Antifungal Activity of Soil Fungus from Shwe Myin Tin area of Pathein Township on *Candida albicans*

Tin Aung Kyaw[1], Khin Min Min Phyo[2]

Abstract

In the isolation procedure of soil fungi, the soil samples were collected from Koesu, Shwe Myin Tin and Payagyi Gone Areas of Pathein Township on May, 2020. Soil fungi were isolated by serial dilution method and direct inoculation method on the nutrient growth medium. In the course of isolation, 31 different soil fungi (TAK-1 to TAK-31) were isolated by two methods at room temperature. Then, 31 isolated soil fungi were tested the antimicrobial activity on 8 different test organisms by paper disc diffusion assay method. Among them, TAK-23 showed the best antifungal activity (27.64 mm of inhibitory zone) on *Candida albicans.* For the investigation of the kinetic growth of the selected soil fungus (TAK-23), 0.54% of packed cell volume was the best at 120 hrs. In the fermentation condition of TAK-23, 5 days of inoculum was the best age (29.87 mm of inhibitory zone) and 20% of inoculum was the best size (29.22 mm of inhibitory zone) on *Candida albicans*. In the fermentation medium composition as carbon sources, the growth condition of the glycerol was the excellent (51.31 mm in diameter of colony) and the soluble starch had the best antifungal activity (28.51 mm of inhibitory zone) on *Candida albicans* among 9 different carbon sources. In the fermentation medium composition as nitrogen sources, the growth condition of $CaNO_3$ was the excellent (52.86 mm in diameter of colony) and the peptone gave the best antifungal activity (26.79 mm of inhibitory zone) on *Candida albicans* among 9 different nitrogen sources. On the effects of the different pH levels (pH 4.5 to 6.5) and temperatures (20 ºC to 40 ºC), pH 5.0 and 30ºC were the best antifungal activity (27.94 mm and 27.79 mm of inhibitory zones) on *Candida albicans* for the production of antifungal metabolite of TAK-23. Moreover, the shaking condition of TAK-23 was more suitable than the static condition of the antifungal activity (28.06 mm of inhibitory zone) on *Candida albicans*.

Keywords: soil fungus, antifungal activity, antifungal metabolite

Introduction

Soil is rich habitat or the main reservoir for the growth of fungi more than any other microorganisms. of fungi. Fungi are an important component of the soil microbiota depending on soil depth and nutrient conditions. The role of fungi in the soil is an extremely complex and is fundamental to the soil ecosystem. They perform ecological services that strongly impact the quality of human life and enormous potential for providing economic benefits (Ramadan and Ismael, 2011).

Fungal antagonism has been reported for a wide variety of pathogens. Many new and interesting bioactive metabolites such as antibiotics, antiviral, anticancer and antioxidant compounds having pharmaceutical, industrial and agricultural importance are isolated and characterized from soil fungi. Soil fungi are also the major sources of other industrially important compounds like enzyme inhibitors, anti-helminthics, antitumor agents, insecticides, vitamins, immunosuppressant and immunomodulators. The antimicrobial properties of secondary metabolites derived from various groups of fungi are widely reported as an important source of bioactive molecules (Bhattacharyya and Jha, 2011).

Therefore, the soil samples were collected from three different places of Pathein Township. The aim and objectives of this research were to isolate the soil fungi, to investigate the antimicrobial activity of the isolated soil fungi on the different test organisms, to study the kinetic growth, age and sizes of the selected soil fungus (TAK-23) for antifungal activity, to find the influences of different carbon

[1] U, Lecturer, Department of Chemisrty, Pathein University
[2] Dr., Professor, Department of Botany, University of Yangon

sources and nitrogen sources and to investigate the effects of pH levels, different temperatures and shaking, static conditions on antifungal metabolite production.

Materials and Methods

Isolation of soil fungi and their antimicrobial activity

The soil samples were collected from Koesu, Shwe Myin Tin and Payagyi Gone Areas of Pathein Township on May, 2020. The collected soil samples were air-dried, grounded and sieved in 2 mm screen.

