ISOLATION AND IDENTIFICATION OF SOIL FUNGI FROM PATHEINGYI TOWNSHIP, MANDALAY REGION

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Abstract

This research deals with the isolation and identification of soil fungi from Patheingyi Township in Mandalay Region during July to September, 2018. The soil samples collected from Yay Kyi and Shin Taw Gone Villages. And then, soil samples were undertaken by chemical treatment dilution method. Four fungi were isolated from Yay Kyi and six fungi were isolated from Shin Taw Gone Villages. The morphology of ten isolated fungi the surface view and reverse view are different color. Ten isolated strains were tested by five test organisms for preliminary study of antimicrobial activities. Among them, four fungi showed the antibacterial activity against on *Bacillus subtilis* and *Xanthomonas oryzae,* three fungi showed the antibacterial activity against on *Escherichia coli* and *Pseudomonas fluorescens,* six fungi showed antifungal activities against on *Candida albicans.* ZN-01, ZN-02, ZN-04, ZN-05, ZN-06, ZN-07 and ZN-10 showed the antimicrobial activity against on all test organisms. Moreover, ZN-03, ZN-08 and ZN-09 cannot show the antimicrobial activity against on all test organisms. Especially, ZN-10 showed highest antimicrobial activity of clear zone (28.12 mm) against on *Pseudomonas fluorescens.* Therefore, this strain ZN-10 was selected for further investigation. According to the morphological character, microscopical character and references keys, the fungus ZN-10 is preliminarily identified as *Purpureocillium* sp.

Keywords: soil fungi, antimicrobial activity, identification

Introduction

Patheingyi Township located at 7.5 miles of Mandalay Region. Yay Kyi and Shin Taw Gone Villages located in Patheingyi Township. The villages have kept some of the traditional lifestyles and the main occupations is agriculture. Therefore, the study on isolation of effective fungi from soil samples in Patheingyi Township. Spoonful of soil contains billions of microorganisms. In general, the majority of microbial population is found in the upper six to twelve inches of soil and the number decreases with depth. All soils contain bacteria, fungi and viruses in varying amounts depending on soil conditions (Blackwell, 2011). Fungi are not only beautiful but play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling as biofertilizers. Soil fungi play an important role as a major decomposer in the soil ecosystem. The soil serves as a reservoir of essential nutrients for many microbial communities, plants and small animal. The microorganisms play major role in soil ecosystem. Microbial composition and functioning changes the soil quality through decomposition of organic matter, recycling of nutrients and biological control (Stefanis *et al*., 2013). Soil fungi are also the major sources of other industrially important compounds like enzyme inhibitors, antitumor agents, insecticides and vitamins (Karthikeyan *et al.,* 2014). *P. fluorescens* encompasses a group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments. *Purpureocillium* sp. has been shown to have potential for biopesticide applications (Singh *et*

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al.,2013). The aim of this study is to isolate soil fungi, their activities and to identify of selected fungus.

Materials and Methods

Collection of Soil Samples

The soil samples were collected from two different places of Patheingyi Township in Mandalay Region, during July to September, 2018 (Table 1). The soil sample were collected from different places (up to 15 cm depth) into sterilized polythene bags after removing the surface soil for the isolation of fungi and brought to the laboratory of Biological Resources and Biotechnology Development Center (BDC) at Pathein University.

Table 1. The collection of soil sample at Patheingyi Township

Soil No.	Collected at		Soil Type	Soil pH	Collected Date
	Places	Location			
S-1	Yay Kyi Village	N 21°57' 11.925, E 96°10'42.324"	Clay Loam	5.6	7.7.2018
S-2	Shin Taw Gone Village	N 21°58' 5.023" E 96°10'5.487"	Clay Loam	6.3	7.7.2018

Chemical Treatment Dilution Method (Hayakawa and Kobayashi, 2005)

