Soil Fungi from Four Different Places at Hinthada Township in Ayeyarwaddy Region

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Abstract

In the study of biological properties of soil fungi, 10 different kinds of fungi were isolated from four different places of soil samples from (Khone Gyi, Tagon Taing, Damar Yone, Myoe Pat Lan) Hinthada Township, Ayeyarwady Region. The isolation of fungi was undertaken by the method of chemical treatment dilution method and serial treatment dilution method. In the present study, fungi MZ-7, MZ-8, MZ-9 having immediately starch hydrolyzing activity fungi, MZ-1, MZ-2, MZ-3, MZ-4, MZ-5 having after 2 hours starch hydrolyzing activity. Isolated fungi was employed for the fermentation of antimicrobial metabolite.

Keywords: Isolation, Morphological characters and Test for Starch Hydrolyzing Activity

INTRODUCTION

Microorganisms in soil are important because they affect soil structure and fertility. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae, algae and protozoa. Each of this group has characteristic that define them and their functions in soil. Fungi thrive in acidic environments, while bacteria and actinomycetes cannot surviven acid, which results in an abundance of fungi in acidic areas. Fungi also grows well in dry, and soil because fungi are aerobic or dependent on oxygen, and the higher the moisture content in the soil, the less oxygen is present for them. Soil fungi are microscopic plant-like cell that growing long thread-like structures or hyphae that make a mass called mycelium. The mycelium absorbs nutrients from the roots it has colony. (Parkinson, 1994)

Various fungi isolated are identified to their generic and specific taxon on the basis of grass colonial and microscopic morphology. Detailed taxonomic study of isolates of soil fungi are made according to the methods describe by Ando, 2004.

Gross colonical morphology is noted and observations are recorded on the size and shape of conidiophores, vesicle, sterigmata, conidia and spores. Gross colonical morphology and microscopic observations are made from days old growth on Czapek's Dox agar medium incubated at 27·C. (Micra and Tind, 1998)

Most scientists performing identification on fungal samples still use traditional method of macroscopic and microscopic examination (Cabot, 2007). Therefore, identification of the antimicrobial metabolites are always in demand for increasing incidents of fungal and bacterial infections and for controlling pathogenic microbes.

10 fungi were isolated from four different places of soil samples. In the course of screening for their biological properties, antifungal metabolite against Candida albicans was found from the soil fungus Aspergillus sp. (MZ-6).

The aim and objectives of these study were to investigate the isolation of microorganisms from 4 different soil places. The investigate the morophological characters of these isolated fungi. The study the amylase activity of isolated fungi.

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MATERIALS AND METHODS

Collection of soil samples

In the present work, the soil sample was used as sources for screening of useful fungi were collected from four different sites. The collected of soil samples from the depth of 6 inches was taken from respectively study sites. The soil samples were air dry at room temperature and unnecessary debris was removed. Then, the soil powder was sieved of 1mm mush.

- **Myoe Pat Lan**
- **Khone Gyi**
- **Hinthada University**
- **Tagon Taing**

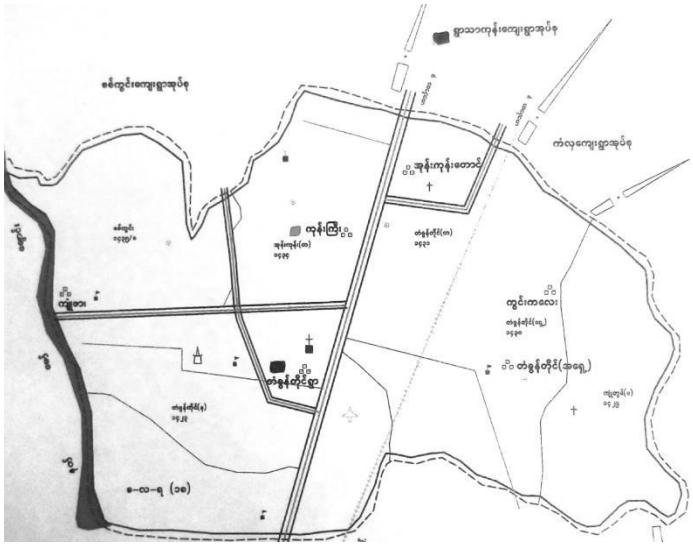


Figure 1. The Map of Hinthada Township

Table.1 Soil sample collected at Hinthada Township

Soil Sample No.	Soil collected at		Soil pH	Collected Date
	Place	Location		
S-1	Khone Gyi	17◦36′ 14.4″N 95◦25′ 44.9″ E	± 6.5	November-January
S-2	Tagon Taing	17◦35′ 27.3 ″N 95◦25′ 28.1″ E	± 6	November-January
S-3	Damar Yone	17◦36′ 63.0″ N 95◦26′ 2.05″ E	± 6.5	November-January
S-4	Myoe Pat Lan	17◦38′ 56.0″ N 95◦26′ 0.64″	±5.5	November-January