The serial dilution method and direct inoculation method were used by soil samples for the isolation of soil fungi on nutrient growth medium (glucose 1.0 g, peptone 0.3 g, agar 1.8 g, distilled water 100 mL at pH 7.0; chloramphenicol was added to the medium for the isolation of fungi). After 7 days, the isolated soil fungi were re-cultured in the petri-dishes at three equidistance points and stored in slant culture at room temperature for further used as the antimicrobial activity.

The isolated soil fungi were inoculated in 50 mL of seed medium (glucose 1.0 g, peptone 0.3 g, $CaCO_3$ 0.01 g, distilled water 100 mL at pH 7.0) for 3 days and fermentation (glycerol 1.0 mL, yeast extract 0.3 g, $CaCO_3$ 0.01 g, $MgSO_4$ 0.01 g, distilled water 100 mL at pH 7.0) was carried out for 12 days at room temperature. Then, the fermented broth was tested the antimicrobial activity on 8 different test organisms by paper disc diffusion assay (glucose 1.0 g, peptone 0.3 g, agar 1.8 g, distilled water 100 mL at pH 7.0) method. The test organisms were incubated in the saline water (1%) for 1 day to 3 days at room temperature.

Fig. 1. Map of collected soil sample (Source from Department of Geography, Pathein University)

Table 1. Collected soil sample location and soil texture

Soil Sample	Collected Area	Location	Soil Type	Soil Moisture	Soil pH
1	Koe Su	16°49.1780′ N 94°47.3820 ′E	Clay	4.301%	4.9
2	Shwe Myin Tin	16°53.6920′ N 94°44.4440′ E	Sandy Clayloam	0.557%	5.8
3	Payagyi Gone	16°46.2610 ′N 94°45.2740′ E	Clay	3.631%	6.1

Antagonistic activity was detected after incubating of 24-48 hrs at room temperature. The presence of zone clearance around the paper disc (6 mm in diameter) on the agar plates was used as an indicator for the antimicrobial activity.

The isolated soil fungi which showed the maximum zone of clearance was chosen for further study.

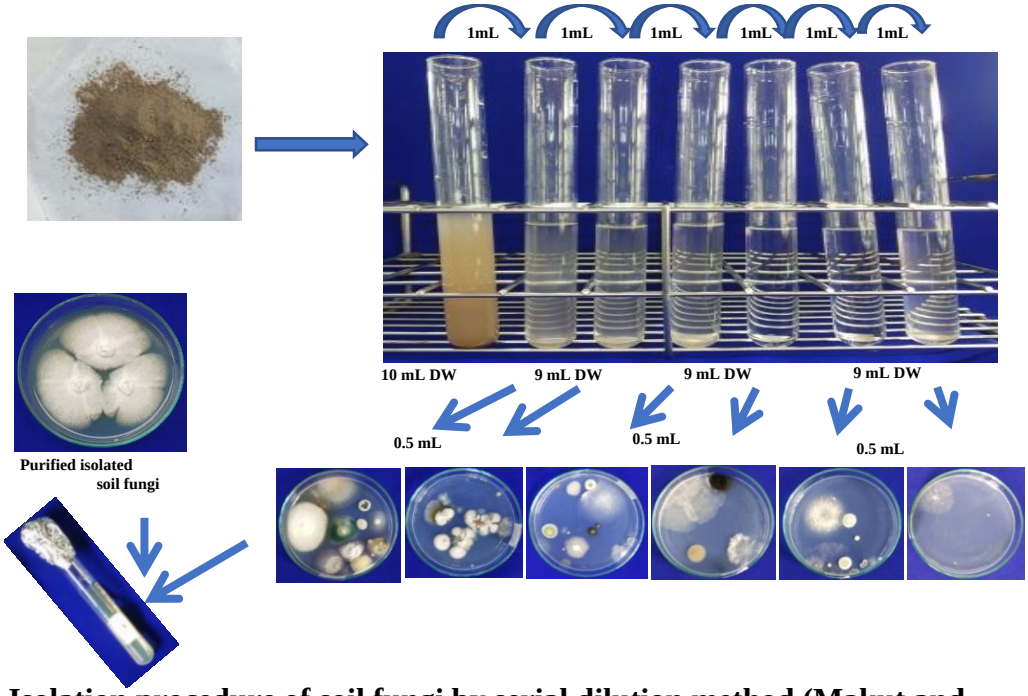


Fig. 2. Isolation procedure of soil fungi by serial dilution method (Makut and Owolewa, 2011)

The kinetic growth, age and sizes of TAK-23

For the investigation of the kinetic growth of the soil fungus (TAK-23), the dry weight of packed cell volume of TAK-23 was measured the time course from 12 hrs to 204 hrs.

