ISOLATION OF FUNGI FROM SOIL SAMPLES AND THEIR ANTIMICROBIAL ACTIVITIES

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Abstract

Ten soil samples were collected from ten different places of Waing Maw areas, Kachin State. 22 fungi were isolated from these ten soil samples. The isolated soil fungi were checked the antimicrobial activity by paper disc diffusion assay method. PPK-01, PPK-03, PPK-04, PPK-10, PPK-14, PPK-18 and PPK-20 showed antibacterial activities. The fungus PPK-10 and PPK-14 showed the activity on Agrobacterium tumefaciens IFO543, Bacillus subtilis KY-327, Micrococcus luteus NITE83297, Pseudomonas fluorescens IFO94307. Among them, PPK-10 showed the highest antibacterial activities (PPK-10, 29.62 mm inhibitory zone) on Agrobacterium tumefaciens IFO543. This fungus PPK-10 was isolated from the soil sample collected from Nawng Hee. Therefore, fungus PPK-10 was selected for further investigations based on the results of antimicrobial activity.

Keywords: isolation, antimicrobial activity of soil fungi

Introduction

Microorganisms are the important sources of bioactive compounds with enormous potential to be developed as new molecules for drug discovery. Microorganisms grow in unique and extreme habitats that provide them the capability to produce unique and unusual metabolites (Thamilvanan, Ram kumar, Ramesh, Balakumar and Kumaresan,2018). Soil are highly complex systems, with many components playing diverse functions mainly due to the activity of soil organisms. Soil microflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth. Microorganisms are beneficial in increasing the soil fertility and plant growth as they are involved in biochemical transformation and mineralization activities in soil (Gaddeyya, Shiny Niharika, Bharathi and Ratna Kumar, 2012).

Fungi are microscopic cells that usually grow as long threads or strands called hyphae. Hyphae interact with soil particles, roots, and rocks forming a filamentous body that promotes foraging for food.

These networks release enzymes into the soil and break down complex molecules that the filaments then reabsorb. Fungus act like natural recyclingbins, reabsorbing nutrients in the soil. Hyphae are usually only several thousandths of an inch (a few micrometers) in deameter. Single hyphae can span in length from a few cells to many yards. Hyphae sometimes group into masses called mycelium or thick, cord-like rhizomorphs that look like roots.

Mushrooms are a special type of fungus with special features (spores, gills, fruiting bodies). A single individual fungus can include many fruiting bodies scattered across a large area (as big as a baseball diamond). They generally make up 10-20% of the total microorganisms in the soil rhizosphere. Fungus generally have a lower number of individuals in a healthy soil, however they dominate the soil biomass due to their larger size fungus biomass in the soil ranges from the equivalent of two to six cows in a healthy soil. There are at least 70,000 different species of fungus but it is estimated that there are at least 20 times that number worldwide. See Understanding Soil Microbes and Nutrient Recycling for more information on microbial numbers.

Fungi perform important services related to water dynamics, nutrient cycling, and disease suppression. Along with bacteria, fungi are important as decomposers in the soil food web,

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converting hard to digest organic material into usable forms. In no-till, fungal population dominate the soil food web (although they are less in number than the bacteria).

Fungi have 40-55% carbon use efficiency so they store and recycle more carbon (C) compared to bacteria. Bacteria are less efficient at retaining C and release more into the air as carbon dioxide. Fungi have higher C content (10:1 C:N ratio) and less nitrogen (N=10%) in their cells than bacteria. Fungus help recycle both N and phosphorus (P) to plants. Due to their smaller size and much greater surface area, fungus can efficiently scavenge for N and P better than plant root hairs and greatly increase the plant root nutrient extraction efficiency. Many plants cultivate certain species of both bacteria and fungus to increase nutrient extraction from the soil.

