Antimicrobial Metabolite Producing Fungi Isolated From Soil at Dala Township

Kay Tha Ye Soe Win[1]
Dr Kathy Myint[2]

Abstract

In this study, five different soil samples were collected at Dala-Township, Yangon Region. In the isolation of fungi, 12 fungi were isolated by dilution method. In the biological properties of isolated fungi, KTY-07 showed high antibacterial activity of clear zone (27.76 mm) against *Staphylococcus aureus*. Therefore, this strain KTY-07 was selected for further investigation. This fungus KTY-07 was isolated from Silty Clay Loam soil pH 6.95. Based on the morphology and microscopical characteristic, this fungus KTY-07 is preliminarily identified as *Fusarium* sp. It is therefore concluded that 72 hrs ages of inoculum and 15% sizes of inoculum were suitable for the fermentation. The fermentation was performed using fermentation medium FM-4 a suitable of pH 6.5 at room temperature for 7 days. Maximum activity of (35.35 mm) reached at 5 days fermentation period with 72 hrs of ages and 15% of sizes of inoculum.

Introduction

The typical materials for microbial sources are soil, living and fallen leaves, leaf litters, dung, inset, fresh water and marine water. Soil is one of the important source for the habitation of fungi and actinomycetes. Fungi also play on important role in medical, food, beverages and agricultural uses. Fungi grow best in environments that are slightly acidic. They can grow on substances with very low moisture. Fungi live in the soil and on your body, in your house and on plants and animals, in freshwater and seawater. A single teaspoon of topsoil contains about 120,000 fungi. Fungi are basically stationary. But they can spread either by forming reproductive spores that are carried by wind and rain or by growing extending their hyphae. Hyphae grow as new cells, form at the tips, creating even chains of cells.

Fungi absorb nutrients from living or dead organic matter that they grow on (Website 1). *Fusarium* is a large genus of filamentous fungi, part of a group often referred as hypomycetes, widely distributed in soil and associated with plants. Most species are harmless saprobes, and relatively abundant members of soil microbial community (Website 2). In the study of antibacterial metabolite producing fungi, twelve fungi were isolated from five different kinds of soil samples by physical treatment dilution method. In the course of the screening for their biological activities, antibacterial metabolite against *Staphylococcus aureus* was found from the soil fungus *Fusarium* sp. The purpose of this study had been aimed to know various isolated fungi, their activities and fermentation conditions of selected fungi.

MATERIALS AND METHODS

Collection of Soil Samples

The soil samples that was collected from five different places were utilized for the isolation of microorganisms especially fungi. The soil samples were collected from five different townships of Dala area, Yangon Region on July, 2017 (Table 1, Figure 1). The soil type as its pH was analyzed by Department of Agriculture (Land Use), Giorgione.

[1] Kay Tha Ye Soe Win, Master of Research Student, Department of Botany, University of Yangon
[2] Dr. Kathy Myint, Lecturer, Department of Botany, University of Yangon

Table 1. Location of the collected soil sample

Soil No.	Collected places	GPS
S -1	Paddy Field	16º 44′ 43º 86″ N, 96º 8′ 29º 56″ E
S-2	Downtown	16º 45′ 12º 02″ N, 96º 8′ 37º 02″ E
S-3	Dump	16º 45′ 32º 06″ N, 96º 7′ 58º 62″ E
S-4	Rice Mill	16º 45′ 32º 06″ N, 96º 7′ 58º 62″ E
S-5	River Bank	16º 45′ 52º 69″ N, 96º 8′ 50º 30″ E

Isolation of fungi from soil samples

The isolation of soil fungi were carried out by physical treatment dilution methods (Phay and Amachi, 2005). The collected soil sample was air dried at room temperature for 2 days. Then the soil sample was ground and sieved. The sample was placed in the hot air oven at 120ºC for 1 hour. Then the dried sample was diluted with sterile water as shown in Figure 2. Thirty µL of sample was cultured on the plate of low carbon agar medium (LCA medium) and incubated for 5-10 days (Ando, 2004).

Screening of effective soil fungi by paper disc diffusion assay (Tomita, 1998)

The isolated soil fungi were grown on PGA medium for 5days. The isolated fungi were inoculated into seed medium and incubated at room temperature for 3days (Ando, 2004). Ten ml of seed culture was transferred into the fermentation medium. The fermentation was carried out for 7 days. After the end of fermentation periods, the fermented broth (20µl) was used to check the antimicrobial activity against test organisms by paper disc diffusion assay. In this study eleven pathogenic microorganisms were utilized for antimicrobial activity.