The seed culture of TAK-23 was undertaken for fermentation from 1 day to 8 days at room temperature as the age of inoculum optimization.

Table 2. Different test organisms used for antimicrobial activity

No	Test organisms	Sources	Infections
1	*Escherichia coli*	AHU 5436	Diarrhea
2	*Micrococcus luteus*	NITE 83297	Skin disease
3	*Candida albicans*	NITE 09542	Candidiasis
4	*Bacillus subtilis*	IFO 90571	DNA topoisomerase I
5	*Staphylococcus aureus*	AHU 8465	Food poisoning, Methicillin Resistance
6	*Pseudomonas fluorescens*	IFO 94307	Rice disease
7	*Malasseia furfur*	AVU 0255	Dandruff, Seborrhea dermatitis
8	*Bacillus pumilus*	IFO 12092	Wound and burn infection, Fever

In the sizes of inoculum optimization, 5 days of seed culture was utilized as 5 % to 40 % for fermentation. Each fermentation was carried out 9 days and tested the antifungal activity by paper disc diffusion assay on *Candida albicans*.

The fermentation medium composition as carbon and nitrogen sources of TAK-23

For the growth conditions and the antifungal metabolite production of soil fungus (TAK-23), 9 different carbon (rice powder, soluble starch, glucose, lactose, maltose, tapioca powder, sugar, potato powder, glycerol) and nitrogen (malt extract, $NaNO_3$, KNO_3, $CaNO_3$, yeast extract, peptone, fish extract, meat extract, NH_4Cl) media sources were utilized for 7 days as the growth conditions and tested the antifungal activity for 7 days by paper disc diffusion assay on *Candida albicans* at room temperature.

The different pH levels and temperatures for fermentation of TAK-23

For the antifungal metabolite production of soil fungus (TAK-23), pH 4.5, 5.0, 5.5, 6.0, 6.5 and temperature 20 ºC, 25 ºC, 30 ºC, 35 ºC, 40 ºC were utilized at 7 days of fermentation for the antifungal activity by paper disc diffusion assay on *Candida albicans*.

The shaking and static conditions for fermentation of TAK-23

The shaking and static conditions of TAK-23 were inoculated for 7 days of fermentation at 100 spm and tested the antifungal activity by paper disc diffusion assay on *Candida albicans* at room temperature.

Results

Total 31 different soil fungi (TAK1 to TAK-31) were isolated from the soil samples of Koesu, Shwe Myin Tin and Payagyi Gone Areas of Pathein Township.

Table 3. Isolated soil fungi from three different areas

Sample	Place	Isolated soil fungi	No. of isolated soil fungi
1	Koe Su	TAK – 1 to TAK - 15	15
2	Shwe Myin Tin	TAK – 16 to TAK - 25	10
3	Payagyi Gone	TAK – 26 to TAK-31	6
		Total	31

According to the antimicrobial activity, 31 isolated soil fungi (TAK-1 to TAK-31) showed the antimicrobial activity on the most of 8 different test organisms. Among them, TAK-23 from Shwe Myin Tin area had the best antifungal activity (27.64 mm of inhibitory zone) on *Candida albicans* for 7 days of fermentation.

The effects of the kinetic growth, age and sizes of TAK-23

In the investigation of the kinetic growth of the selected soil fungus (TAK-23), 0.54% of packed cell volume with dry weight was the best at 120 hrs.

