ISOLATION OF SOIL BACTERIA FROM NGATHAICHAUNG TOWNSHIP AND THEIR ANTIMICROBIAL ACTIVITIES

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Abstract

Soil samples were collected from three different areas of Ngathaichaung Township, Ayeyawady Region. These samples were cultured on Glucose Peptone Agar medium, Flo Agar medium and Dextrose Casein Peptone Agar medium. A total of 15 bacterial colonies were isolated from these soil samples. 5 isolates were obtained from Glucose Peptone Agar medium, 7 isolates from Flo Agar medium and 3 isolates from Dextrose Casein Peptone Agar medium. Isolated bacteria were designated as KM. In the colony morphology, the size of isolated bacteria were medium, large and very large. The margin of colonies were entire, lobate and undulate. Cell morphology of isolated strains were studied by Gram staining, colony morphology and shape of cell strains were short rod, other strains were long rod chain and rod chain. Three strains were Gram positive and other strains were Gram negative. Moreover, antimicrobial activities of all isolated strains were carried out by agar well diffusion assay with seven test organisms. Among them, 6 isolated strains showed the antimicrobial activity. KM-13 showed the higher antibacterial activity (27.77 mm and 27.63 mm) against *Escherichia coli* and *Bacillus pumilus.* Especially this strain exhibited the highest antifungal activities (31.17 mm) on *Malassezia furfur* followed by (25.46 mm) on *Candida albicans.* And then, KM-11 also showed the antibacterial activity (21.40 mm) on *Bacillus subtilis.*

Keywords: Soil bacteria, culture medium, antimicrobial activity

Introduction

Soil is a primary source of microorganisms. Soil bacteria and fungi have played a significant and an important role in antibiotic discovery.

The numbers and species of microbes in soil is depend on environmental conditions like nutrient avability, soil texture, presence of moisture in soil and type of vegetation cover and their number varies according to the type of environmental condition (Atlas and Bartha, 1998).

Natural products having novel structures have been observed to possess useful biological activities, soil is a natural reservoir for microorganisms and their antimicrobial products (Dancer, 2004).

Natural products from microorganisms have been the most successful source that has found many applications in the fields of medicine, pharmacy and agriculture. Most of the antibiotics in current use for the treatment of various infectious diseases are microbial products (Tawiah *et al.,* 2012). Studies on soil bacteria and fungi have shown that these microorganisms are potentially rich source of unique bioactive substances (Fenical, 1993).

Numerous antibiotics have been isolated from a variety of microorganisms, however studies are still being conducted to identify novel antibiotics effective against pathogenic fungi and bacteria (Cavalcanti, *et al.,* 2006).

Antimicrobial agents play the most important role in the treatment of bacterial infections and wide spread efforts have been carried out by many scientists in order to screen for novel antibiotic producing microbes (Oskay, *et al.,* 2004).

The aim and objectives of this research were to collect the three different soil samples from Ngathaichaung Township, Ayeyawady Region, to isolate the diversity of bacteria from soil samples on three different culture media, to investigate the colony characters of isolated bacteria,

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to observe the microscopic characters of isolated soil bacteria and to study the preliminary study of antimicrobial activity of isolated bacteria on seven test organisms.

MATERIALS AND METHODS

Collection of Soil Samples

Three different soil samples were collected from three different places of Ngathaichaung Township, Ayeyawady Region in July, 2018. These soil samples were carried out at the laboratory of Biotechnology and Development Centre of Pathein University.

Isolation of the collected soil samples was done in laboratory as soon as possible after soil collection in fields. Serial dilutions of plating and streaking techniques were used to isolate the microorganisms from soil according to Salle (1948); Collins (1965) and Pelezer and Chan (1972).

Isolation of Bacteria from Soil Samples by Serial Dilution Method

Serial dilutions of plating and streaking techniques described by Salle (1948), Collins (1965), and Pelezar and Chan (1972) were used for the isolation of bacteria species from soil.

The sterilized medium in conical flask was cooled down to about 45ºC and separately poured into each of the petridish containing the respective soil dilution. The inoculated plates were shaken clock-wise and anticlockwise direction for about 5 minutes so as to make uniform distribution of the bacterial inoculums. When the agar was solidfied, the inoculated plates were inverted and incubated at room temperature for 3-5 days.. Various types of colonies developed on the inoculated plates. They 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 respective medium.