Soil samples were air-dried at room temperature for a week. Soil samples were ground and sieved in 2 mm screen. Two g of sieve soil was put into the test tube. Four mL of sterile water was poured into the test tube containing soil and settle for 6 hours to germinate early germinating soil microorganisms. Fourteen mL of 70 % ethanol was added into the test tube containing soil suspension and shaken for 1 minute and dilution with sterile water. The dilution series were cultured on LCA medium. These culture plates were incubated at room temperature 2-5 days. After 2-5 days of isolation, microorganism colonies were appeared. The observed colonies were culture separately on LCA medium. Culture of isolation fungi was carried out 3-5 times with PGA medium until the pure culture was obtained. Then, isolated pure fungi were preserved into slant culture on PGA medium for further experimentation. LCA medium-Glucose:0.2g, Sucrose:0.2 g, K_2HPO_4:0.1 g, KNO_3:0.1 g, $MgSO_4.7H_2O$:0.05 g, KCl:0.05 g, Agar:1.8 g, pH:6.5 and DW: 100 mL. PGA medium - Potato Glucose Agar: 3.9 g, DW: 100 mL, pH: 6.5. LCA = Low Carbon Agar medium, PGA = Potato Glucose Agar medium

Screening of effective soil fungi by paper disc diffusion assay (NITE, 2005)

The isolated fungi were grown for 7 days on PGA medium at room temperature. The isolated fungi were inoculated on seed medium. Seed medium-Glucose:2.5g, Yeast extract: 0.8g, $MgSO_4$:0.02 g, K_2HPO_4:0.01 g, DW:100 mL, pH-6.5 (Nakagawa, 1995) and incubated at room temperature for 3 days. Five mL of seed culture was transferred into the fermentation medium. Fermentation medium-Glucose: 1.5 g, Yeast extract: 0.6 g, Soluble Starch:0.3 g, K_2HPO_4:0.01 g, $MgSO_4$:0.02 g, DW:100 mL, pH-6.5 and incubated at room temperature for 3-7 days. Twenty µL of fermented broth was put on paper disc and placed on assay plate containing test organisms. Assay medium-Glucose:1.0 g, Polypeptone 0.3 g, KNO_3:0.01 g, Agar: 1.8 gm, DW: 100 mL, pH-6.5 (Tomita, 1988).

Test Organisms

This method was carried out at Central Research and Development Center (CRDC) Ministry of industry, lower Myanmar. The test organisms used for this experiment were *Bacillus subtilis* IFO 90571, *Candida albicans* NITE 09542, *Escherichia coli* AHU5436, *Pseudomonas fluorescens* IFO94307, *Xanthomonas oryzae* NITE 09582.

Results

Isolation of Soil Fungi

In the course of investigation of fungi, ten fungi were isolated from two different soil samples by using chemical treatment dilution method which collected from Patheingyi Township, Mandaldy Region. Four fungi were isolated from Yay Kyi Village and six fungi were isolated from Shin Taw Gone Village respectively as show in (Table 2 and Figure 1).

Table 2. Isolated Fungi from Two Different Soil Sample

Soil No.	Collected Place	Soil pH	Isolation Method	Isolated Fungi
			Chemical Treatment Dilution	
S-1	Yay Kyi Village	5.6	4	ZN- 01, 02, 03, 04
S-2	Shin Taw Gone Village	6.3	6	ZN- 05, 06, 07, 08, 09, 10
Total isolated soil fungi			**10**	