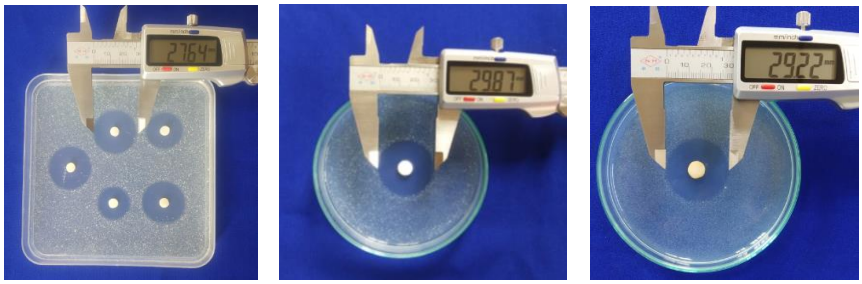
Five days of inoculum was the best age (29.87 mm of inhibitory zone) on *Candida albicans* for 7 days of fermentation at room temperature.

For the production of antifungal metabolite, 20% of seed culture (5 days age) was the best size (29.22 mm of inhibitory zone) among 5% to 40% for fermentation on *Candida albicans* at room temperature.

Table 4. Antimicrobial Activity of Soil Fungi on Test Organisms

No.	Isolated soil fungi	*Escherichia coli*	*Micrococcus luteus*	*Candida albicans*	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas fluorescens*	*Malasseia furfur*	*Bacillus pumilus*
1	TAK-1	20.78 mm	10 mm	22.15 mm	21.65 mm	19.69 mm	10 mm	16.62 mm	24.36 mm
2	TAK-2	23.65 mm	18.58 mm	22.21 mm	23.03 mm	20.49 mm	15.12 mm	20.63 mm	21.43 mm
3	TAK-3	19.06 mm	-	20.39 mm	17.59 mm	18.44 mm	16.16 mm	12.30 mm	18.27 mm
4	TAK-4	16.80 mm	10 mm	20.28 mm	23.56 mm	23.80 mm	-	16.46 mm	15.51 mm
5	TAK-5	18.25 mm	10 mm	22.24 mm	22.09 mm	25.07 mm	10 mm	15.33 mm	11.65 mm
6	TAK-6	20.03 mm	10 mm	23.13 mm	18.33 mm	21.42 mm	20.14 mm	17.42 mm	20.20 mm
7	TAK-7	17.08 mm	10 mm	21.27 mm	+	24.16 mm	10 mm	19.40 mm	23.54 mm
8	TAK-8	17.63 mm	10 mm	21.60 mm	25.00 mm	23.07 mm	10 mm	16.17 mm	17.11 mm
9	TAK-9	14.53 mm	-	25.05 mm	21.91 mm	17.65 mm	10 mm	14.53 mm	15.50 mm
10	TAK-10	13.77 mm	-	20.64 mm	21.91 mm	19.03 mm	-	11.00 mm	14.11 mm
11	TAK-11	13.70 mm	-	22.36 mm	22.61 mm	15.78 mm	-	13.37 mm	15.31 mm
12	TAK-12	10 mm	-	24.13 mm	22.10 mm	18.57mm	-	16.20 mm	17.34 mm
13	TAK-13	20.16 mm	10 mm	24.65 mm	21.09 mm	20.70 mm	20.15 mm	17.80 mm	23.50 mm
14	TAK-14	10 mm	-	20.85 mm	23.83 mm	17.36 mm	-	15.21 mm	14.63 mm
15	TAK-15	16.00 mm	-	20.11 mm	20.11mm	17.03 mm	10 mm	17.67 mm	15.43 mm
16	TAK-16	20.17 mm	20.69 mm	20.96 mm	20.05 mm	20.91 mm	18.26 mm	21.03 mm	15.44 mm
17	TAK-17	20.75 mm	23.32 mm	22.19 mm	21.48 mm	22.12 mm	18.26 mm	22.04 mm	16.44 mm
18	TAK-18	19.20 mm	21.78 mm	23.04 mm	21.48 mm	20.47 mm	10 mm	23.26 mm	17.50 mm
19	TAK-19	17.36 mm	-	15.03 mm	16.76 mm	16.64 mm	10 mm	18.26 mm	13.66 mm
20	TAK-20	20.16 mm	23.28 mm	21.97 mm	21.08 mm	18.88 mm	10 mm	22.92 mm	15.50 mm
21	TAK-21	24.16 mm	21.28 mm	**25.58 mm**	23.61 mm	22.04 mm	10 mm	23.03 mm	22.42 mm
22	TAK-22	16.91 mm	-	14.12 mm	14.88 mm	20.95 mm	18.46 mm	21.25 mm	13.59 mm
23	TAK-23	**26.13 mm**	11.60 mm	**27.64 mm**	22.18 mm	19.63 mm	13.30 mm	16.84 mm	25.09 mm
24	TAK-24	16.19 mm	-	14.12 mm	19.27 mm	20.21 mm	18.46 mm	20.03 mm	13.61 mm
25	TAK-25	16.54 mm	-	13.19 mm	13.09 mm	18.60 mm	16.04 mm	10 mm	13.61 mm
26	TAK-26	21.09 mm	13.14 mm	21.13 mm	20.34 mm	21.23 mm	-	17.05 mm	22.12 mm
27	TAK-27	11.12 mm	-	10 mm	12.17 mm	13.30 mm	-	-	10.11 mm
28	TAK-28	11.00 mm	-	12.45 mm	11.11 mm	13.30 mm	-	-	10.11 mm
29	TAK-29	15.21 mm	-	12.45 mm	14.21 mm	19.36 mm	13.30 mm	15.69 mm	+
30	TAK-30	12.12 mm	-	11.60 mm	12.95 mm	18.20 mm	11.20 mm	10 mm	+
31	TAK-31	23.85 mm	10 mm	**27.03 mm**	**25.94 mm**	25.05 mm	20.00 mm	24.12 mm	**27.24 mm**

