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Research Article

ISOLATION OF ANTIBIOTIC PRODUCING BACTERIA FROM POND SOIL, GUDLAVALLERU

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ABSTRACT

Soil being a major reservoir for microorganisms it is a source of interest for isolation of antibiotic producing organisms. The emergence of antibiotic resistance and need for better, broad spectrum antibiotics is always in high demand. In the present study, antibiotic producing bacteria were isolated from a local soil sample. Total ten soil samples were collected from local pond aseptically and subjected to serial dilution. Crowded plate technique was employed for the isolation of the colony. Total five isolated were isolated which exhibited zone of inhibition around the colony. The isolated colonies were subjected to morphological, microscopical and biochemical characterization. All five colonies were found to be gram positive, non-sporulating organisms and found they belong to the *Actinobacteria* class. The isolated colonies were subjected to screening for antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Yeast* by perpendicular streak method. The primary screening results conclude that except one colony all have good antimicrobial activity. One colony found to be highly potential activity which had inhibition towards gram positive, gram negative, sporulating and fungal activity. This study may contribute in providing information on the antibiotic producing microorganisms in soil. Further characterization, purification, and structural elucidation are recommended to know the novelty, quality and commercial value of these antibiotics.

INTRODUCTION

Micro-organisms and their activities are crucially essential for all intents and purposes all procedures on Earth. They play a major role in human life. One such application is antibiotic production. Antibiotics are chemotherapeutic agents, which are powerful tool in the clinical management of diseases. Of all antibiotics available in nature only few tend to be useful based on their toxicity. In addition, the infectious bacteria tend to develop resistance for antibiotics in use. This makes an urge to discover new antibiotics which have clinical application⁽¹⁻³⁾.

Soil being a major reservoir for microorganisms it is a source of interest for

isolation of antibiotic producing organisms. Antibiotics are low molecular-weight (non-protein) molecules produced as secondary metabolites, mainly by microorganisms that live in the soil. While many antibiotics are known to exist, efforts to discover new antibiotics still continue. Hence, species such as *Streptomyces*, *Bacillus* and *Penicillium* have been researched constantly for their antibiotic production capability. *Bacillus* species, the predominant soil bacteria because of their resistant endospore formation and production of vital antibiotics⁽⁴⁾. The major antibiotics reported till date are from actinomycetes. With large number of genes encoding they offer a wide scope for exploring

novel antibiotics. It is advisable to screen antibiotic producing bacteria and to improve the strains⁽⁵⁻⁹⁾. Based on the review- the present study aims to isolate antibiotics producing strains and screening of isolates against test organisms.

Material and Methods

Collection of Soil Sample

From local pond of Gudlavalleru 10 samples were collected aseptically from various locations. The collected samples were transferred to the laboratory, where entire work is carried. The composite sample was preserved aseptically for the further work⁽¹⁰⁻¹¹⁾.

Isolation

For isolation of strains serial dilution method was employed. Different dilutions i.e., 10^{-3} , 10^{-4} and 10^{-5} were used as inoculum and nutrient agar is used for isolation of colonies. Spread plate method was employed. The plates were incubated at 37°C for 48hrs for bacterial growth. The incubated plates were observed for the colonies with zone of inhibition. The colonies with zone of inhibition were isolated and subjected for purification by multiple streak technique. Repeated streaking on starch case in agar plates led to purify bacterial colonies that showed actinomycetes like appearance. The isolated strains are preserved at 4°C for further study. Thus, isolated colonies were subjected to morphological and biochemical screening⁽¹²⁾.

Identification of Isolates

The microbial isolates were subjected for gram staining, spore staining and motility testing. In gram staining a clean glass slide was taken. Smear was prepared by heat fixing and air drying. Drop of crystal violet was added on smear and allowed to stand for 60 sec and then washed with the distilled water. Then gram iodine was added and stands for 30 sec. After though decolorizer was added to title the slide followed by safranin and stand for 60 sec and was washed and dried and viewed under oil immersion lens whereas in

spore staining a clean glass slide was taken. For spore staining malachite green was added on smear and steam for 5-7 min. Safranin was added which act as counter strain and was allowed to stand for 60 sec. Slide was then washed and dried and viewed under oil immersion microscope lens. The motility test is performed by hanging drop technique⁽¹³⁾.

Further identification biochemical tests were performed as described by the Bergey's manual i.e., IMViC tests (indole test, citrate utilization test, catalase test, MR-VP test), triple sugar iron test (TSI), oxidase, urease, nitrate reduction and Gelatine hydrolysis⁽¹⁴⁾.

Screening for Antimicrobial Activity

Primary screening for evaluating the antimicrobial potential of the isolated cultures was performed by perpendicular streak method. Isolates were screened for antagonism studies by inoculating a single streak of the pure producer organism in the middle of the assay media plate. The plates were incubated for 4 days at 28°C and subsequently seeded with test organism by a single streak at a 90° angle to the streak of the "producer strain" and finally the plates were incubated for 1-2 days at 28°C. The microbial interactions were analyzed by observing the distance of inhibition⁽¹⁵⁾.