Genetically, fungi are closely related to plants and animals. Membrane bound organelles present in each cell are similar to insects, plants, and animals. They evolved about a billion years ago and are equal in rank to plants and animals. In fact, fungi have 80% or more of the some genes as humans. They generally reproduce by spores (microscopic parts similar to plant seeds). The longevity of fungus has not been measured in many species but their open ended growth suggests that they have a longevity measured in millions of years, because they are basically the same organism. For example, fairy fungal rings grow in ever widening circles, much like rings on a tree, and are measured in decades and centuries instead of days and weeks for most microbes (James. Hoorman, 2011).

The aim and objectives of this study were to isolate the growth of fungi occurring inactively in the soil, to study the morphology of isolated fungi and to observe the preliminary study of antimicrobial activity.

Materials and Methods

Collection of soil samples

Ten different soil samples were collected from Waing Maw areas (Nang War, Aung Myay-1, Ma Hkan Tee, San Ka, Nawng Hee, Mai Na, Nawng Ta Law, Mading, Wu Yang, Inn Lay) in Kachin state during 2018. (Table 1) (Fig. 1).

Table 1 Soil samples collected from different places of Waing Maw areas

Soil No.	Collected place		Collected Date	pH	Soil type
S-1	Nang War	N 25° 22' 37.340" E 097° 33' 30.361"	27.7.2017	6.04	Sandy loam
S-2	Aung Myay-1	N 25° 14' 32. 857" E 097° 26' 13.489"	27.7.2017	5.76	Sandy loam
S-3	Ma Hkan Tee	N 25° 19' 32.186" E 097° 22' 50.874"	27.7.2017	5.26	Sandy loam
S-4	San Ka	N 25° 18' 59 337" E 097° 19 10.735"	27.7.2017	5.62	Sandy loam
S-5	Nawng Hee	N 25° 21' 01.656" E 097° 20' 49.448"	27.7.2017	4.53	Clay loam
S-6	Mai Na	N 25° 24' 56.400" E 097° 25' 48.221"	27.7.2017	5.59	Loam
S-7	Nawng Ta Law	N 25° 23' 18.045" E 097° 25' 29.132"	27.7.2017	4.62	Silt loam
S-8	Mading	N 25° 21' 18.149" E 097° 27' 19.737"	27.7.2017	5.75	Loam
S-9	Wu Yang	N 25° 21' 51.369" E 097° 29' 33.962"	27.7.2017	5.88	Sandy loam
S-10	Inn Lay	N 25° 17' 19.245" E 097° 26' 30.820"	27.7.2017	6.04	Loamy sand

Source: Department of Geography Pathein University

Figure 1 Map of soil samples collected area

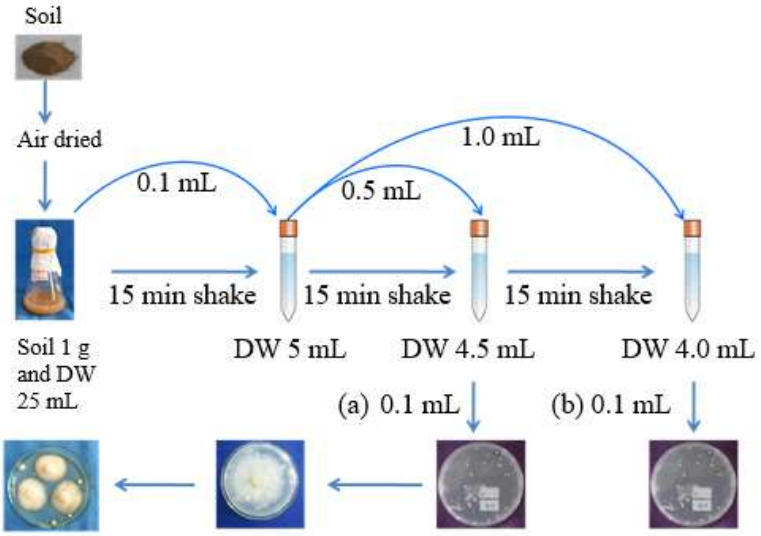

Figure 2 Dilution method (Phay and Yamamura, 2005)

Dilution method (Phay and Yamamura, 2005)

Soil was air dried at room temperature for three days. Grounded and sieved of 1 mm. 1 g of soil sample put into 25 mL of sterilized water. 0.1 mL of diluted soil suspension was put into 5 mL of sterilized water. 0.5 mL of diluted soil suspension was put into 4.5 mL of sterilized water. 1.0 mL of diluted soil suspension was put into 4.0 mL of sterilized water. Cultured onto PGA medium. Transferred onto GYP medium.