- No activity



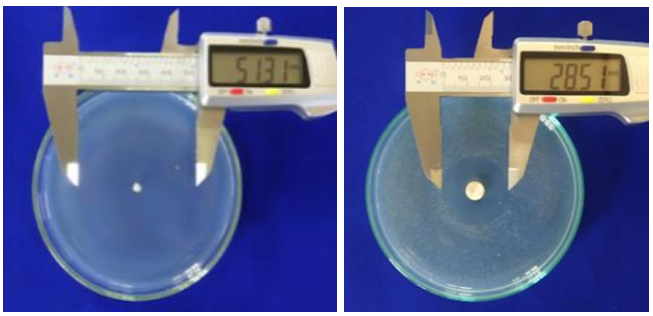
7 days of fermentation **5 days** **20%**

Fig. 3. Antifungal activity of TAK-23 on *Candida albicans* by age and size

The effects of carbon and nitrogen sources of TAK-23

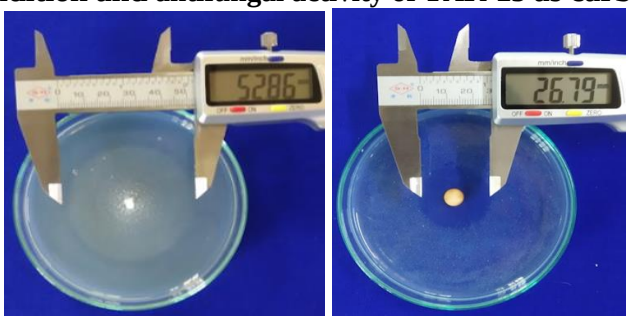
Although the growth condition of the glycerol was the excellent (51.31 mm in diameter of colony) among 9 different carbon sources, the antifungal activity of the starch was the best (28.51 mm of inhibitory zone) on *Candida albicans* at 7 days of fermentation for the production of antifungal metabolite.

Although the growth condition of $CaNO_3$ was the excellent (52.86 mm in diameter of colony) among 9 different nitrogen sources, the antifungal activity of the peptone was the best (26.79 mm of inhibitory zone) on *Candida albicans* at 7 days of fermentation for the production of antifungal metabolite.



growth condition on glycerol antifungal activity of **starch** on *Candida albicans*

Fig. 4. Growth condition and antifungal activity of TAK-23 as carbon source



growth condition on $CaNO_3$ antifungal activity of **peptone** on *Candida albicans*

Fig. 5. Growth condition and antifungal activity of TAK-23 as nitrogen source

The effects of different pH levels and temperatures of TAK-23

In the different pH levels and temperatures for fermentation of TAK-23, pH 5.0 and 30ºC had the highest antifungal activity (27.94 mm and 27.79 mm of inhibitory zones) at 7 days on *Candida albicans* for the production of antifungal metabolite.