Results and Discussion

Isolation of Microorganisms

Plates were observed for the presence of any colony with a clear zone around it. Plates with approximately 300-400 colonies were selected that showed crowd but well demarcated colonies. Five different types of colonies from dilutions 10^{-3} and 10^{-4} were found to show clear zone of inhibition around them. The five isolated colonies were subjected to morphological characterization. Like their colony shape, colony colour, staining techniques and motility. The observations are recorded in table-1.

Table 1: Morphological Characterization

Name of colony	Colony Features		Cell Features			Motility
	Colour of colony	Nature of colony	Gram Staining	Shape	Endospore	
Colony 1	White	Circular, flat & smooth	Gram positive	Cocci	Non sporulating	Non motile
Colony 2	Red	Rough, irregular & raised	Gram positive	Cocci	Non sporulating	Non motile

Colony 3	Yellowish orange	Irregular, convex & lobate	Gram positive	Rod	Non sporulating	Non motile
Colony 4	White	Circular large colony, raised undulate	Gram positive	Cocci	Sporulating	Non motile
Colony 5	White	Punctiform, flat & entire	Gram positive	Cocci	Non sporulating	Non motile

Biochemical Characterization

The isolated colonies were further subjected to biochemical testing. Biochemical tests are the tests used for the identification of bacteria species based on the differences in the biochemical activities of different bacteria. These differences in carbohydrate metabolism, protein metabolism, fat metabolism, production of certain enzymes, ability to utilize a particular compound, etc., help them to be identified by the biochemical tests. The observation of biochemical characterization was compiled in table-2. By the observation of morphological and biochemical characterization and comparing to standard literature the organisms were found to belong to *Actinobacteria*.

Table 2: Biochemical Characterization

Biochemical Test	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
Catalase test	(+) ve				
Oxidase test	(+) ve	(+) ve	(-) ve	(-) ve	(+) ve
Nitrate reduction test	(+) ve	(+) ve	(-) ve	(+) ve	(+) ve
Gelatin hydrolysis test	(-) ve	(+) ve	(+) ve	(+) ve	(+) ve
Casein Hydrolysis	(+) ve				
Urease test	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve
Triple sugar iron test	A/A	A/A	A/A	K/A	A/A
Indole test	(+) ve	(-) ve	(-) ve	(-) ve	(+) ve
Methyl Red test	(+) ve	(-) ve	(+) ve	(-) ve	(+) ve
Voges Proskauer test	(-) ve	(-) ve	(-) ve	(+) ve	(-) ve
Citrate utilization test	(-) ve	(-) ve	(+) ve	(-) ve	(+) ve
Starch Hydrolysis test	(+) ve	(-) ve	(-) ve	(+) ve	(+) ve

(+) ve = Positive, (-) ve = Negative, A/A-acid slant and acid butt, K/A- Alkaline slant and Acid Butt

Screening for Antimicrobial Activity

Primary screening is carried out by using *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Yeast* by Isolates were screened for antagonism studies by inoculating a single streak of the pure producer organism in the middle of the assay media plate. The plates were incubated for 4 days at 28°C and subsequently seeded with “test” organism by a single streak at a 90° angle to the streak of the “producer strain” and finally the plates were incubated for 1–2 days at 28°C. The results are presented in table -3.

From the observation it is clear that colony 3 exhibited antimicrobial properties towards both bacteria and fungi. Colony 4 do not possess antibiotic release capability and remaining 3 colonies i.e., colony 1, 2 & 5 limited antibacterial properties.

Table 3: Antimicrobial activity of isolated colonies

Test organism Colony	Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus	Bacillus subtilis	Yeast
Colony 1	No Inhibition	Inhibited	No Inhibition	Inhibited	No Inhibition
Colony 2	Strong Inhibition	Strong Inhibition	Strong Inhibition	Moderate Inhibition	No Inhibition
Colony 3	Strong Inhibition	Strong Inhibition	Strong Inhibition	Strong Inhibition	Strong Inhibition
Colony 4	No Inhibition	No Inhibition	No Inhibition	No Inhibition	Moderate Inhibition
Colony 5	Moderate Inhibition	Strong Inhibition	Strong Inhibition	No Inhibition	Strong Inhibition

CONCLUSION

The present study was an attempt to identify and characterize versatile strains of bacteria and to check their ability for antibiotic production. By performing crowded plate technique, five potential colonies with zone of inhibition were isolated. By performing morphological characterization, they all five colonies were found to be bacteria, of which four colonies were found to be gram positive cocci and one colony gram positive Rod. By performing morphological study and biochemical characterization they belong to the *Actinobacteria* class. The primary screening results conclude that the except one colony all have good antimicrobial activity. Isolated colony 4 was found to be highly potential activity which had inhibition towards gram positive, gram negative, sporulating and fungal activity. This study may contribute in providing information on the antibiotic producing microorganisms in soil. Further characterization, purification, and structural elucidation are recommended to know the novelty, quality and commercial value of these antibiotics.

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