Media used for the isolation of soil fungi (Ando, 2004)

Glucose Yeast Peptone Medium (GYP medium)

Glucose	1.3 g
Yeast extract	0.7 g
Peptone	0.7 g
K_2HPO_4	0.05 g
$MgSO_4$	0.01 g
Agar	1.8 g
DW	100 mL

Potato Glucose Agar Medium (PGA medium)

Components per liter

Potato	20 g
Glucose	2 g
Agar	1.8 g
DW	100 mL

Seed Medium (Components of per liter)

Glucose	1.0 g
Yeast extract	0.7 g
K_2HPO_4	0.01 g
$MgSO_4$	0.01 g
DW	100 mL

Fermentation Medium (Components of per liter)

Glucose	1.5 g
Yeast extracta	0.5 g
Peptone	0.5 g
K_2HPO_4	0.01 g
$MgSO_4$	0.01 g
DW	100 mL

After autoclaving, chloramphenicol 25mL/100mL was added to this medium.

Assay medium

Glucose	0.8 g
Yeast extract	0.4 g
Peptone	0.4 g
KNO_3	0.1 g
Agar	1.6 g

Table 2 Test Organisms utilized for antimicrobial activities

No.	Test Organisms	Diseases
1	*Aspergillus flavus* IFO3290	Fruits diseases
2	*Bacillus subtilis* KY-327	Fever
3	*Micrococcus luteus* NITE83297	Skin disease
4	*Escherichia coli* AHU5436	diarrhea
5	*Pseudomonas fluorescens* IFO94307	Rice pathogen
6	*Salmonella typhi* AHU7943	Typhoid fever
7	*Agrobacterium tumefaciens* IFO543	Tumour cell in plant

Results

Table 3 Fungi isolated from different soil samples by physical treatment methods

Soil No.	Collected place	Total Isolation fungi
S-1	Nang War	PPK-01
S-2	Aung Myay-1	PPK-02
S-3	Ma Hkan Tee	PPK-03, PPK-04, PPK-05
S-4	San Ka	PPK-06, PPK-07
S-5	Nawng Hee	PPK-08, PPK-09, PPK-10,
S-6	Mai Na	PPK-11, PPK-12
S-7	Nawng Ta Law	PPK-13, PPK-14, PPK-15
S-8	Mading	PPK-16
S-9	Wu Yang	PPK-17, PPK-18, PPK-19,
S-10	Inn Lay	PPK-20, PPK-21, PPK-22

Table 4 Morphological color of isolated fungi

No.	Isolated fungi	Character			No.	Isolated fungi	Character	
		Surface color	Reverse color				Surface color	Reverse color
1	PPK-01	White	Cream		12	PPK-12	Yellowish white	Yellowish
2	PPK-02	White	White		13	PPK-13	Greenish white	Yellow
3	PPK-03	White	Cream		14	PPK-14	Greenish white	Cream
4	PPK-04	Gray	Cream		15	PPK-15	Greenish white	Cream
5	PPK-05	White	Yellow		16	PPK-16	White	White
6	PPK-06	White	Cream		17	PPK-17	Greenish white	Cream
7	PPK-07	White	Cream		18	PPK-18	Greenish white	Cream
8	PPK-08	White	Cream		19	PPK-19	White	Cream
9	PPK-09	White	Cream		20	PPK-20	Greenish white	Cream
10	PPK-10	Greenish	Cream		21	PPK-21	Greenish white	Cream
11	PPK-11	Greenish	Yellow		22	PPK-22	White	Cream