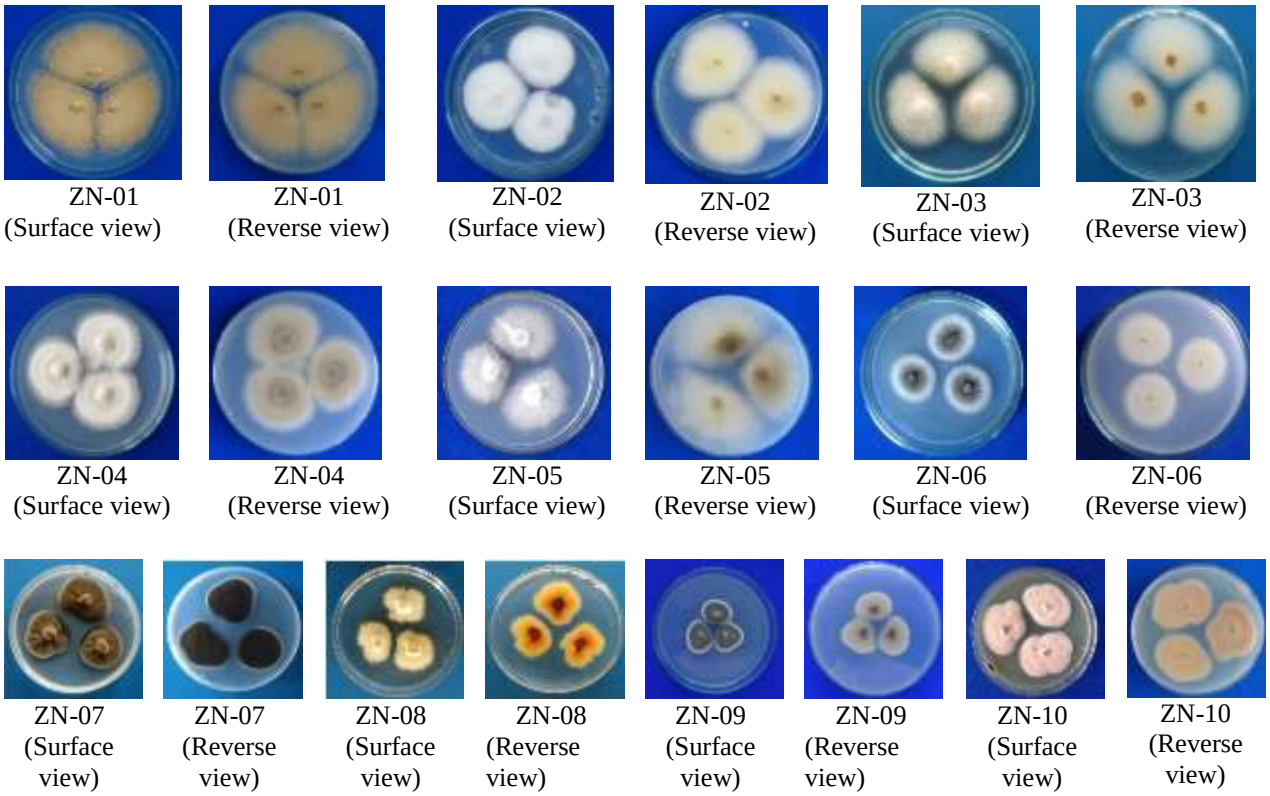


Figure 1. Morphological character of isolated fungi ZN-01 and ZN-10 on PGA medium

Antimicrobial activities of isolated soil fungi

All fungal strains were tested by five test organisms for preliminary study of antimicrobial activities. Among them, ZN-10 exhibited the highest activities as shown in (Table 3 and Figure 2)

Table 3. Antimicrobial Activities of Isolated Soil Fungi (ZN-01 to ZN-10) at 5 days period

Isolated Fungi	Antimicrobial Activity (mm)				
	Antibacterial				Antifungal
	B. subtilis	*E. coli*	*P. fluorescens*	*X. oryzae*	*C. albicans*
ZN-01	17.27	22.58	14.21	20.34	12.33
ZN-02	-	-	-	11.53	13.35
ZN-03	-	-	-	-	-
ZN-04	14.10	-	15.12	-	17.10
ZN-05	-	-	-	14.38	17.35
ZN-06	14.37	-	-	-	16.39
ZN-07	-	23.10	-	24.12	-
ZN-08	-	-	-	-	-
ZN-09	-	-	-	-	-
ZN-10	17.00	24.11	**28.12**	24.60	14.67

(-) = no activity, paper disc = 8mm

Bacillus subtilis *Escherichia coli* *Pseudomonas fluorescens* *Xanthomonas oryzae* *Candida albicans*