Isolation of Pure Culture from Plate to Slants

For pure culture from plate to test tube, about 100 mL of culture media were separately distributed into test tubes. These tubes were plugged with cotton wool and sterilized by autoclaving then at 15 pounds pressure per square inch for 15 minutes at 121ºC. The sterilized media were cooled down. Each of the separate colonies on petri-dish was taken out to streak on the slant medium to obtain pure cultures (Atlas, 1993).

Gram Staining Method (Collins, 1965)

A smear of bacterial cells was prepared on a clean glass slide and the smear was then allowed to air-dry followed by a mild heat fixation. Crystal violet solution was added onto bacterial smear and incubated for one minute. The smear was washed with water Mordant Gram's iodine Solution was then added on bacterial smear and incubated for one minute. The smear was decolonized by washing with 95% ethyl alcohol and rinsed with water. Finally, safranin was used as counter for one minute and washed with water. Cell were then air dried and studied under microscope.

Preliminary Study on Antimicrobial Activities of Isolated Bacteria

The isolated soil bacteria were inoculated into seed medium and incubated for 1 day at room temperature. Seed culture were transferred to the fermentation medium. After one day, the

seed cultured (1%) was transferred into the fermentation medium and carried out by static culture. Then, the fermented broth was used to check the antimicrobial activity by agar well method (Collins, 1965). Agar well having (8 mm in diameter) were utilized for antimicrobial activity.

Test Organisms

Escherichia coli AHU 5436, *Bacillus subtilis* IFO 90571, *Bacillus pumilus* IFO 90571, *Candida albicans* NITE 09542, *Pseudomonas fluorescens* IFO 94307, *Staphylococcus aureus* AHU 8465, *Malassezia furfur* UY were obtained from NITE (National Institute of Technology and Evaluation, Kisarazu, Japan), PRD (Pharmaceutical Research Development, Ministry of Industry) and UY (University of Yangon).

Agar well method (Collins, 1965)

This method was used for the antimicrobial activity by seven test organisms. The assay medium (peptone 0.5 g, NaCl 0.5 g, yeast extract 0.2 g, beef extract 0.1 g and agar 1.5 g) was utilized for these bacteria. Isolated strains were subjected with antimicrobial activity by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclave basal antimicrobial test medium.

Well impregnated with 1-5 days fermented broth (0.1 mL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. Clear zone surrounding the wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

Results

The total of 15 bacterial strains such as KM-1, KM-2, KM-3, KM-4, KM-5, KM-6, KM-7, KM-8, KM-9, KM-10, KM-11, KM-12, KM-13, KM-14 and KM-15 were isolated from the three different area of Ngathaichaung Township. The results showed that the colonies morphology of those isolated strains (KM-1-15) were medium and large in sizes; undulate, lobate, entire in margins; cream, white, pale brown and purple in color; raised and flat in elevation and form; mucous, dry and greasy in pigments on agar, respectively. Those bacterial strains were short-rod, rod-chain and long rod chain in their cell morphologies and their antimicrobial activities were also tested.

Table 1 Colony morphology of the isolated strains

Isolated strains	Size of colony	Margin	Color	Elevation and form	Pigment on agar
KM-1	Large	Undulate	Mucous	Flat	Mucous
KM-2	Medium	Lobate	Mucous	Raised	Mucous
KM-3	Large	Entire	Dry	Raised	Dry
KM-4	Large	Undulate	Dry	Flat	Dry
KM-5	Large	Undulate	Dry	Flat	Dry

Isolated strains	Size of colony	Margin	Color	Elevation and form	Pigment on agar
KM-6	Large	Entire	Purple	Flat	Mucous
KM-7	Large	Undulate	White	Flat	Dry
KM-8	Medium	Entire	White	Raised	Dry
KM-9	Large	Undulate	Cream	Flat	Dry
KM-10	Large	Undulate	Cream	Flat	Mucous
KM-11	Large	Undulate	Cream	Flat	Greasy
KM-12	Large	Undulate	Pale brown	Flat	Greasy
KM-13	Large	Undulate	Cream	Flat	Mucous
KM-14	Large	Entire	Cream	Flat	Mucous
KM-15	Medium	Entire	Cream	Flat	Mucous

