig. 1: Zone of inhibition by I-5 and I-6 against S. aureus

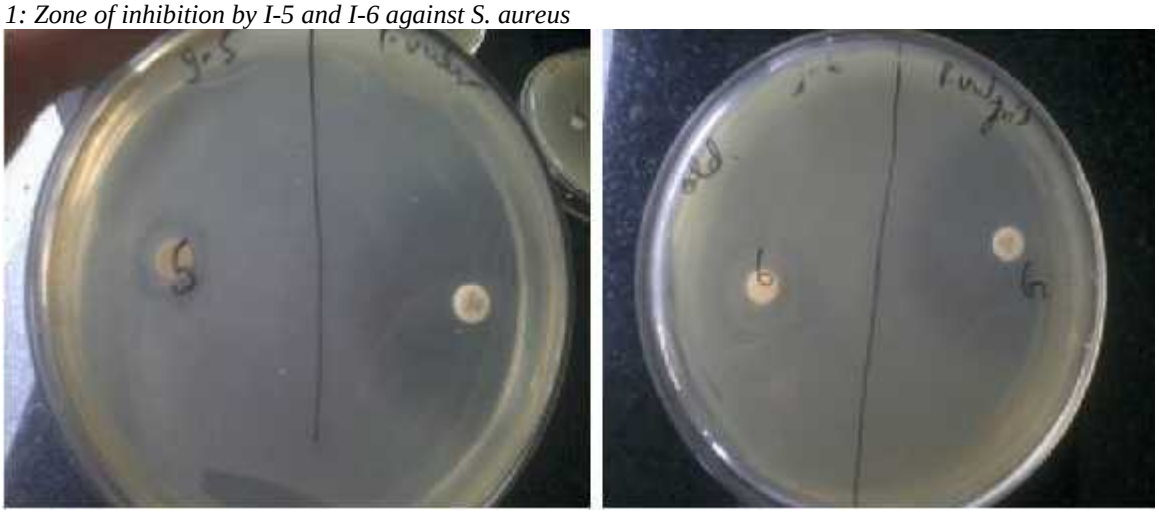

Fig. 2: Zone of inhibition by I-5 and I-6 against P. vulgris

Table 3. Biochemical characterization of I-5 and I-6

S. No.	Biochemical test	Isolate-5	Isolate-6
1.	Catalase	+	+
2.	Indole	+	-
3.	Methyl red	+	+
4.	VP	-	-
5.	Citrate	-	-

Good inhibition++; No inhibition -

streak agar method, disc diffusion method and agar well method. In streak agar method each of the isolate was streaked as a straight line on SBCD medium and incubated at 27°C for 6 days (144 hours). After the 6th day, different strains of test microorganisms were streaked at right angle without touching each other and then incubated at 37°C for 24 hours in the case of bacteria and 27°C for 48 hours in the case of fungi. If the organism is susceptible to the antibiotic produced by isolates, then it will not grow near the isolates. The zone of inhibition against each test organism was noted. In disc diffusion method, nutrient agar medium was prepared and sterilized followed by proper pouring into petri plates under aseptic conditions in a laminar air flow. Petri plates were kept for setting of medium for some time. The supernatant of production medium was collected and stored at 4^0C. Antibiotic activities were assayed for the supernatants using whatman paper discs. Test organisms cultures were aspirated out and the cultures were swabbed on petri plates followed by a proper placement of the antibiotic discs on the nutrient agar medium using forceps. Further, these Petri plates were incubated overnight without inverting at optimum temperature of 37^0C for 24 hours (Ahmed and Sani, 2013). Filter paper discs were taken in a petri dish and sterilized in oven at 110°C for 1 hour. The stock solution was placed on paper discs separately by micropipette so that antibiotic would be absorbed on discs. Gentamycin (50 µg/disc) was used as a standard disc. The sample impregnated discs and standard discs were placed gently on the solidified agar plates seeded with the test organisms to ensure contact with the medium. The plates were then kept in a refrigerator at 4°C for 4-6 h in order to provide sufficient time for diffusion of the compounds into the medium. After incubation, the antibacterial activities of the test samples were determined by measuring the radius of the inhibition zones. In agar well method, media plates were prepared, swabbed by the test organisms and wells were bored by preheated glass vials. These wells were filled with the supernatants of soil isolates which were centrifuged at 3000 rpm for 30 minutes followed by the incubation at 37^0C (NA) for 24 hours and 27^0C for (PDA) 48 hours without inverting them. The efficacy of antibiotic produced by soil isolates agents against the test organisms were assessed through measurement of zones of inhibition (Bizuye et al., 2013). Test organisms: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Mycobacterium smegmatis, Proteus vulgaris and Bacillus subtilis.

Gram staining: A smear of selected strain was prepared on a clean glass slide and the smear was allowed to air-dry and then heat-fixed. The heat-fixed smear was flooded with crystal violet and after one minute, it was washed with water and flooded with mordant Gram's iodine. The smear was decolorized with 95 % ethyl alcohol, washed with water and then counter-stained with safranin for 45 sec. After washing with water, the smear was dried with tissue paper and examined under oil immersion (100 X) (Williams et al., 1993).

