STUDY ON ANTIMICROBIAL ACTIVITY OF BACTERIA ISOLATED FROM THE SOIL OF MEIKTILA UNIVERSITY CAMPUS

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Abstract

Naturally soil is rich in microorganisms capable of antibiotic synthesis but the frequency which synthesis occurs at ecologically significant levels has been much less clear. Five bacterial species were isolated. The bacterial species were identified by their cellular characteristics, colonial morphology and biochemical tests. Most antibiotic used today are isolated and extracted from microbial sources. In the present study, antibiotic producing bacteria were isolated from a local soil sample collected from the soil of Meiktila University Campus. The study area were selected and soil samples were collected from different parts at 6"depth. Antibiotic is one of the most important commercially exploited secondary metabolites produced by bacteria and employed in a wide range. Most of the antibiotics used today are from the microbes. Bacteria are easy to isolate, culture, maintain and to improve their strain. In the present research study, soil bacteria with the antibiotic activity was screened and isolated. The media used in this research was nutrient agar medium by serial dilution method. In finding out their biological properties, five test organisms were used to study the presence of antimicrobial activities. Isolated TM-5 showed high antibacterial activity.

Keywords: Soil bacteria, Antimicrobial activity, Meiktla University

Introduction

The soil contained millions of microorganisms and approximately more than 85% of them are important for plant life and provide precious life to soil systems. Moreover, soil microorganisms that are closely associated with roots play a vital role in stimulating plant growth (Aly et al., 2012)

The term soil refers to the outer loose material of the earth crust. It may be regarded as a three phase's system composes of solids, liquids and gases, dispersed to form a heterogeneous matrix. On the whole the soil is composed of five major components, including Mineral matter, Water, Organic matter, Air and living Organisms.

The bacteria are the most abundant group usually more numerous than the other four combined. Soil bacteria can be rod (bacilli), cocci (spherical), spirilla (spirals), of these, bacillus are more numerous than the others. They are one of the major groups of soil bacteria population and are very widely distributed. The number and type of bacteria present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matters contents, cultivation, aeration and moisture content.

In this study, the soil bacteria were collected from two different places, location 1(in front of the Botany Department) and location 2 (near the Geography Department). To study morphological characters, biochemical characters and antimicrobial activities of isolated bacterial strains, the isolated strains were cultured in nutrient agar media (Atlas, 1993), one day and two days old culture of isolated strains were used.

The aim and objectives are to isolate and characterize the soil microorganisms, to determine the variety of microorganisms that live in soil and to study antimicrobial activities of isolated bacterial strains.

Materials and methods

Collection of soil samples

Soil sample was collected from location 1(in front of the Botany Department) and location 2 (near the Geography Department). These samples were taken and the experiments were carried out at the microbiology laboratory of Botany Department Meiktila University from June to August 2016.

Isolation of species from soil sample

Serial dilutions of fermented, plating and streaking techniques described by Salle (1948) Collins (1964) and Pelezar and Chan (1972) were used for the isolation of species from soil. An appropriate amount (1g) of soil was introduced into a conical flask containing 99mL of distilled water to make a soil water dilution ratio of 1:100. The flask was then shaken for about 30 minutes in order to make the soil particles free from each other. This solution was then serially diluted into 10^{-1} to 10^{-5} dilution in separate test tubes and 1mL each of the above dilutions was separately transferred into sterile petridishes under aseptic condition. A sterile pipette was used for each transfer. An appropriate amount (10mL) of the medium was separately into test tubes and plugged with non-absorbent cotton wool. They were sterilized by autoclaving at 15 pounds pressure per square inch for 15 minutes at 121ºC. The sterilized medium in each conical flask was cooled down to about 45˚C and separately poured into the petridishes containing the

respective fermented soil dilutions. The inoculated plate was shaken clock-wise and anti clock-wise direction for about 5 minutes so as to make uniform distribution of the bacterial inoculums. When the agar was solidified, the inoculated plates were inverted and incubated at 30˚C for 24 hours. Various types of colonies developed on the inoculated plates were separately streaked over another set of petridishes containing the same sterile medium. Each of the discrete colonies visible in the second set of inoculated plates was separately transferred to sterile nutrient medium cultured repeatedly so as to obtain a pure culture of isolated species. The isolates were maintained in nutrient the media for further experimentations.