Isolation of fungi from soil samples

The isolation of fungi were undertaken by chemical treatment dilution method and physical treatment serial dilution method. (Phay and Yamaura, 2005)

Physical treatment serial dilution method

The collected soil was air-dried at room temperature. Soil was grounded and sieved. Two gram of the sieved soil was put into a tube containing distilled water. The tube was shaken for about 30 mins. Two ml of this suspension was transferred to next tube containing 18 ml of distilled water. And then this suspension was done serially diluted into next two tubes containing 9 ml of distilled water. Finally, 0.1 ml of soil suspension was cultured on LCA (Low Carbon Agar) medium for 5-7 days.

Method 1

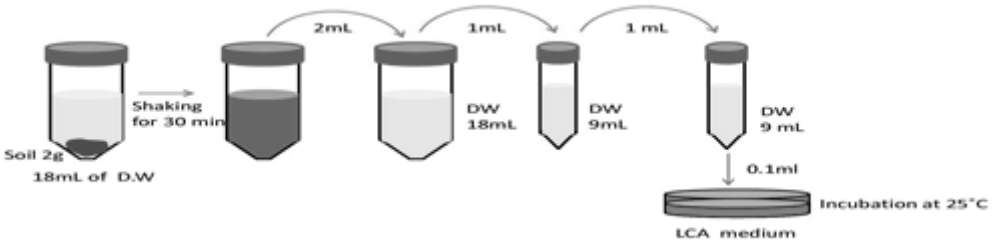


Figure 2. Physical treatment serial dilution method

Chemical treatment dilution method

Soil was air-dried at room temperature. After dried soil was grounded and sieved. Two gram of the sieved soil was put into test tube. Four ml of sterilized distilled water was put into the tube containing soil, and settle for 6 hours. 70% Ethanol solution (14ml) was then added into the tube containing soil suspension, and shaken for 1min. And then 1ml of soil suspension was transferred to next tube containing 5 ml of distilled water. 0.5 ml of soil suspension from these tube was put into the next tube containing 4.5 ml of distilled water.1 ml of soil suspension was transferred to next tube containing 4 ml of distilled water. Finally, 30 µl of soil suspension was cultured on LCA (Low Carbon Agar) and incubated for 5-7 days.

Method 2

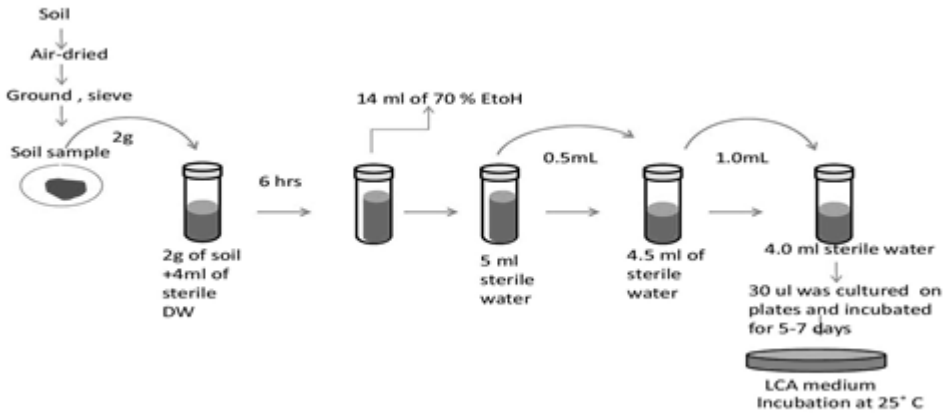


Figure 3. Chemical treatment dilution Method (+EtOH Treatment MethodNITE 2005)

Medium used for the isolation of fungi
LCA medium (Low Carbon Agar medium, Ando, 2004)

Sucrose	3.0g
$NaNo_3$	0.2g
$MgSo_4.7H_2O$	0.05g
KCL	0.05g
$FeSo_4$	0.001g
K_2HPO_4	0.001g
Agar	1.8g
DW	100ml
pH	±6.5

(after autoclaving chloramphenicol were added to the medium)

Starch Medium (For starch hydrolyzing activity test)

Soluble starch	1.0 g
K_2HPO_4	0.1g
$MgSO_4$	0.1 g
NaCL	0.1 g
$(NH_4)_2SO_4$	0.2 g
$CaCO_3$	0.1 g
DW	100 ml
pH	±6.5