Table 2. Test organisms utilized in the antimicrobial activities of isolated soil fungi

No.	Test organisms	Source	Diseases
1.	*Agrobacterium tumefaciens*	-	Plant tumor cell
2.	*Bacillus pumalis*	IFO 1202	Eye infections, soft tissue infections
3.	*Bacillus subtilis*	JAP 0225025	Fever
4.	*Escherichia coli*	ATCC 25922	Cholera, diarrhea and vomiting, urinary tract infections
5.	*Klebsiella pneumonia*	-	Pneumonia like illness, meningitis, liver abscess
6.	*Micrococcus luteus*	ATCC 23840	Skin disease
7.	*Proteus mirabilis*	-	Urinary tract infection
8.	*Pseudomonas aeruginosa*	IFO 3080	Urinary infection, respiratory system infection, soft tissue infection
9.	*Salmonella typhi*	ST.3 / Sep.69	Typhoid
10.	*Staphylococcus aureus*	ATCC 12877	Skin disease, food poison, boils, wound infection
11.	*Xanthomonas oryzae*	-	bacteria for leaf blight

Effect of ages of culture on the fermentation by isolated soil fungi KTY 07 (Omura, 1985)

The selected fungus KTY 07 was inoculated into the seed medium and then transfer to fermentation medium 24 hrs intervals. Age of culture with (24 hrs, 48 hrs, 72 hrs, 96 hrs, 120 hrs, 144 hrs, and 168 hrs) were utilized for fermentation 7 days. The antibacterial activity was carried out paper disc diffusion method by using with *Staphylococcus aureus* test organisms.

Effect of sizes of inoculum for the fermentation medium of isolated soil fungi KTY 07 (Omura, 1985)

In this study 5%, 10%, 15%, 20%, 25% and 30% of 72 hrs of seed culture were utilized for the fermentation. Fermentation was carried out 7 days antibacterial activity was tested by paper disc diffusion assay.

Effect of fermentation period on the production of antimicrobial activity against *Staphylococcus aureus*

Fermentation was undertaken by FM 4 medium with the optimum of ages of culture. Size of inoculum was fermented for 1,2,3,4,5,6 and 7 day. The antimicrobial activity was carried out paper disc diffusion method using *Staphylococcus aureus* as test organisms.

Results

Isolation of Fungi

In the course of investigation of fungi, 12 fungi were isolated from five different soil samples which collected from Dala Townships. Two fungi were isolated from soil 1, two fungi were isolated from soil 2, three fungi were isolated from soil 3, three fungi were isolated from soil 4 and two fungi were isolated from soil 5 respectively (Table 3).

Table 3. Analyzed soil type, its pH and isolated fungi from soil samples of five different places

Sample No.	Soil Type	pH	Isolated Fungi
S-1	Silty Clay Loam	5.29	KTY-01, KTY-02
S-2	Silty Clay	5.99	KTY-03, KTY-04
S-3	Silty Clay Loam	6.95	KTY-05, KTY-06, KTY-07
S-4	Silty Clay	4.88	KTY-08, KTY-09, KTY-10
S-5	Silty Clay Loam	7.22	KTY-11, KTY-12

Table 4. Morphological characters of isolated fungal strains

No.	Strain	Cultural characters	
		Front side	Reverse side
1.	KTY 01	Pale yellow-spread	White
2.	KTY 02	White mycelium are raised above	Basal pink
3.	KTY 03	White center raised	White
4.	KTY 04	Inside pale brown outside creamy	Inside brown, outside creamy
5.	KTY 05	Yellow	Pale yellow
6.	KTY 06	Center green surround white layer	Yellowish pigmentation
7.	KTY 07	Pink cottony raised in the center	The center is yellowish and pinky mycelium around the colony
8.	KTY 08	Center gray, green patches	Creamy pale
9.	KTY 09	Center gray, margin white	Yellowish pigmentation
10.	KTY 10	Brown	Pale yellow
11.	KTY 11	White, margin	White
12.	KTY 12	White raise up cottony	White

Screening of effective soil isolate fungi by paper disc diffusion assay

The study of biological properties of these fungi, 3 fungi exhibited antibacterial against *Bacillus pumaliss,* 2 fungi against *B. subtilis,* 4 strains exhibited antibacterial againt *Escherichia coli*, 3 strains against *Klebsiella* pneumonia and 2 strains showed antibacterial activity against *Staphylococcus aureus*. Among them KTY-07 showed more highly antibacterial activity agains *staphylococcus a*ureus. (Table 5 and Figure 1)

Table 5. Antimicrobial activities of isolated soil fungi 4 days after fermentation by paper disc diffusion method