Figure 2. Antimicrobial activities of isolated soil fungi (ZN-01 to ZN-10)

Identification of selected fungus ZN-10

Morphological characters of fungus ZN-10

Colonies are fast growing, suede like, violet-colored on malt extract agar, vinaceous or pale violet and pink to deep purple on PGA medium for 7 days at 25°C. The vegetative hyphae are smooth-walled, hyaline, and violet rough-walled

Microscopical characters of fungus ZN-10

Conidiophores give rise from denser mycelium, bearing branches with densely cluster phialides. Phialides developed at the end of the conidiophore were in whorls and swollen at their bases, gradually tapering into a slender neck. Conidia are divergent chains, oval to ellipsoidal in shape, and smooth walled, lacking septum, violet/purple.

On the basis of morphological-microscopical characters and references key (Luangsa *et al*., 2011; Perdomo *et al*., 2013 & 2017; Dan *et al*, 2015; Sunil *et al*., 2015), it is assumed that ZN-10 strain may be *Purpureocillium* sp.

Classification -

Kingdom - Fungi

Division - Ascomycota

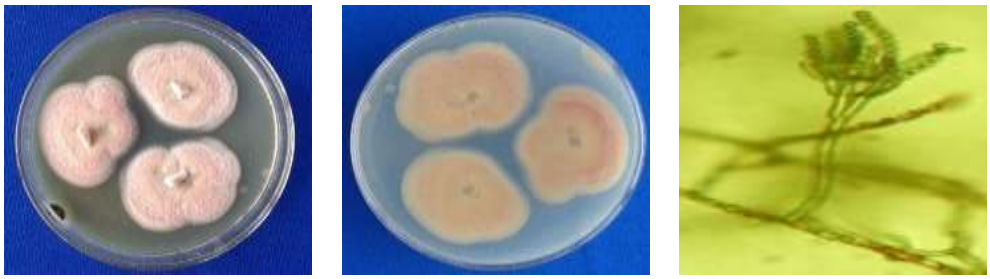
Class - Sordariomycetes

Order - Hypocreales

Family - Ophiocordycipitaceae

Genus - *Purpureocillium*

Species - *Purpureocillium* sp.



Morphology of fungus ZN-10 (Surface view) | Morphology of fungus ZN-10 (Reverse view) | micrograph of fungus ZN-10

Figure 3. Colony of morphology and micrograph of selected fungus ZN-10 (400

Discussion

In the study of isolation of soil fungi, ten different fungi were isolated from two different soil samples collected at Patheingyi Township in Mandalay Region. The isolation of soil fungi was undertaken by chemical treatment dilution method (Hayakawa and Kobayashi, 2005) were used for the isolation of soil fungi. The reported by the soil analytical data of the Department of Agriculture (Land Use) analysis of these different soil were show that the acid condition pH range (5.6 and 6.3). The soil texture was determined soil sample No.1 and No.2 soil type are Clay loam respectively.

Four fungi were collected at Yay Kyi Village and six fungi were collected at Shin Taw Gone Village. The morphological characters of four fungi (ZN-02, ZN-03, ZN-04 and ZN-05) were white colors, ZN-01 was greenish yellow color, ZN-06 was edge white, center black color, ZN-07 was brown color, ZN-08 was cream color, ZN-09 was edge white, center green color and ZN-10 was pink color in surface view. ZN-02, ZN-03 and ZN-06 were white color, ZN-04, ZN-05 and ZN-09 were cream color, ZN-01 was greenish yellow color, ZN-07 was black color, ZN-08 was orange color and ZN-10 was pink color in reverse view. In the preliminary study of ten

soil fungi were tested the paper disc diffusion assay on five test organisms. Among them, four fungi (ZN-01, ZN-04, ZN-06, and ZN-10) against on *B. subtilis*, six fungi (ZN-01, ZN-02, ZN-04, ZN-05, ZN-06 and ZN-10) against on *C. albicans*, three fungi (ZN-01, ZN-07 and ZN-10) against on *E. coli*, three fungi (ZN-01, ZN-04 and ZN-10) against on *P. fluorescens*, four fungi (ZN-01, ZN-05, ZN-07, and ZN-10) against on *X. oryzae*.