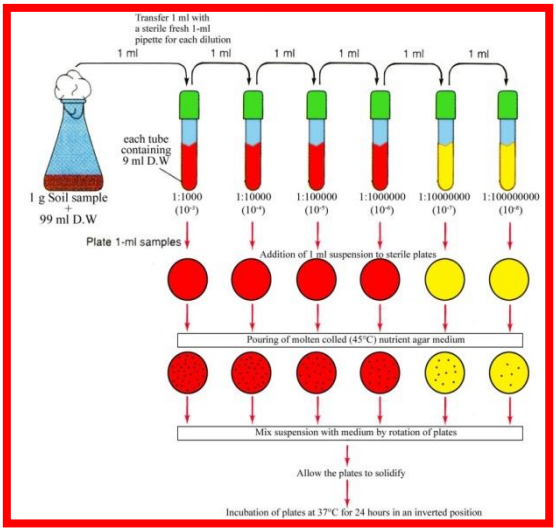


Figure 1. Serial dilution method used for the isolation of spp.

Isolation of pure culture from plates

Nutrient agar medium was used for the isolation of bacterial colonies from soil samples.

About 100mL of nutrient media were separately distributed into test tubes. The test tubes were plugged with cotton wool and sterilized by autoclaving them at 15 pounds pressure per square inch for 15 minutes at 121˚C. The sterilized media were cooled down. The separate colonies appear and the different types of bacterial colonies were cultured in test tubes. The slants of medium were repeatedly sub-cultured to obtain pure culture (Atlas, 1993).

Preparation of culture medium

Nutrient medium (Atlas, 1993)

Yeast	5.0 g
NaCL	5.0 g
Beef extract	5.0 g
Agar	25 g
Distilled water	1000 mL
pH	7.0 ± 0.2

After autoclaving, Nystatin (1.5 mL) was added to the medium.

Test bacteria

The test bacteria used in this study were obtained from DCPD, Yangon.

Antimicrobial activity estimation

The study of antimicrobial activity was performed by paper disc diffusion method. Nutrient agar was prepared according to the method described by Cruickshank (1975). After autoclaving, 20 -25 ml of nutrient agar was poured into each petridish and made plating by using 0.1 to 0.2 ml of five test organisms (*Pseudomonas aeruginosa*, *Saccharomyces cerevisae, Staphylococcus aureus*, *Agrobacterium tumefaciens and Escherichia coli*). This respective plate were allowed to set for 2-3 hours. And then, 6mm paper disc was made with the help of sterilized. After that, about 0.2 ml of sample was introduced into the paper disc and incubated at room temperature for 24 hours. The clear zones appeared around the paper disc, indicated the presence of antimicrobial activity secreted by respective isolated strains. The extent of antimicrobial activity shown by clear zone was measured with the help of clipper.

Results

Collection of soil sample

The samples were collected from location 1 and location 2.

Figure 2. Two different positions of Soil samples

Isolation of bacteria from soil

A total of five strains were obtained from soil samples by using nutrient medium and designated them into TM - 1 to 5 respectively as shown in Table (1) and Figure (2 - 4).