No.	Test organisms	KTY 1	KTY 2	KTY 3	KTY 4	KTY 5	KTY 6	KTY 7	KTY 8	KTY 9	KTY 10	KTY 11	KTY 12
1.	*Agrobacterium tumefaciens*												
2.	*Bacillus pumalis*	18.24		24.53	22.03								
3.	*Bacillus subtilis*										14.51		14.20
4.	*Escherichia coli*							16.7	14.8		11.2		13.8
5.	*Klebsiella pneumoniae*									14.82		17.91	14.43
6.	*Micrococcus luteus*												
7.	*Proteus mirabilis*												
8.	*Pseudomonas aeruginosa*												
9.	*Salmonella typhi*												
10.	*Staphylococcus aureus*							27.76					22.60
11.	*Xanthomonas oryzae*												

Bacillus pumalis *Bacillus subtils* *Escherichia coli* *Klebsiella pneumonia* *Staphylococcus aureus*

Fig. 1 Antimicrobial activities of isolated soil Fungi

Identification of selected fungus KTY-07 by morphology and microscopic character

Colony morphology (Macroscopic Characters)

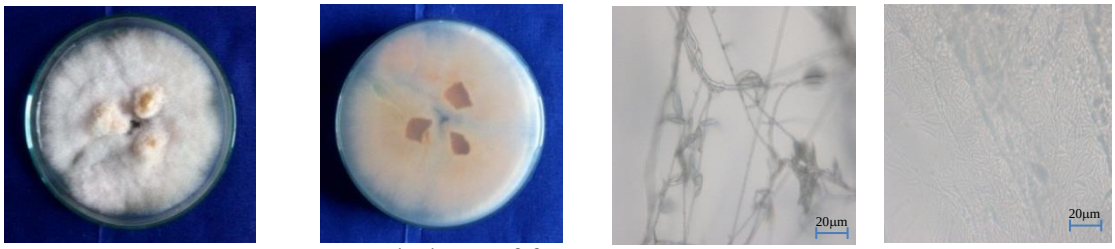
Fungi isolated are usually incubated at three points on potato dextrose agar and incubated at 25°C. Most species sporulated within 7 days. Mycelium extensive and cotton-like culture, tinge of pink in the mycelium on medium (Fig-2).

Microscopic Morphology

Septate hyphae, conidia hyaline, large sickle or canoe-shaped, multiseptate, macroconidia produced from phialides on unbranched conidiophores. According to the macroscopical and microscopical characteristic features and based on the reference keys of Barnett 1972, Davise 1987, Colin *et al.*, 2013, it is assumed that KTY 07 strain may be *Fusarium* sp.

Scientific Classification

Kingdom	-	Mycota
Division	-	Eumycota
Sub-division	-	Deuteromycotina
Class	-	Hypomycetes
Order	-	Moniliales
Family	-	Tuberculariaceae
Genus	-	*Fusarium*
Species	-	*Fusarium* sp.



Morphology of fungus KTY 07(Front side)

Morphology of fungus KTY 07 (Reverse side)

Micrograph of fungus KTY 07

5 days old culture on PGA medium

Fig. 2 Colony morphology and micrograph of isolated fungi KTY 07

Study on the effects of ages of culture on the fermentation

According to Cruger (1989), age of culture (24, 48, 72, 96, 120, 144, 168 hrs) were utilized for the fermentation. In the experiment it was observed that 72 hrs ages of seed culture were suitable for the fermentation. (Table 6, Figure 3)

Table 6. Effects of ages of inoculum on fermentation by isolated soil fungi KTY 07

Culture time (Ages of culture, hours)	Antibacterial activity (mm)
24	-
48	11.84
72	18.34
96	18.26
120	12.49
144	10.46
168	9.94