Table 5 Preliminary studies of antimicrobial activities

No.	Isolated fungi	Test organisms and Inhibitory zone (mm)						
		A. tumefaciens	***B. subtilis***	***M. luteus***	***E. coli***	***P. fluores***	***S. typhi***	***A. flavus***
1	PPK-01	19.00	-	-	-	18.40	-	-
2	PPK-02	-	-	-	-	-	-	-
3	PPK-03	20.00	17.44	18.44	-	18.44	-	-
4	PPK-04	-	17.18	17.18	-	-	-	-
5	PPK-05	-	-	-	-	-	-	-
6	PPK-06	-	-	-	-	-	-	-
7	PPK-07	-	-	-	-	-	-	-
8	PPK-08	-	-	-	-	-	-	-
9	PPK-09	-	-	-	-	-	-	-
10	PPK-10	29.62	27.04	27.06	-	20.00	-	-
11	PPK-11	-	-	-	-	-	-	-
12	PPK-12	-	-	-	-	-	-	-
13	PPK-13	-	-	-	-	-	-	-
14	PPK-14	27.62	27.04	27.00	-	23.03	-	-
15	PPK-15	-	-	-	-	-	-	-
16	PPK-16	-	-	-	-	-	-	-
17	PPK-17	-	-	-	-	-	-	-
18	PPK-18	-	-	-	-	19.44	-	-
19	PPK-19	-	-	-	-	-	-	-
20	PPK-20	16.08	-	-	-	20.00	-	-
21	PPK-21	-	-	-	-	-	-	-
22	PPK-22	-	-	-	-	-	-	-

(-) no activity

Table 6 Preliminary studies of antimicrobial activities

No.	Isolated fungi	Test organisms and Inhibitory zone (mm)						
		A. tumefaciens	***B. subtilis***	***M. luteus***	***E. coli***	***P. fluores***	***S. typhi***	***A. flavus***
1	PPK-01	19.00	-	-	-	18.40	-	-
2	PPK-03	20.00	17.44	18.44	-	18.44	-	-
3	PPK-04	-	17.18	17.18	-	-	-	-
4	PPK-10	29.62	27.04	27.06	-	20.00	-	-
5	PPK-14	27.62	27.04	27.00	-	23.03	-	-
6	PPK-18	-	-	-	-	19.44	-	-
7	PPK-20	16.08	-	-	-	20.00	-	-