Table 2 Cell morphology of the isolaed strains

Isolated strains	Gram staining	Cell morphology
KM-1	-	Long rod chain
KM-2	-	Short rod
KM-3	-	Rod chain
KM-4	-	Long rod chain
KM-5	-	Rod chain
KM-6	-	Rod
KM-7	-	Rod chain
KM-8	-	Short rod
KM-9	-	Rod
KM-10	-	Short and chain
KM-11	-	Short rod
KM-12	-	Short rod
KM-13	+	Rod
KM-14	+	Short rod
KM-15	+	Short rod

(-) = Gram negative (+) = Gram positive

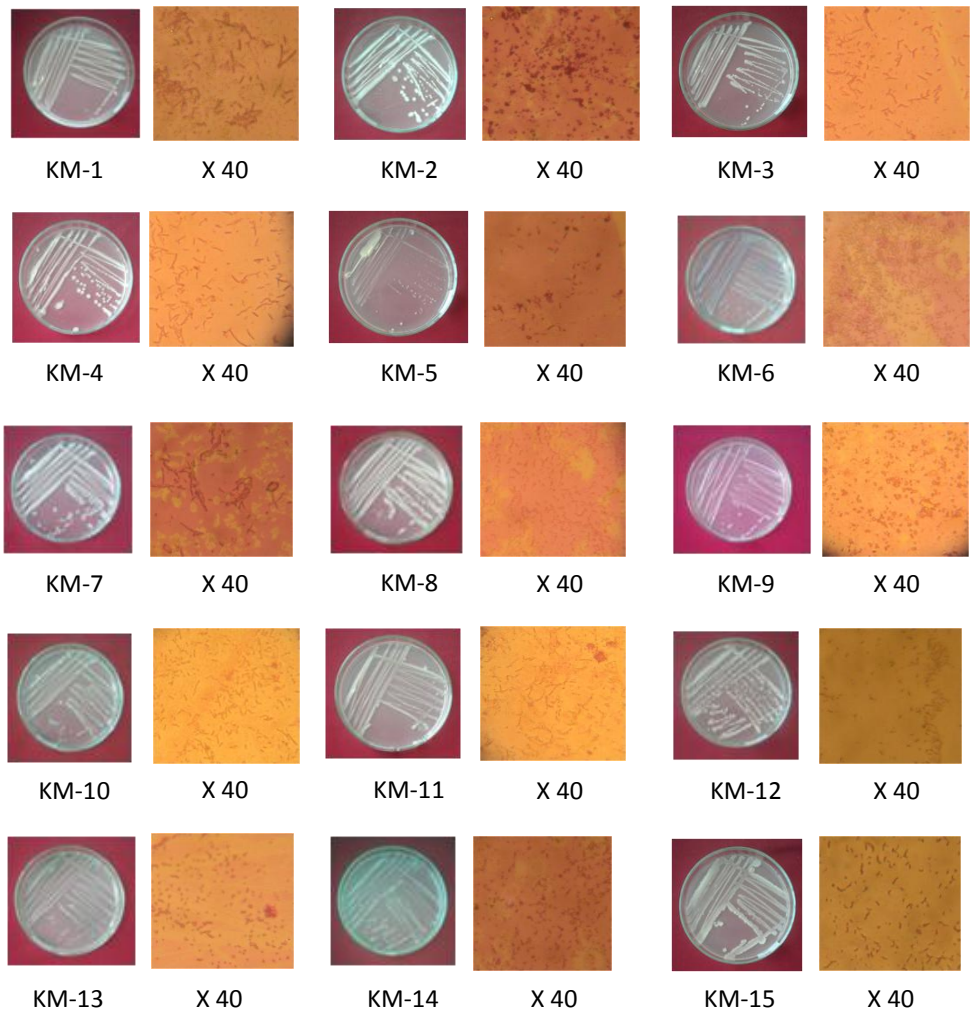


Figure 1 Cultural characteristic and cell morphology of isolated bacteria

Antimicrobial Activities of Isolated Bacterial Strains

Ten isolated bacterial strains were tested from 15 isolated bacteria. Among them, 6 isolated bacterial strains showed the different level of antimicrobial activities on 5 test organisms, except *Pseudomonas fluorescens* and *Staphylococcus aureus.*