The effects of shaking and static conditions of TAK-23

The selected soil fungus (TAK-23**)** exhibited the more antifungal activity on the shaking condition (28.06 mm of inhibitory zone) than on the static condition (27.00 mm of inhibitory zone) on *Candida albicans* at 7 days of fermentation for the antifungal metabolite production.

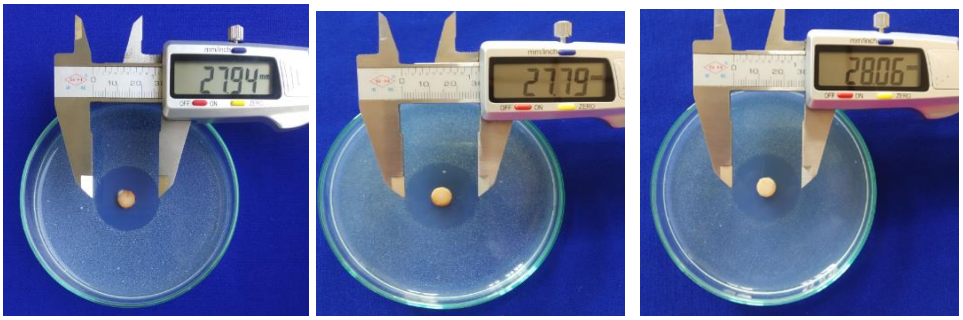


Fig. 6. Antifungal activity of TAK-23 on *Candida albicans* by pH, temperature and shaking condition

Discussion and Conclusion

This study raises the possibility of exploring fungal species having antifungal activity and used them as antibiotics. Furthermore, our observations indicated that fungi from pharmaceutical soil sample had pharmaceutical potential by antifungal compounds and then the medicinal properties of these fungi can be produced the active secondary metabolites (Bhardwaj *et al.*, 2017).

The mycelial growth and the production of antifungal metabolite by the antagonistic strain isolated from the subsurface soils of Brahmaputra plains, Assam, India, are mainly influenced by various nutrient supplements in the culture media. The maximum production of the antifungal metabolites by our antagonist, could be achieved in Potato dextrose broth medium with sucrose at 2.0 g/L as carbon and asparagine at 1.0 g/L as nitrogen source *in vitro*. Further process parameters, like incubation time and temperature at 25ºC, pH 5.5 and 16 days of incubation period under shaking condition are found for the maximal production of bioactive metabolite (Bhattacharyya and Jha, 2011).

In this investigation, 31 different soil fungi were isolated from the soil samples. According to the antimicrobial activity, TAK- 23 showed the best antifungal activity (27.64 mm of inhibitory zone) on *Candida albicans*. As the kinetic growth of TAK-23, the packed cell volume with the dry weight had the best at 120 hrs. According to the age and sizes of inoculum, the highest activity of TAK-23 reached for 7 days of fermentation period with 5 days age and 20 % size of inoculum on *Candida albicans*. In the optimum fermentation compositions as carbon and nitrogen sources, the starch and the peptone were the highest antifungal activity with pH 5.0 at 30 ºC on the shaking condition on *Candida albicans* for the production of antifungal metabolite.

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References

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

Bhardwaj, A., S.Chaman and S.Verma, 2017: **Production of antibacterial agent from fungi isolated from pharmaceutical soil sample by fermentation under optimized conditions** (Asian Journal of Pharmaceutical and Clinical Research) Sri Guru Gobind Singh College, Chandigarh, India.

Bhattacharyya P. N. and D. K. Jha, 2011: **Optimization of cultural conditions affecting growth and improved bioactive metabolite production by a subsurface aspergillus strain TSF 146,** Gauhati University, International Journal of Applied Biology and Pharmaceutical Technology, India.

Makut, M. D. and O. A. Owolewa, 2011: **Antibiotic-production fungi present in the oil environment of Keffi metropolis, Nasarawa state, Nigeria**, Trakia Journal of Sciences, Vol. 9, No. 2, pp 33-39, Nasarawa State University, Keffi, Nigeria.

Ramadan, N. A. and H. M. Ismael, 2011: **Isolation of Fungi Seasonally from Soil, Air and Water in Villages in "Binary Safeen",** 4th International Scientific Conference of Salahaddin University-Erbil, Irag.