

Production and Qualitative Analysis of Secondary Metabolites of Staphylococcus Bacteria having Antibiotic Activity

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Abstract:

Antibiotics are one of the most important commercially exploited secondary metabolites produced by the bacteria and employed in a wide range. Most of the antibiotic producers used today are the soil microbes and the antibiotics are the secondary metabolites of these cells. With the increasing applications of these commercially available antibiotics they are becoming more expensive and hence it is the time to discover a more cost effective method for their production. The current work aims to use the soil bacteria in the field of antibiotic production due to their abundant availability. The work involves the isolation of the bacteria from the selected area and the production of pure cultures. As all the bacteria are not capable of antibiotic secondary metabolite production, the isolates would be subjected for the initial screening for the sensitivity to the test antibiotic ampicillin. Here ampicillin is selected as the test or reference antibiotic to which the activity of the metabolites produced is compared. Ampicillin resistant strains were selected as ampicillin is the referenced antibiotic. The antibiotic production was further confirmed by Zone of Inhibition test in comparison with the commercial ampicillin. Once the bacteria capable of antibiotic secondary metabolite production are isolated its identification is done based on Biochemical tests. Finally the antibiotic metabolites are produced which can be further purified.

Keywords: antibiotic, soil bacteria, isolation, production

Introduction:

Antibiotics are chemical compounds used to kill or inhibit the growth of infectious organisms. All antibiotics share the property of selective toxicity. They are more toxic to an invading organism than they are to an animal or human host. Penicillin is the most well-known antibiotic and has been used to fight many infectious diseases, including syphilis, gonorrhea, tetanus, and scarlet

fever. Another antibiotic, streptomycin, has been used to combat tuberculosis. Antibiotics can be classified in several ways. The most common method classifies them according to their action against the infecting organism. Some antibiotics attack the cell wall; some disrupt the cell membrane; and the majority inhibits the synthesis of nucleic acids and proteins, the polymers that make up the bacterial cell. Most antibiotics act by selectively interfering with the synthesis of one of the large-molecule constituents of the cell—the cell wall or proteins or nucleic acids. Some, however, act by disrupting the cell membrane. Many antibiotics operate by inhibiting the synthesis of various intracellular bacterial molecules, including DNA, RNA, ribosomes, and proteins. The synthetic sulfonamides are among the antibiotics that indirectly interfere with nucleic acid synthesis.

The production of a new antibiotic is lengthy and costly. First, the organism that makes the antibiotic must be identified and the antibiotic tested against a wide variety of bacterial species. Then the organism must be grown on a scale large enough to allow the purification and chemical analysis of the antibiotic and to demonstrate that it is unique. This is a complex procedure because there are several thousand compounds with antibiotic activity that have already been discovered, and these compounds are repeatedly rediscovered. After the antibiotic has been shown to be useful in the treatment of infections in animals, larger-scale preparation can be undertaken.

The use of antibiotics is limited because bacteria have evolved defenses against certain antibiotics. One of the main mechanisms of defense is inactivation of the antibiotic. This is the usual defense against penicillin and chloramphenicol, among others. An antibiotic is a selective poison. It has been chosen so that it will kill the desired bacteria, but not the cells in body. Antibiotics work to

kill bacteria. Bacteria are single-cell organisms. If bacteria make it past our immune systems and start reproducing inside our bodies, they cause disease. We want to kill the bacteria to eliminate the disease. Each different type of antibiotic affects different bacteria in different ways. For example, an antibiotic might inhibit a bacterium's ability to turn glucose into energy, or its ability to construct its cell wall. When this happens, the bacterium dies instead of reproducing. At the same time, the antibiotic acts only on the bacterium's cell-wall-building mechanism, not on a normal cell's.

Material and methods:

1. Sample Collection:

The soil sample was collected from a hospital, LB Nagar, Hyderabad. One gram of soil was used for the isolation of bacteria.

2. Isolation of bacteria from soil by serial dilution method:

10 clean and sterilized test tubes were taken and to them 9ml distilled water was added. In order to decrease the bacterial concentration and obtain isolated colonies on the NAM serial dilution was performed for 10 old dilutions. From the last tube 0.1 ml of the inoculums was used to spread plate on a fresh NAM plate. The plate was incubated at 37°C for 24hrs. The plate thus obtained would serve as the master plate.

3. Preparation of Pure Cultures:

80ml of nutrient agar was prepared and kept in autoclave for sterilization. Two test tubes were taken to which 20ml of nutrient agar was added. To one of the four tubes 10ml of ampicillin (reference antibiotic) was added whereas the other was left as control. All the tubes were sterilized and the media was poured into sterilized Petri plates. Selected colonies from the master plate were picked and streaked onto the above plates. The plates were incubated at 37°C for 24hrs.

4. Screening test for Antibiotic Production in the presence of reference antibiotic Ampicillin:

40ml of nutrient broth media was prepared. Four sterilized test tubes were used into which 10ml of nutrient broth was poured. In two of the tubes, 5ml of ampicillin was added and the other tubes were left without ampicillin (controls). Each tube was inoculated with different bacteria under laminar air

flow. Tubes were incubated at 37 °C overnight. The Growth of the Organisms indicated their Resistance to the ampicillin, reference antibiotic.