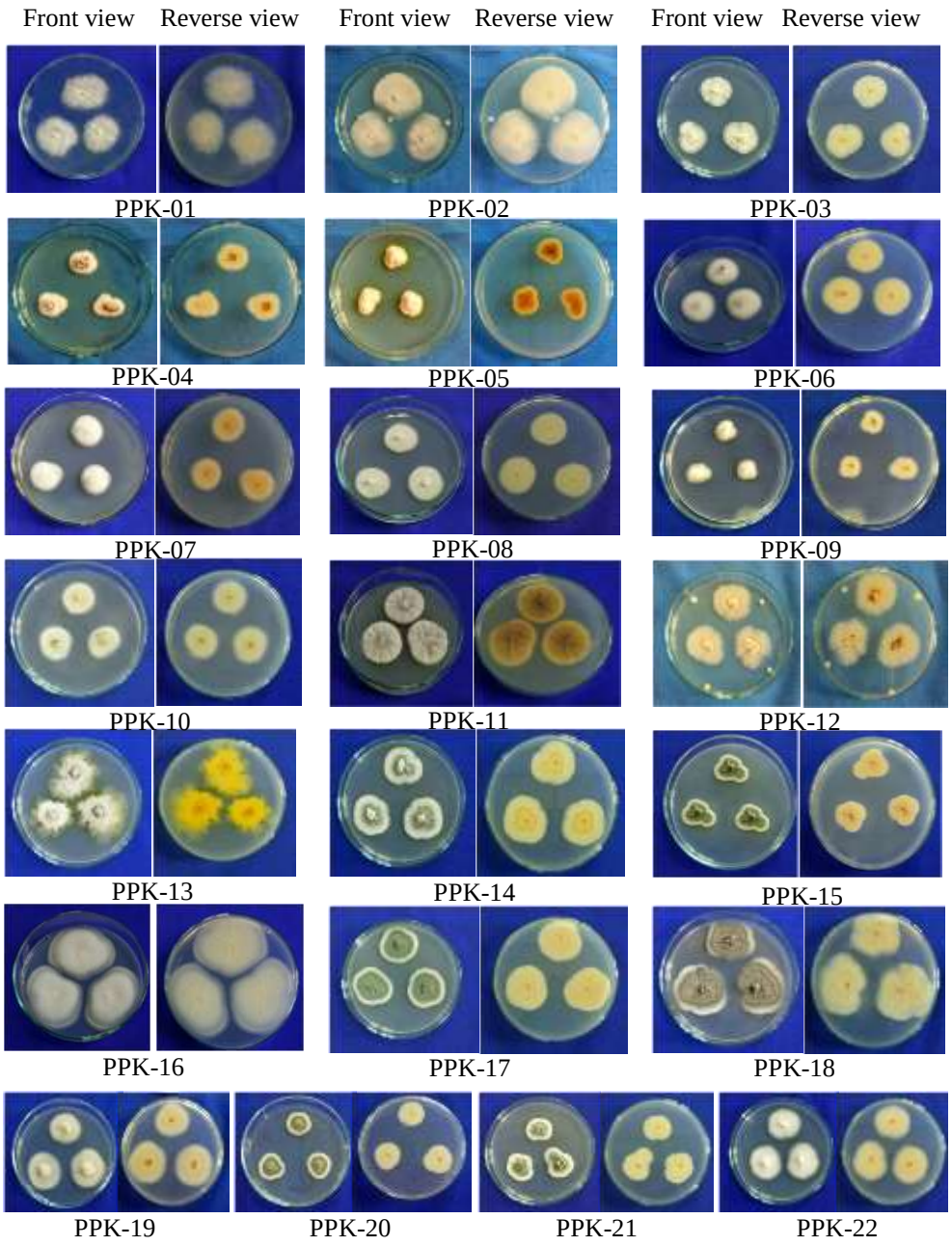

Figure 3 Morphologies of soil fungi (7 days after culture)

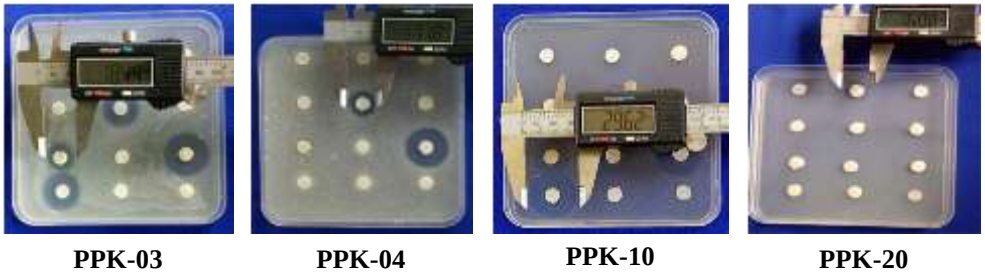

Figure 4 Antimicrobial activity of isolated fungi against *Agrobacterium tumefaciens*

PPK-10
Antimicrobial activity of isolated fungi against *Micrococcus luteus*

PPK-14
Antimicrobial activity of isolated fungi against *Bacillus subtilis*

PPK-14
Antimicrobial activity of isolated fungi against *Pseudomonas fluorescens*

Figure 5 Antimicrobial activity of isolated fungi against *Micrococcus luteus, Bacillus subtilis* and *Pseudomonas fluorescens*

Discussion and Conclusion

Fungi grow on diverse habitats in nature and are cosmopolitian in distribution requiring several specific elements for growth and reproduction. In laboratory, these are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmosphere gas mixture (Northolt and Bullerman, 1982).