Czapek medium (Atlas. RM, 1993) (For Pure fungi culture)

Glucose	0.2g
Sucrose	0.2g
K_2HPO_4	0.1g
$MgSO_47H_20$	0.05g
KNO_3	0.1g
KCL	0.05g
Agar	1.8g
DW	100 L
pH	±6.5

RESULTS

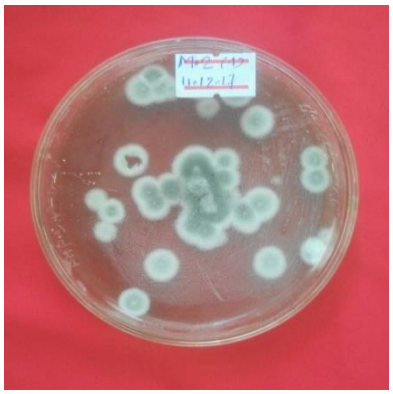
Isolation of Fungi from Soil Sample

In the isolation of fungi from four different soil samples, 10 fungi were isolated showed in Table and figure.

Table.2 Fungi isolated from four different soil sample by physical treatment serial dilution method and chemical treatment dilution method

Collected No	Collected place	Soil pH	Total isolated fungi			
			Serial dilution method	Chemical treatment dilution method	Total fungi	Fungi No.
S-1	Khone Gyi	±6.5	2	0	2	MZ-1,2
S-2	Tagon Taing	±6	1	1	2	MZ-3,4
S-3	Damar Yone	±6.5	2	0	2	MZ-5,6
S-4	Myoe Pat Lan	±5.5	3	1	4	MZ-7,8,9,10

Morphological characters of isolated fungi



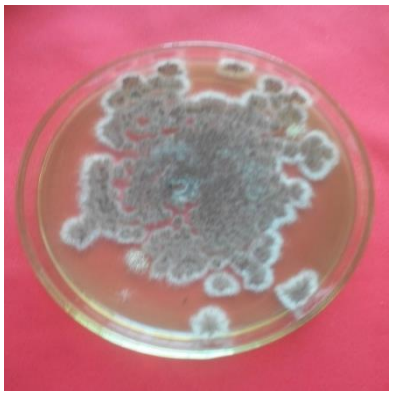
MZ.1

MZ.2

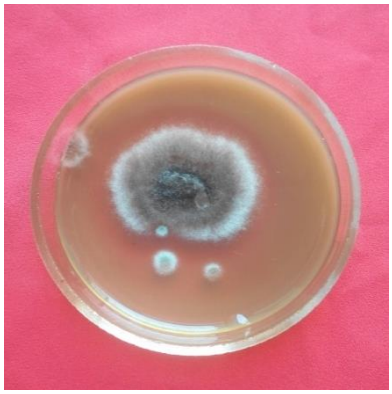
MZ-3

MZ-4

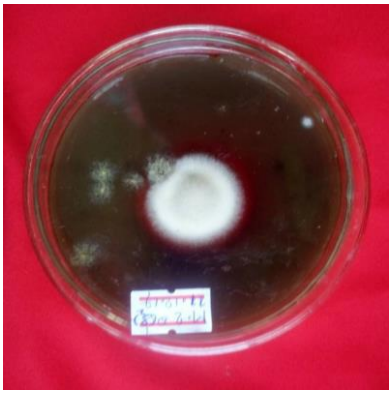
MZ-5



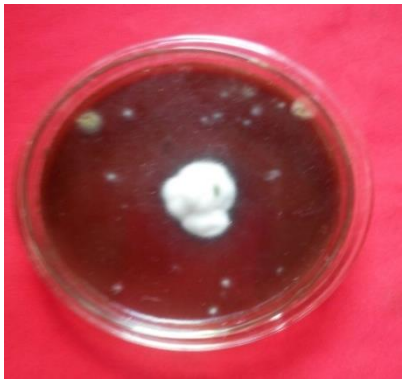
MZ-6



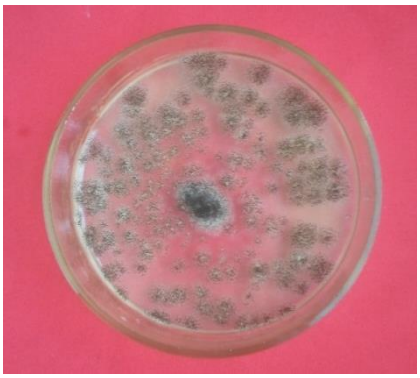
MZ-7



MZ-8



MZ-9



MZ-10

Figure 4. Morphological characters of isolated fungi

Figure 5. Pure culture of isolated fungi MZ-1 to MZ-10 preserved in test tube

Test for Starch Hydrolyzing Activity

In the course of the starch hydrolyzing activity, MZ-1, MZ-2, MZ-3, MZ-4, and MZ-5 can hydrolyze the starch after 2 hours, MZ-7, MZ-8, MZ-9 can hydrolyze the starch immediately.