5. Identification of the Isolated Bacteria based on Gram's Nature and Biochemical tests:

To identify the bacteria that was resistant to the ampicillin, reference antibiotic Gram's test was performed. Gram's test identifies the gram nature of the organisms. Once the test is performed based on the results the other biochemical characterization can be made.

Biochemical tests: Most bacteria are identified and classified largely on the basis of their reactions in a series of biochemical tests. Some tests are used routinely for many groups of bacteria (oxidase, nitrate reduction) other are restricted to a single family, genus, or species (coagulase test for staphylococci).

Catalase test: To a drop of hydrogen peroxide on the glass slide one loop full of the test culture was added. Bubble formation indicates the positive test for catalase.

Mannitol fermentation test: Mannitol fermentation broth was prepared as per the composition and sterilized by autoclaving. Each of specified fermentation tubes were labeled as per the organisms to be inoculated with the test bacterium and one uninoculated tube of fermentation broth was kept as a comparative control. Durham's tube was inserted in the test tube gently.

6. Qualitative test to identify the Zones of inhibition:

40ml of nutrient agar was prepared and autoclaved. To this 20ml of ampicillin was added and poured in two sterilized Petri dish. Bacterial inoculum from master plate was taken and spread on agar culture. The plates were incubated at 37°C overnight.

7. Qualitative test for the Production of Antibiotic Secondary Metabolites based on Zone of Inhibition:

20ml of nutrient agar was prepared, after autoclaving, it was poured into Petri dish and allowed to solidify. With the help of a spreader an ampicillin sensitive culture was spread onto the medium. Now with the help of a sterilized boarer three wells were punched into the plate. Into one of the wells standard ampicillin liquid was added, and into the second well the test broth containing the secondary metabolites was added. The third well must be added with

sterilized distilled water that acts as a control. The plates were at 37°C for 24 hours. Observed the zone pattern.

Results and discussion:

Colony Morphology on Agar Paltes:

The shape, margin and pigmentation of the colonies developed were noted down as follows:

Colony1: white colored colonies in irregular shape were observed.

Colony2: cream colored, circular, flat colonies

Colony3: white colored, small

Colony4: cream colored, irregular

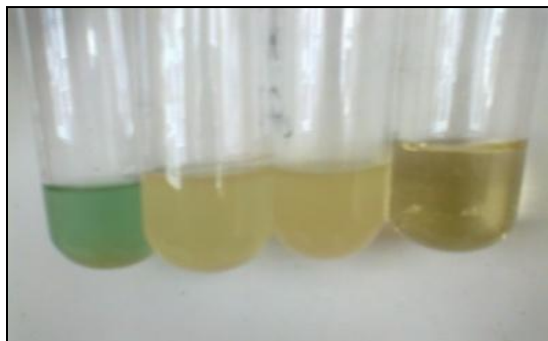
Fig 1: Master plate with Isolated Colonies:



Screening Test for Antibiotic Production:

From the results of the screening test it was found that the bacteria 1 was capable of growing in the presence of ampicillin whereas the other bacteria were incapable, which indicates that only the first bacteria is ampicillin resistant. Further tests were performed to confirm the identity of this bacterium.

Fig 2: Test Tubes Showing the Broth in the Screening Media with 4 isolates:



Biochemical test: As per the Bergey's Manual the Biochemical tests were performed. The tests to which the bacteria responded were shown below:

The Bacteria were gram positive cocci in chains; the first test performed was **Catalase test** which gave a positive response. The bacteria were capable of producing the enzyme Catalase. The bacteria were **Catalase Positive**.

The Next test in the series is the **Mannitol fermentation test**. The Bacteria was proved to be **Mannitol Fermentation positive**.

From the above two test results the bacteria is identified as **Streptococcus aureus**.

Screening for the Zone of Inhibition:

From the results of the qualitative inhibition test it can be concluded that the zone of inhibition was seen in both standard antibiotic well as well as the broth well. There was no zone around the distilled water well. This concludes that the broth do contain the antibiotic produced which inhibits the bacterial growth around its well. The Fig 3 displays the zones formed

Fig 3: The zones of inhibition formed by Standard antibiotic and Sample broth:



W= standard Ampicillin, C = Test Broth, E= Sterile water

Conclusion:

The current work aimed to identify the ability of the commonly found soil bacteria to resist to the antibiotics and the use of these bacteria in the industrial production of the antibiotic secondary metabolites. The organism that was isolated was first subjected for antibiotic sensitivity against the reference antibiotic ampicillin. The organisms were further subjected for Gram's staining and biochemical tests for their identification. The bacteria were found to be *staphylococcus aureus*. This bacterium was further employed for the production of

antibiotic metabolites that can inhibit the growth of the selected bacteria. Here we have selected the ampicillin sensitive bacteria as the target species. The efficacy of the bacteria was tested based on zones of inhibition. This was further compared with the commercially available ampicillin. It was concluded that these secondary metabolites can be used in place of ampicillin as they exhibit equivalent inhibitory activity. The work has to be further extended by increasing the number of target organisms and the antibiotic range.

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