Table 3 Antimicrobial Activities of 6 Isolated Bacteria Against Five Test Organisms

No	Isolated bacteria	*Escherichia coli*	*Bacillus subtilis*	*Bacillus pumilus*	*Candida albicans*	*Malassezia furfur*
1	KM-6	13.28 mm	+	18.01 mm	19.61 mm	19.73 mm
2	KM-9	22.72 mm	19.98 mm	+	21.14 mm	23.77 mm
3	KM-10	27.21 mm	19.95 mm	26.62 mm	+	19.38 mm
4	KM-11	17.78 mm	**21.40 mm**	16.74 mm	+	27.76 mm
5	KM-13	**27.77 mm**	+	**27.63 mm**	**25.46 mm**	**31.17 mm**
6	KM-15	14.73 mm	+	26.15 mm	16.07 mm	21.69 mm

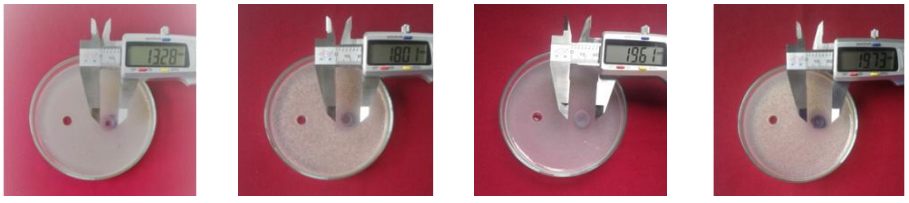


Figure 2 Antimicrobial activities of KM-6 against (A) *Escherichia coli*, (B) *Bacillus pumilus*, (C) *Candida albicans* and (D) *Malassezia furfur*

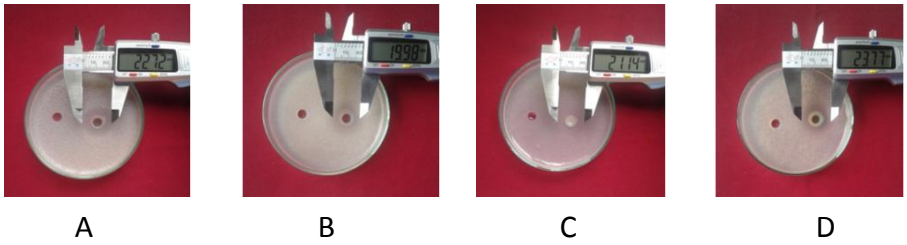


Figure 3 Antimicrobial activities of KM-9 against (A) *Escherichia coli*, (B) *Bacillus subtilis*, (C) *Candida albicans* and (D) *Malassezia furfur*

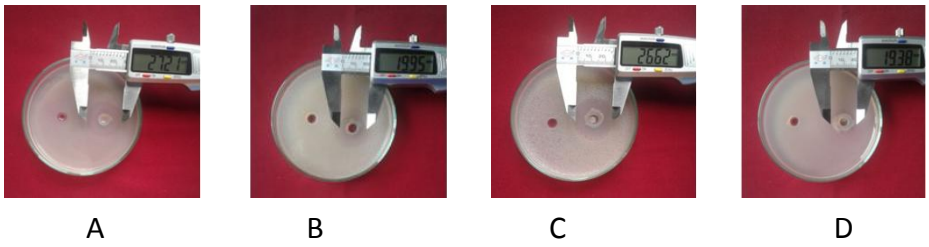


Figure 4 Antimicrobial activities of KM-10 against (A) *Escherichia coli*, (B) *Bacillus subtilis*, (C) *Bacillus pumilus*, and (D) *Malassezia furfur*