Biochemical test-

Indole test: Indole test detects the production of indole from the amino acid tryptophan. One percent tryptophan broth in test tube glucose was inoculated with bacteria colony. After incubation period of 37°C for 48 hours, one mililitre (1ml) of kovac's reagent was added to the broth. The test tube was shaken gently and then allowed to stand for 20 minutes. The formation of red colouration at the top layer indicated positive and yellow colouration indicates negative test (Fay and Barry, 1974).

Catalase test: Catalase test used to detect the presence of catalase which converts hydrogen peroxide to water and oxygen. This test was carried out by putting a drop of hydrogen peroxide on a clean slide. With the edge of another slide, a colony of the microorganism was picked and allowed to contact to hydrogen peroxide. Presence of bubbles indicates positive reaction while absence of bubble indicates negative reaction (Chester, 1979).

MRVP test: Methyl red (MR) is a pH indicator to determine whether the bacterium carries out mixed acid fermentation. VP (Voges-Proskauer) used to detect the production of acetoin. Five milliliters (5ml) of MR VP broth was inoculated with the test organism and incubated for 48-72 hours at 37°C after which, one milliliter (1ml) of broth was transferred into a small tube. Small amount (2-3 drops) of methyl red was added. A red colour formation on addition of the indicator signified a positive methyl red test while yellow colour signified a negative test. To the rest of the broth in the original tube 4-5 drops of 4% KOH were added followed by 5% naphtol in ethanol. The test tube (sealed with cotton plug) was shaken and placed in a sloping position. The development of red colour started to appear from the liquid-air interface within 1 hour indicated a VP positive test while no colour change indicated a VP negative test (MacFaddin, 1980).

Citrate utilization test: This test determines whether or not the bacterium can use sodium citrate as a sole source of carbon. This is carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated for 24 hours to 72 hours. The development of deep blue colour after incubation indicated a positive result (Abdulkadir and Waliyu, 2012).

RESULTS AND DISCUSSION

A total of 12 isolates were obtained on the basis of inhibition. Out of these there were 5 bacteria and 7 fungi. Isolation was followed by the screening of isolates to test whether they inhibit the growth of test organisms or not. All these 12 isolates were screened against test organisms

E. coli, P. aeruginosa, S. aureus, M. smegtitis, P. vulgris and B. subtilis. Only Isolate-5 and Isolate-6 inhibited the growth of S. aureus and P. vulgris and these isolates were selected for further work (Table 1). Sensitivity of test organisms against various isolates has been shown in Table 2. The Biochemical characterization for Isolate-5 and Isolate-6 is presented in Table 3. Bacteria from soil sample are commonly employed for the antibiotic production. Bacteria are ubiquitous in nature. Antibiotic isolated may be bactericidal or bacteriostatic in nature. Production of antibiotic by microorganisms from soil is affected by nitrogen and carbon source.

CONCLUSION

In the present work, 12 antibiotics producing microorganisms were isolated. Out of these isolates, Isolate-5 and Isolate-6 were observed to inhibit the growth of S. aureus and P. vulgris while others test microorganisms were resistant towards the antibiotic. Both the isolates were confirmed to be gram positive. Results clearly unmasked antibiotic producing microorganisms from soil samples and warrants further investigation regarding characterization using molecular techniques for their identification.

REFERENCES

1. Abdulkadir, M., & Waliyu, S. (2012). Screening and isolation of the soil bacteria for ability to produce antibiotics. European Journal of Applied Sciences, 4(5), 211-215.
2. Ahmed, R. N., & Sani, A. H. (2013). Soil Screening For Antibiotic-Producing Microrgansms. Advances in Environmental Biology, 7(1).
3. Bizuye, A., Moges, F., & Andualem, B. (2013). Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia. Asian Pacific Journal of Tropical Disease, 3(5), 375-381.
4. Chester, B. (1979). Semiquantitative catalase test as an aid in identification of oxidative and nonsaccharolytic gram-negative bacteria. Journal of clinical microbiology, 10(4), 525-528.
5. Euanorasetr, J., Nilvongse, A., Tantimavanich, S., Nihira, T., Igarashi, Y., & Panbangred, W. (2010). Identification and characterization of soil-isolated Streptomyces SJE177 producing actinomycin. Southeast Asian Journal of Tropical Medicine and Public Health, 41(5), 1177.
6. Fay, G. D., & Barry, A. L. (1974). Methods for detecting indole production by gram-negative nonsporeforming anaerobes. Applied microbiology, 27(3), 562-565.
7. MacFaddin, JF. (1980). Biochemical Tests for Identification of Medical Bacteria, 2nd ed. Williams and Wilkins, Baltimore
8. Russell, A. D. (2004). Types of antibiotics and synthetic antimicrobial agents.Hugo and Russell's, 152.
9. Walsh, C. (2003). Antibiotics: actions, origins, resistance. American Society for Microbiology (ASM).
10. Williams ST., Locci R., Beswick A., Kurtboke DI., Kuznetsov VD., Lemonnior FJ., & Long PF. (1993). Detection and identification of novel actinomycetes. Res Microbiol, 144(8): 653-656.