Before reaction with iodine solution

After reaction with iodine solution

After reaction with iodine solution (immediately)

After reaction with iodine solution (after 2 hours)

After reaction with iodine solution (no change)

Figure 6. Starch hydrolyzing activity

Table 3. Test for Starch hydrolyzing activity

Inoculation Period (7 days)		
Isolated fungi	Color after reaction with iodine solution (immediately)	Color after reaction with iodine solution (after 2 hours)
MZ-1	No reaction (negative)	Positive
MZ-2	No reaction (negative)	Positive
MZ-3	No reaction (negative)	Positive
MZ-4	No reaction (negative)	Positive
MZ-5	No reaction (negative)	Positive
MZ-6	No reaction (negative)	No reaction (negative)
MZ-7	Positive	No reaction (negative)
MZ-8	Positive	No reaction (negative)
MZ-9	Positive	No reaction (negative)
MZ-10	No reaction (negative)	No reaction (negative)

DISCUSSION AND CONCLUSION

In the study of biological properties of soil fungi, 10 different kinds of fungi isolated were from four different places. MZ-1and MZ-2 was isolated from Khone Gyi, MZ-3, MZ-4 were isolated from Tagon Taing, MZ-5, MZ-6 were isolated from Damar Yone and MZ-7, MZ-8, MZ-9, MZ-10 were isolated from Myoe Pat Lan. It was observed that fungi MZ-7, MZ-8, MZ-9 having immediately starch hydrolyzing activity fungi MZ-1, MZ-2, MZ-3, MZ-4, MZ-5 having after 2 hours starch hydrolyzing activity.

In conclusion, the diversity of fungi is reflected in the diversity of fungal metabolities which are the products of secondary metabolism (Maurice, 2011), Starch degrading amylolytic enzymes are most important in the biotechnology industries with huge application in food fermentation textile and paper. The effectives microorganisms can be used in the medicinal fields as antibiotic metabolities.

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References

Ando, K-2004, **isolation and identification of fungi**. Workshop, BRBDC.

Ando, K. (1999). **Microbial aspests of useful microorganism**, confereces in Malaysia.

Atlas, R.M.1993. **Microbiological media CRC Press Printed in the United of America**.

Ando, K, M. Suto and S. Inaba. 2004; **Sampling and Isolation methods of fungi Workshop at Pathein University**.

Alexandar, M. (1961). **Introduction to Soil Microbiology**. John Wiley and sons. Inc, New York and London.

Biotechnology cells AL Demain JE Davie, **ASM Press**. Washington, DC

Cruegar, W., and A Cruegar.1989; **Methods of fermentation in Biotechnology A Textbook of Idustrial Microbiology** , 64-74.

Cabot, 2007, **Fungi identification Test**. In Biology 21124

Domash K.H and W.Gems 1980; **Compendium of Soil Fungi Vol. I**, Institute of Soil Biology.

Dube, H.C(1983). **An Introduction of Fungi**. pp-14

Gupte , M., P. Kalkaeni, B.N. anguli.2002: **Antifungal antibiotis**. Apply microbial technol .

Hunter- Cevera J.C and A Belt 1999. **Isolation of cultures**. In; Manual of Industrial microbiology and

NITE (National Institue of Technology and Elevation) 2004: **Amylase enzymes test activities methods**.

Omura, S, 1985: **Microbial growth kinetic and secondary metabolites**. J.Fermentation technology.

Phay, N and H Yamamura , 2005: **Approach method for rare microorganisms from soil sources**. J. Microbial.

Parkinson, D. 1994 filamentous fungi, **Method of Soil Analysis**, Part 2, 329-350.

Pathrick, F. Gailiot, 1998 **Initial extraction and product capture. In Natural Products Isolation**, by R.J.P. Cannell, 53-89.

Smith J.L and J.W Doran, 1996 **Measurement and see of pH and conductivity for soil quality analysis**. Soil science Society of America Special Publication 49:169-182.

Schlegal, H.G, 1993. **General Microbiology**. Cambridge, UK: Cambridge University Press. P. 360.

Tomita, F, 1998: **laboratory Method**, ltokkaido University, Japan.