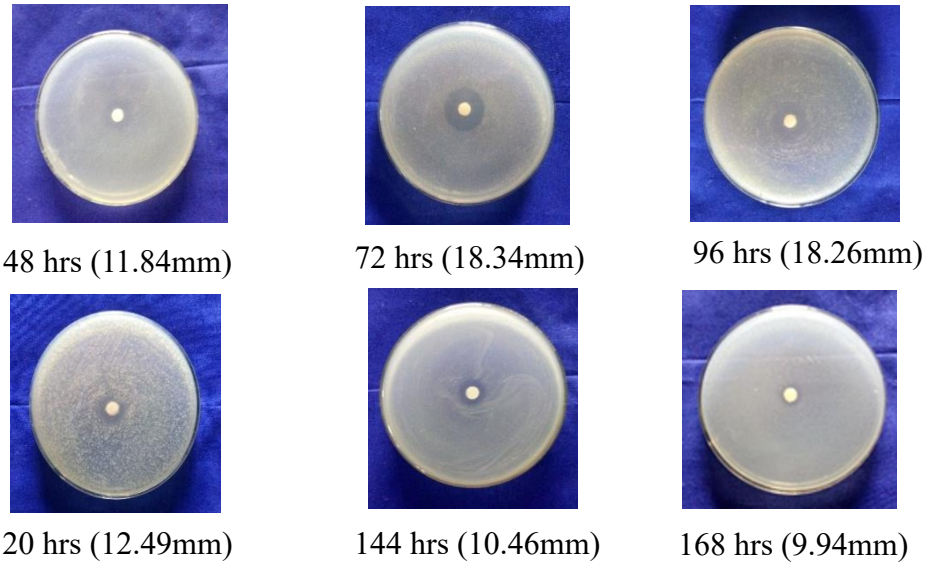


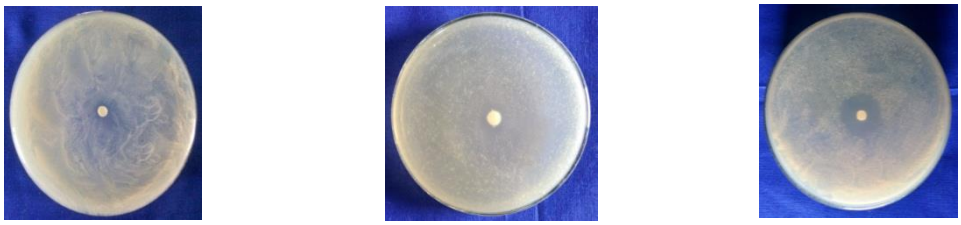
Fig. 3 Effects of ages of inoculum on fermentation by isolated soil fungi KTY 07

Effects of size of inoculum on the fermentation by isolated soil fungi KTY 07

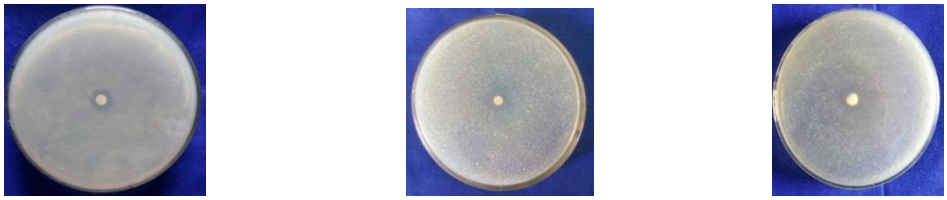
In the study of size of inoculum, different size of inoculum (5%, 10%, 15%, 20%, 25% and 30%) were tested, 15% isolated fungi sizes of inoculum concentration was the best for the fermentation. The size of inoculum isolated soil fungi was 15% most suitable for fermentation period 7 days for 72 hours age of culture isolated soil fungi (Table 7, Figure 4).

Table 7. Effects of sizes of inoculum on fermentation by isolated soil fungi KTY 07

Sizes of inoculum at 72 hrs (%)	Clear zone (mm)
5	13.15
10	15.31
15	20.67
20	18.10
25	16.5
30	12.5



5% (13.15mm) 10% (15.31mm) 15% (20.67mm)



20% (18.10mm) 25% (16.5mm) 30% (12.5mm)

Fig. 4 Effect of sizes of inoculum on fermentation by isolated soil fungi KTY 07

Table 8. Antibacterial activity from four different fermentation method by isolated soil fungi KTY 07 against *Staphylococcus aureus* (Atlas, 1993)

Fermentation media	Inhibitory Zone(mm)
FM1	-
FM2	-
FM3	-
FM4	19.2

Age of inoculum - 72 hrs
Size of inoculum - 15 %
Medium - Assay medium
Fermentation Time - 4 days

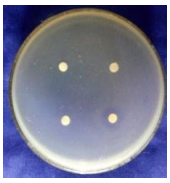


Fig. 5 Effect of medium on the fermentation of antibacterial activity

Study on the fermentation periods for the production of antibacterial metabolite against *Staphylococcus aureus*

Fermentation was carried out by using FM 4 using optimum condition. Antibacterial activity showed 3-7 days with 72 hrs of ages of culture and 15% of size of inoculum. Maximum activity reached at 5 days fermentation period.