Table 1. Designation of isolated bacterial strains from nutrient medium

Culture media	Designated strains
Nutrient Medium (Atlas, 1993)	TM - 1 to 5

Table 2. Biochemical characteristics of all isolated strains

No.	Tests	Strain No.				
		1	2	3	4	5
1	Gram Stain	+	-	-	-	-
2	Shape	cocci	bacillus	cocci	cocci	cocci
3	Motility	+	+	+	+	+
4	Aerobic/Anaerobic	aerobic	aerobic	aerobic	aerobic	aerobic
5	Casein hydrolysis	-	-	-	-	-
6	Catalase	+	+	+	+	+
7	Citrate Utilization	+	+	+	-	+
8	Glucose hydrolysis	+	+	+	-	-
9	H_2S Production	-	-	-	-	-
10	NaCl tolerance (2%)	+	+	+	+	+
11	NaCl tolerance (6%)	+	+	+	+	+
12	Nitrate Reduction	+	-	+	+	+
13	Starch Hydrolysis	+	-	+	+	+
14	Urease	-	-	+	-	-

+ = positive result, - = negative result

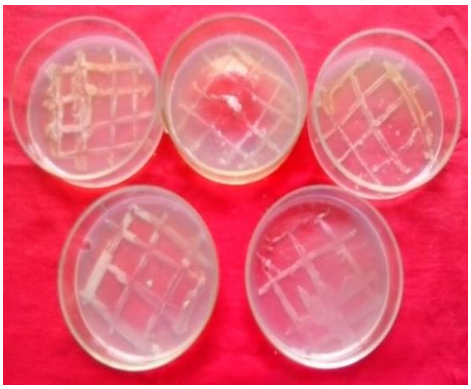
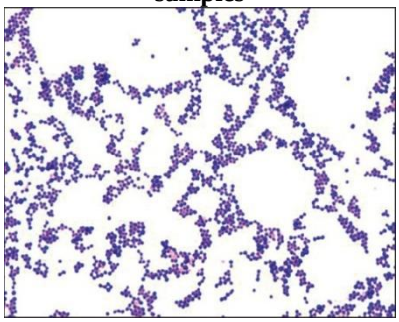
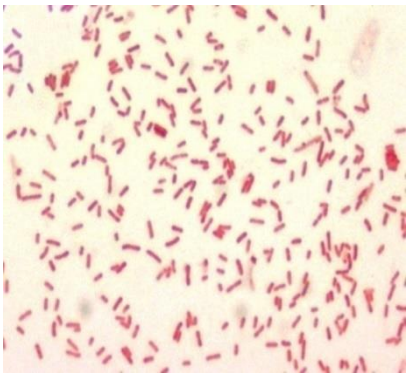


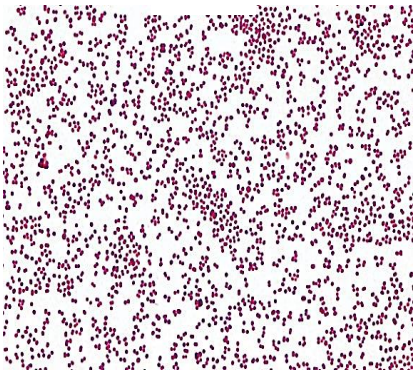
Figure 3. Pure culture of isolated bacteria from soil samples



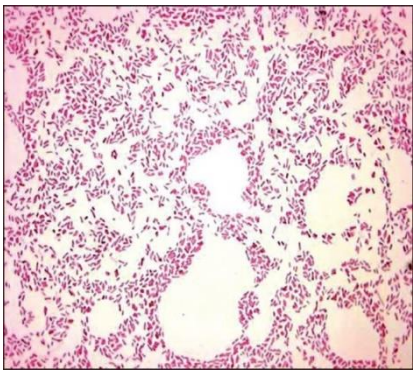
TM-1



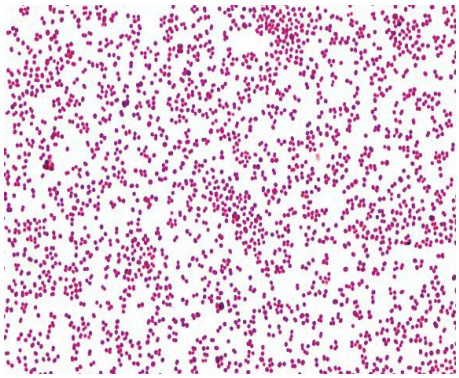
TM-2



TM-3



TM-4



TM-5

Figure 4. Staining of bacteria from isolated bacteria

Figure 5. Catalase test of the isolated strains

Figure 8. Nitrate test of the isolated strains of the isolated strains

Figure 6. Citrate test of the isolated strains

Figure 9. Starch hydrolysis of the isolated strains

Table 3. Antimicrobial activities of all isolated strains (paper disc– 6mm)