Especially, ZN-01, ZN-02, ZN-04, ZN-05, ZN-06, ZN-07 and ZN-10 showed the antimicrobial activity against on all test organisms. Moreover, ZN- 03, 08 and 09 cannot show the antimicrobial activity against on all test organisms. ZN-10 showed highest antibacterial activity against on *P. fluorescens*. The selected fungus was isolated from soil sample. No.2. The soil was collected from the place of Shin Taw Gone Village. Soil type is clay loam, pH-6.3 and N 21˚ 58′ 5.023″ and E 96˚ 10′5.487″. While selected fungus ZN-10, it was observed that colonies are fast growing, suede like. The vegetative hyphae are smooth-walled, hyaline. Conidiophores give rise from denser mycelium and bearing branches with densely cluster phialides. Phialides developed at the end of the conidiophore were in whorls and shapes are swollen at their bases, gradually tapering into a slender neck. Conidia are divergent chains, oval to ellipsoidal in shape, and smooth walled, lacking septum. On the basis of morphological-microscopical characters and references key (Luangsa *et al*., 2011; Perdomo *et al*., 2013 & 2017; Dan *et al*., 2015; Sunil *et al*., 2015), the selected fungus ZN-10 may be identified as *Purpureocillium* sp.

Eilers *et al*., (2012) reported that soil microbial abundance and diversity are highest in the top 10 cm and decline with depth. Soil samples were collected from 0-15 cm depth after removing the surface soil for the isolation of fungi. Wingfield *et al*., 2023 were stated that soil samples collected from Pattani Province, Thailand. Twenty-four fungi were isolated among them N-01, N-02, N-04, N-05, N-06, N-07, N-10, N-19 and N-24 were against on *E. coli* and *C. albicans*. In the present study, ZN-01, ZN-02 and ZN-04 were isolated from Yay Kyi Village, Patheingyi Township, Mandalay Region, that exhibit the antimicrobial activity against on *E. coli* and *C. albicans*. The morphological character of N-18 is similar to ZN-02. This is an agreement with the observation of Wingfield *et al*., 2023. Bebric *et al*., 2012 were stated that soil samples collected from Serbia. One hundred-eighteen fungi were isolated among them S- 6, S-8, S-11, S-12, S-17, S-18 and S-24 were against on *B. subtilis* and *X. oryzae*. In the present study, ZN-05, ZN-06, ZN-07 and ZN-10, were isolated from Shin Taw Gone Village, that exhibit the antibacterial activity against on *B. subtilis* and *X. oryzae*. This is an agreement with the observation of Beric *et al*., 2012.

Bhattacharyya and Jha, 2012 were described the endophytic fungi *Purpureocillium* sp. showed the antimicrobial activity against on *X. oryzae.* Kar and Chakraboty., 2018 reported that isolated fungus *Purpureocillium* sp. from the soil showed that the antimicrobial activity against on *P. fluorescens.* Dash and Dangar, 2020 were stated that isolated fungus *Purpureocillium* sp. from the soil showed that against on *B. subtilis, C. albicans, E. coli.* The results of the present work agree with Dash and Dangar, 2020.

Conclusion

The isolated fungus *Purpureocillium* sp. will further investigation to clarify the identification of isolated fungus up to species level and ages of culture, sizes of inoculums, effect of carbon source, nitrogen source, temperature, fermentation periods and time course. Majority of fungi produces secondary metabolites which may be beneficial towards pharmaceutical chemist

as these metabolites are widely used in medicine and agriculture. The results seen in the present study also support the medicinal usage as antibacterial agents in new drugs for therapy infection diseases caused by pathogens and undergo further pharmacological screening that can be used as sources for new drugs.

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