Table 9. Duration of fermentation for the production of antibacterial metabolite against *Staphylococcus aureus*

Fermentation Times (Days)	Clear zone (mm)
1	-
2	-
3	18.26
4	19.96
5	35.35
6	33.35
7	18.28

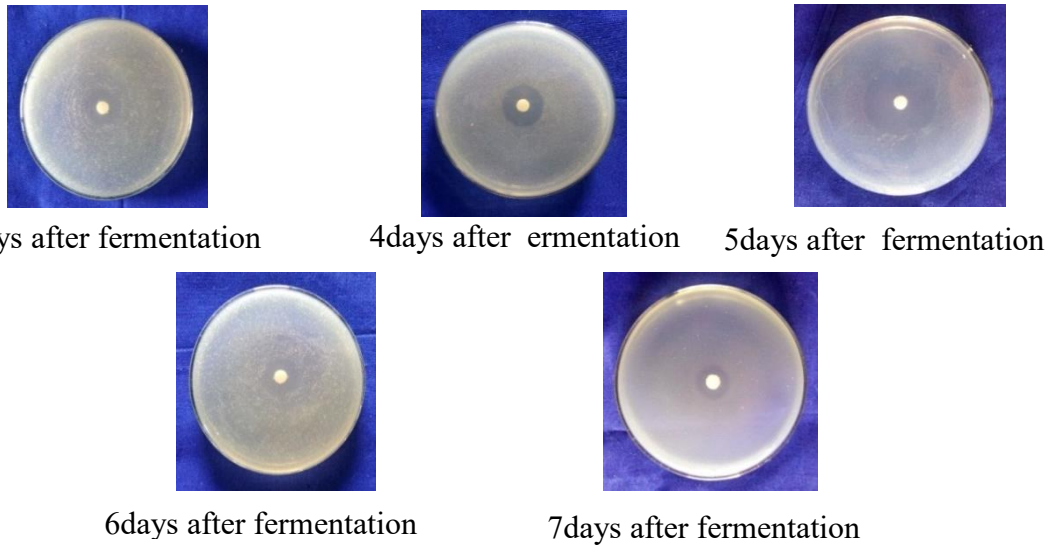


Fig. 6 Duration of fermentation for the production of antibacterial metabolite against *Staphylococcus aureus*

Discussion and Conclusion

In the course of investigation of fungi, 12 fungi were isolated from five different soil samples which were collected from Dala Township, Yangon Region. Isolated twelve fungi their morphological character and reverse color were found to be different. Two fungi were isolated from soil 1(Silty Clay Loam), two fungi were isolated from soil 2 (Silty Clay), three fungi were isolated from soil 3 (Silty Clay Loam), three fungi were isolated from soil 4 (Silty Clay) and two fungi were isolated from soil 5 (Silty Clay Loam). During the study of biological properties of these fungi, 3 fungi exhibited antibacterial activity against *Bacillus pumalis*, 2 fungi against *Bacillus subtilis*, 3 strains against *Escherichia coli*, 3 strains exhibited *Klebsiella pneumoniae* and 2 strains exhibited *Staphylococcus aureus*. Among them KTY 07 and KTY 12 showed the antibacterial activity against *Staphylococcus aureus*. However, isolated soil fungus KTY 07 (27.76 mm) showed more highly antibacterial activity them KTY 12 (22.06 mm). Therefore, this strain KTY 07 was selected for further investigation such as ages of culture sizes of inoculums and fermentation periods. This isolated soil (pH 6.95) fungus KTY 07 was collected from the Dump S3 (Silty Clay Loam) of Dala Township, Yangon Region.

While selected fungus KTY 07, it was observed that mycelium are filamentous like a patch of wool and raised in the center but from the reverse view the center is yellowish and pinky mycelium around the colony. Hyphae are septate, conidia (spore) are colourless, canoe-shaped in side view and are divided by several cross-walls. Based on these morphology and microscopical characteristic and the references of Barnett (1972), Davise (1987) and Dube (1957), this isolated soil fungus KTY-07 may be identified as *Fusarium* sp. It was found that the most suitable parameter such as 72 hrs of ages of culture and 15% sizes of inoculum were optimized to produce the antibacterial activity during fermentation. Maximum activity reached at 5 days after fermentation.

Zhang *et.al* (1915) reported that endophytic fungi *Fusarium* showed the strongest antimicrobial activity on *Pseudomonas aeruginosa*. Musavi and Balakrishan (2014) reported that isolated fungus *Fusarium* from soil showed the highest activity

against four pathogenic strains *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The results of the present work agree with Musavi and Balakrishan (2014).

Fermentation was carried out with four different media and antibacterial activity test with paper diffusion assay method with test organisms used an *Staphylococcus aureus.* FM 4 medium was the best fermentation condition of isolated soil fungus KTY 07 for the production of antibacterial activity.

In conclusion, the isolated *Fusarium* will further studies to clarify the identification of isolated fungus up to species level and to find out the nature of metabolites those can kill the test organism.

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