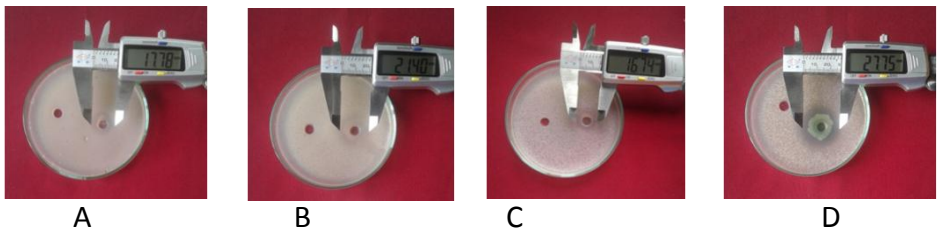
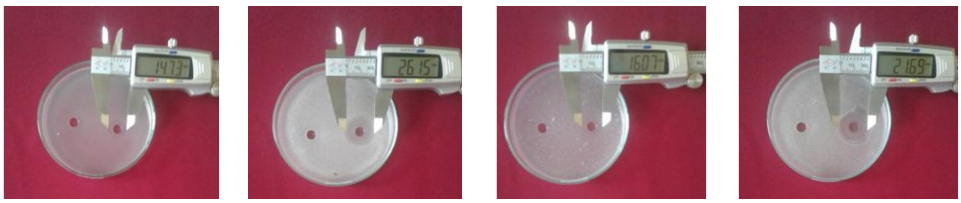


Figure 5 Antimicrobial activities of KM-11 against (A) *Escherichia coli*, (B) *Bacillus subtilis*, (C) *Bacillus pumilus*, and (D) *Malassezia furfur*

A B C D

Figure 6 Antimicrobial activities of KM-13 against (A) *Escherichia coli*, (B) *Bacillus pumilus*, (C) *Candida albicans* and (D) *Malassezia furfur*



A B C D

Figure 7 Antimicrobial activities of KM-15 against (A) *Escherichia coli*, (B) *Bacillus pumilus*, (C) *Candida albicans* and (D) *Malassezia furfur*

Discussion and Conclusion

Bacteria grow in many different environments and specific niches in the soil. 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 matter contents, cultivation, aeration and moisture content (Davies, 1999).

Microorganisms which have capacity to produce more antibiotics can survive for longer time than the other producing antibiotics in fewer amounts. Antibiotics produced by microorganisms have been very useful for the cure of certain human diseases caused by bacteria, fungi and protozoa (Walsh, 2003).

The World Health Organization stated that there is a serious lack of new antibiotics to fight the increasing risk of antimicrobial resistance, which represents a global health emergency (Kmietowicz, 2017). Antibiotics and other bioactive compounds have been isolated from microorganisms in different environments (Charousova *et al.*, 2017).

In the present study of the isolation of bacteria, 15 strains were isolated from three different soil samples collected from Ngathaichaung Township, Ayeyarwady Region. Three different media were employed in the isolation of bacteria and 15 isolates were obtained. These bacterial strains were designated as KM-1 to KM-15.

In the colony morphology, all isolated bacteria were medium and large and the color were cream, white, purple and pale brown. The margin of colonies were entire, lobate and undulate.

Cell morphology of isolated strains were studied by Gram staining, colony characters and shape of cell. Among them, all strains were short rods, long rods, short rods chain and long rods chain. Three strains were Gram positive and other strains were Gram negative.

In the preliminary antimicrobial activity, 6 isolated strains showed different levels of antimicrobial activity on five test organisms except *Pseudomonas fluorescens* and *Staphylococcus aureus.* KM-13 showed the higher antibacterial activity (27.77 mm and 27.63 mm) against *Escherichia coli* and *Bacillus pumilus* than other strains. Especially, this strain exhibited the highest antifungal activity (31.17 mm) on *Malassezia furfur* followed by (25.46 mm) on *Candida albicans.* And then, KM-11 also showed the antibacterial activity (21.40 mm) on *Bacillus subtilis.*

Antibiotics and other bioactive compounds have been isolated from microorganisms in different environment (Charousova *et al.*, 2017; Devi *et al.*, 2017).

It was concluded that the present research was to isolate the diversity of bacteria from soil samples on three different culture media and to study the preliminary study of antimicrobial activity of isolated bacteria on seven test organisms.

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