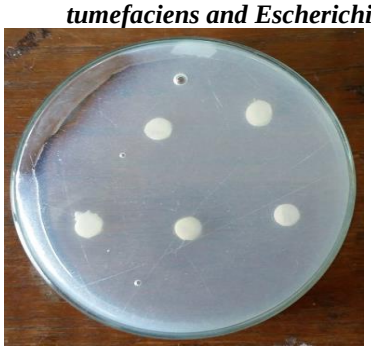
Strain No.	*Agrobacterium tumefaciens*	*Escherichia coli*	*Pseudomonas aerugesinosa*	*Saccharomyces sp.*	*Staphylococcus aureus*
TM-1	20mm	-	-	-	-
TM-2	_	_	_	_	_
TM-3	_	_	_	_	_
TM-4	_	15mm	22mm	23mm	20mm
TM-5	17mm	17mm	25mm	20mm	22mm

Figure 7. Glucose fermentation

Agrobacterium tumefaciens

Escherichia coli

Figure 10. Antimicrobial activity of all isolated bacteria Strains on *Agrobacterium tumefaciens and Escherichia coli*



Pseudomonas aerugesinosa

Saccharomyces sp.

Staphylococcus aureus

Figure 11. Antimicrobial activity of all isolated bacteria Strains on *Pseudomonas aerugesinosa, Saccharomyces sp.* and *Staphylococcus aureus*

Discussion and conclusion

In this investigation, five bacteria were isolated from Meiktila University Campus, collected soil samples culture on Nutrient Agar (NA) medium. Location of possible microbes, and soil sample were collected and chosen from this soil. The soil samples were subjected in the screening procedure, mainly by serial dilution method of Atlas (1988). Detection and estimation of the ability of microorganisms have been used to streak on agar and paced made/cut well methods. Based on the methods obtained with conventional analysis, five isolated bacteria from these soil were identified.

As a result of some biochemical characters such as cell morphological characters, gram-strain, catalase test, Lactose, Glucose, NaCl (2%,6%) tolerance , Ureases, Citrate, Soluble starch hydrolysis , geletinase and growth on 24˚C and 46˚C. All of the isolated strains from strain No.1 is gram positive(Cocci) , strain No 2 is gram negative (short-rod) and other are gram negative (Cocci). All of the isolated strains from strain No2 was given negative reaction and other are positive reaction in soluble starch hydrolysis and Nitrate reduction testing. All of the isolated strains was given negative in Catalase and Hydrogen sulphide testing. In Citrate and Glucose fermentation testing strain TM-1, TM-2 and TM-3 are positive and TM- 4 are negative except from strain TM-5 is negative in citrate and positive in glucose fermentation testing .

All of the strains had very active motility. Among them, not all these strains show Caseinase activity. According to Park *et al.,* (2010), production of halo zones (Clear zones) on solid Nutrient agar (NA) media is attributed to the release of organic acids such as Citric, Glyoxalic , Malic , Keto butyric and Succinic. Likewise, in present work, the clear zone was formed in the same way probably due to acid production. In this study, TM-1, TM-2, TM-3 and TM-4 strains did not showed antibacterial activities. But *Agrobacterium tumefaciens* was shown on antibacterial activity in TM-1 strain. TM-4 and TM-5

strains showed antibacterial activities, especially TM-5 against five test organisms. In TM-4 strains, *Agrobacterium turmefaciens* did not show antibacterial activity. TM-5 strains showed largest area of (25mm) clear zone, against the *Pseudomonas aerugesinosa*. Therefore TM-5 stains were found to exert prominent and largest clear zone which indicated highest antibacterial activity.

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