In this study, 22 fungi were isolated from ten soil samples collected at Waing Maw areas, Kachin State. It was observed that one fungus (PPK-01) was isolated from the soil sample No.1, one (PPK-02) from soil sample No.2, three (PPK-03-05) from soil sample No.3, two (PPK-06,07) from soil sample No.4, three (PPK-08-10) from soil sample No.5, two (PPK-11,12) from soil sample No.6, three (PPK-13-15) from soil sample No.7, one (PPK-16) from soil sample No.8, three (PPK-17-19) from soil sample No.9 and three (PPK-20-22) from soil sample No.10 receptivity.

In studying the morphological colours of fungi, the front colours of PPK-01, 02, 03, 05, 06, 07, 08, 09, 16, 19 & 22 were the same white. The reverse colours of PPK-02,16 were the same white. The reverse colours of PPK-01,03, 06, 07, 08, 09, 19 & 22 were the same cream. The revese colour of PPK-05 was yellow. The front colour of PPK-04 was gray and it reverse colour was cream. The front colour of PPK-10, 11, 13, 14, 15, 17, 18, 20 & 21 were the same greenish white. The reverse colours of PPK-10, 14, 15, 17, 18, 20 & 21 were the same cream. The reverse colours of PPK-11,13 were the same yellow. The front colour of PPK-12 was yellowish white and its reverse colour was yellowish cream.

In the investigation of antimicrobial activities, soil fungi were tested with seven test organisms by using paper disc diffusion assay method. Seven fungi (PPK-01, PPK-03, PPK-04, PPK-10, PPK-14, PPK-18 and PPK-20) showed the antimicrobial activity. All of them, PPK-10 showed the highest activity (29.62mm) followed by PPK-14 (27.62mm),PPK-03 (20.00mm), PPK-01 (19.00mm) and PPK-20 (16.08mm) in 5 days old culture. Among them, the fungus PPK-10 showed the highest activity on Agrobacterium tumefaciens IFO543 (29.62 mm inhibitory zone). Therefore, fungus PPK-10 was selected for further investigations.

In the investigation, soil fungus PPK-10 showed the antibacterial activity on Agrobacterium tumefaciens. PPK-10 was isolated from the S-5 (Loamy sand, pH- 6.04). This soil samples was collected from Nawng Hee Village, Waing Maw areas, Kachin State. Soil fungus

PPK-10 will further studies to clarify the fermentation optimization, 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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Appendix

DEPARTMENT OF AGRICULTURE (LAND USE)
SOIL ANALYTICAL DATA SHEET

Division - ကချင်ပြည်နယ်
Township - ဗန်းမော်

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Sheet No. 1
Sr No. S 1-10 / 17-18

Sr No.	Sample plot	Moisture %	pH Soil : Water 1 : 2.5	Texture				SOIL INTERPRETATION OF RESULTS	
				Sand %	Silt %	Clay %	Total %	pH Soil : Water 1 : 2.5	Texture
	မြို့နယ်များ								
1	Nang War	4.27	5.59	46.75	36.50	15.10	98.35	Moderately acid	Loam
2	Aung Myay-1	6.80	4.62	19.30	54.30	24.90	98.50	Strongly acid	Silt Loam
3	Ma Hkan Too	4.33	5.75	47.35	42.50	8.40	98.25	Moderately acid	Loam
4	San Ka	4.40	5.88	54.05	38.30	5.70	98.05	Moderately acid	Sandy Loam
5	Naung Hee	2.70	6.04	75.05	21.25	2.10	98.40	Slightly acid	Loamy Sand
6	Mai Na	4.01	6.04	60.35	31.90	6.15	98.40	Slightly acid	Sandy Loam
7	Naung Ta Law	3.25	5.76	67.85	18.20	12.55	98.60	Moderately acid	Sandy Loam
8	Mading	3.93	5.26	51.80	39.75	6.65	98.20	Strongly acid	Sandy Loam
9	Wu Yang	3.53	5.62	51.40	22.75	24.35	98.50	Moderately acid	Sandy Clay Loam
10	Inn Lay	6.35	4.53	25.75	38.40	34.30	98.45	Strongly acid	Clay Loam