2nd International Conference of Multidisciplinary Approaches (iCMA), 2015
Faculty of Graduate Studies,
University of Sri Jayewardenepura,
Sri Lanka

ISSN: 2386 – 1509
Copyright © iCMA

ISOLATION OF ANTIBIOTIC PRODUCING BACTERIA FROM SOIL AND STUDYING THE IMPACT OF CARBON SOURCE ON ANTIBIOTIC YIELD

M.M. Gunawardena, S.K.Jayasekara

Department of Microbiology, University of Kelaniya, Kelaniya, Sri Lanka

sandhyajayasekara@gmail.com

Antibiotics are required for the treatment of infectious diseases caused by microorganisms. Many causative agents have developed resistance against antibiotics that have been used against them .Therefore, it is vital to look for new antibiotic producers.This study was done to screen for antibiotic producing bacteria in soil.

Samples collected from different soils were analyzed for the presence of antibiotic producing bacteria. First, soil bacterial colonies were obtained on nutrient agar plates by pour plate method. For this purpose, a di lution series from 100 to 10-7 was prepared using sterile distilled water. After a 48-hour incubation period, well-separated and morphologically different colonies on these plates were given a number for easy recognition. Then the antibiosis of these suspected colonies against selected known bacterial species was tested. *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* were used as known test bacteria.

For testing antibiosis of selected colonies, both primary and secondary screening was done. Primary screening was done by spot test. In this test, first of all overnight broth cultures of each known bacterium were prepared. This was done by inoculating nutrient broth (50 ml broth in 250 ml flasks) with loopful of bacterium and incubating it overnight in a shaker. Then they were introduced onto nutrient agar surface by spread plate method. After that suspected soil bacteria were spotted on each test bacterial spread using a sterile inoculating loop. The formation of inhibitory zones around the suspected colonies was observed. The pure cultures of the colonies that gave inhibitory zones were obtained by streak isolation method.

Next, secondary screening was done. Both cross streak method and agar well diffusion method were used for this. In the cross streak method, known bacterium was first introduced onto nutrient agar surface as a streak line and then the suspected soil bacterium was inoculated over it as a single line. The formation of inhibitory zones along the isolate was observed. Agar well diffusion method was performed in Muller Hinton agar plates. The known bacterium in overnight broth was introduced onto the surface by spread plate method and small wells were formed on agar. Filtered solution of an overnight broth culture of suspected soil bacterium was used as the

antibiotic solution and it was filled into wells. The formation of inhibitory zones around the wells was observed. The antibiosis of each suspected soil bacterial isolate was confirmed by this screening method.

Using morphology as well as biochemical testing, suspected soil bacterial cultures were identified up to species level.mThe most promising antibiotic producer was further investigated in order to determine the effect of carbon source on the antibiotic production using different carbon sources; glucose, sucrose, fructose, starch, peptone, xylose and mannitol. M9 minimal medium which is a liquid medium containing all elements required for microbial growth was used here. It was treated with the relevant carbon source. The initial concentration of carbon source in this medium was 1%.Loopful of the isolate was inoculated into a 250 ml flask containing 50 ml of above medium. It was incubated in a shaker at 150rpm for more than 18 hours. This broth was used as the antibiotic solution for the analysis. Before further analysis this solution was plate in nutrient agar by pour plate method just to determine whether a microbial growth has occurred.

After that the filtered broth was diluted using twofold broth dilution method (from 1:1 to about 1:128).This was used as the antibiotic solution series with different concentrations .If the antibiotic is present its concentration is reduced along the series. Then the known test bacterium (*S.aureus* and *E. coli* were tested separately*)* was inoculated as an overnight broth (0.10 ml) into each tube in the dilution series. The tubes were incubated for 18 hours at 37 0C temperature and compared the growth with a control which was prepared by inoculating known bacterium into nutrient broth. The turbidity was compared here. Then the highest dilution at which the growth was not observed was reported. According to the carbon sources used, this point up to which the growth was not given was different. If the inhibition was reported in higher dilution that could be due to the presence of higher yield in the initial solution and although it was highly diluted the active concentration still remained and *vice versa.*

According to the results, four bacterial isolates were identified as *Pseudomonas aeruginosa, P. putida, Chromobacterium violacium* and *Alcaligenes faecalis.* All isolates exhibited inhibitory activity against *S. aureus*. Except the antibacterial compound produced by *P. putida*, the compounds produced by all other were inhibitory against *B.subtilis* as well. Among all the isolates, *P. aeruginosa* was the most promising antibiotic producer, in which the inhibitory substance was effective against *E. coli* also. Therefore *P.aeroginosa* was used for further analysis.

When *P.aeroginosa* was introduced into different carbon sources, the growth was not observed in starch, fructose and sucrose containing media. Not surprisingly, the inhibition was not observed in test tubes. Even in the initial tubes the turbidity was observed clearly. Therefore, it was concluded that these carbon sources were not utilized by the microorganism for antibiotic production.

When glucose, peptone, xylose and mannitol were used as the carbon source, bacterial colonies were observed in nutrient agar plates. That means these carbon sources were utilized by bacterium for growth .In the two fold dilution series of the antibiotic solution, when glucose was used, the growth was inhibited up to 1: 64 dilution and after that the turbidity was observed. That means from the initial tube up to that dilution, the antibiotic is present in the medium in an active concentration against the test organism. In other words, in the initial broth there was a considerable yield of the antibiotic and it was diluted in the series up to 1:64 levels. When peptone, xylose

mannitol were used this level was consecutively reduced (1:16, 1:4 and 1:1 consecutively) Therefore it was concluded that glucose is the carbon source that gave the highest antibiotic yield. This could be explained as due to rapid population increments that took place in the glucose containing medium. When the population gradually increases in the medium the nutrient limitations could be expected with times which lead to antibiotic production in the relevant medium in a higher yield than with other carbon sources.

Keywords: *optimum antibiotic production, Pseudomonas aeruginosa, antibiotic resistance, antibiosis, antibiotic yield*