FERMENTATION, EXTRACTION AND IDENTIFICATION OF SELECTED SOIL FUNGUS (YTF 8) FROM KAN GYI DAUNG TOWNSHIP, AYEYARWADY REGION

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Abstract

Soil samples were collected from 5-different places of Kan Gyi Daung Township. Eighteen fungi were isolated from these 5-different soil samples. Soil was analyzed at the Applied Geology Department, Yangon University. Isolation of fungi was undertaken by the physical and chemical treatment method. The soil fungi were isolated by using Low Carbon Agar medium for first culture and Potato Glucose Agar medium for pure culture. The isolated fungi were given as temporary names YTF 1 to YTF 18. The purpose of this study was to screen for antimicrobial metabolite production and effect of variation of carbon and nitrogen sources. In this study, thirteen fungal strains were tested by agar well diffusion method with three kinds of test organisms. YTF 1, 2, 7, 8, 9, 11, 12, 15, 16, 17 and 18 showed antimicrobial activity and the remaining five fungi YTF 3, 4, 10, 13 and 14 did not show the activity. The selected fungi (YTF 1, 7, 8, 11, 15 and 16) showed antimicrobial activity against on *Agrobacterium tumefaciens,* while YTF 1, 2, 7, 8, 15 and 16 showed the activity on *Candida albicans.* Especially YTF 8 gave the best activities on *Candida albicans*. The fermentation conditions of YTF 8 was carried out by the studies of proper age, size and different carbon and nitrogen sources on antimicrobial metabolite production on *Candida albicans*. The 5 days was found to be optimum fermentation period. In the size of inoculum, 5 %, 15 % and 20 % were used and 20 % was suitable condition. Moderate growth and the best antimicrobial production of selected fungus (YTF 8) was observed in both carbon and nitrogen sources. The addition of potato as a carbon source resulted in the maximum growth and the inhibition zones reached 33.96 mm in YTF 8. YTF 8 showed the moderate growth on almost all nitrogen sources. The inhibition zone of YTF 8 was 32.90 mm on peptone. The present study was focused on fermentation, extraction and identification of selected soil fungus. Firstly, fermentation was carried by the fermentation medium (FM) with the different concentrations of carbon and nitrogen sources. In the different carbon concentrations, FM 6 (glucose 1.5 g) gave the moderate antifungal activity (39.62 mm) followed by using (glucose 1 g) in FM 5 (31.52 mm) and (glucose 2 g) in FM 7 (31.51 mm) against

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Candida albicans respectively. In the nitrogen concentrations, FM 14 (peptone 1 g) exhibited the highest activity (30.83 mm). And then, the effect of pH was studied by varying pH of 4, 5, 6, 7, 8, 9 and 10. In extraction of antifungal metabolites, YTF 8 was observed by four solvents. Among the four solvents, ethyl acetate was found as the most suitable solvent and R_f value had 0.81. And then, the fungal culture filtrates were studied by various ratios (1:1, 2:1, 3:1 v/v) of ethyl acetate and various pH. Among them, the ethyl acetate extract (1:1) demonstrated the highest activity (30.45 mm) at pH 6 showed highest inhibitory effect against *Candida albicans.* Finally, the selected fungus YTF 8 was identified. According to the results, the surface color of YTF 8 was pale yellow and the reverse colour was yellow. The microscopic character of YTF 8 was conidial heads on PGA radiate, conidiophores: long, vesicle: pyriform, sterigmata: uniseriate, phialides: ampliform. Therefore, YTF 8 was identified as *Aspergillus clavatus.*

Keywords: antimicrobial metabolite, fungi, fermentation medium, soil, inhibition zone

Introduction

Microorganisms live in every part of the biosphere including soil, hot spring, seven miles deep in the ocean, 40 miles high in the atmosphere and inside rocks far down within the Earth's crust. Soil is the largest source of microorganisms. There are billions to hundreds of billions of soil microorganisms in a mere handful of a typical garden soil. That single handful might well contain thousands of different species of bacteria, hundreds of different species of fungi and protozoa. Almost all of these countless soil organisms are not only beneficial, but essential to the life giving properties of soil.

Fungi are very abundant in the soil and may represent up to 80% of soil microbial biomass. Fungi constitute a group of microorganisms that are widely distributed in environment especially in soil (Boer *et al.,* 2005). Fungi are one of the dominant groups present in soil which strongly influence ecosystem structure and functioning and thus playing a key role in many ecological services. (Orgiazzi *et al.,* 2012).

The genus *Aspergillus* belongs to a group of filamentous *Deutromycetes* (Guarro, 1999) which lacks sexual reproductive phase or is

neither known nor discovered. They are characterized by having a spore bearing structure called the conidia head, a basal foot referred to a "foot cell" but not septated.

This research paper aims to investigate the isolation of soil microorganisms and to know the different soil microorganisms from various soil samples. Studies were, therefore, carried out to investigate the effect of fermentation, pH, temperature, static, shaker culture and to extract the antifungal compound and identify of potential fungi.

Materials and Methods

Collection of Soil Samples

Soil samples were collected from five different places S-I to S-V (Table 1) of Kan Gyi Daung Township, Ayeyarwady Region. Soil was analyzed by Applied Geology Department, University of Yangon. Soil samples of five different places were collected during July 2015 to August 2015.

Table 1: Five Different Soil Samples Collected from Kan Gyi Daung Township

Soil samples	Sample collected areas	pH	Soil Type	Location
S-I	Kan Gyi Daung (Community and Government)	6.40	Clay Loam	N 16˚ 55' 05.649" E 0.94˚ 54'12"
S-II	Ta Kone Gyi (Basic Education High School	6.08	Loam	N 16˚ 51' 07.699" E 094˚ 50' 19.345"
S-III	Kyon Pa Doke (Basic Education School)	5.47	Clay	N 16˚ 50' 21.941" E 094˚ 51' 06.449"
S-IV	NyaungGyaung (Monastery)	4.01	Silty clay	N 16˚ 47' 29.297" E 094˚ 51' 59.458"
S-V	Kyaik Lat (Police Station)	4.31	Silty clay loam	N 16˚ 46' 19.946" E 094˚ 51' 41.303"

Isolation of Fungi from the Soil Samples

The soil fungi were enumerated by physical and chemical treatment dilution method or media such as Low Carbon Agar (LCA) medium and Potato Glucose Agar (PGA) medium.

Physical and chemical treatment dilution method

The collected soil was air-dried at room temperature for 5 days. The soil sample was grounded and sieved in 2 mm screen. The sample was placed in the hot air oven at 120 ˚C for 1 h. The dried soil sample was suspended with 1.5 % phenol and diluted with sterile water. The dilution series were cultured on LCA medium. 30 µL of soil suspension was cultured on plates containing LCA medium and incubated for 5-10 days. Pure colonies were picked up to start containing in PGA medium (Phay and Yamamura, 2005). From this experiment, a total of 18 fungi (YTF 1- YTF 18) were isolated.

Preliminary Study for Antimicrobial Activity

The isolated fungi were grown on PGA medium for 5 days. The isolated fungi were inoculated into 25 mL seed medium and incubated at room temperature for 3 days. After 3 days, 20 mL of seed culture was transferred into the 80 mL of fermentation medium and incubated at room temperature. Fermentation was carried for 3-9 days. Test organisms such as *Agrobaterium tumefaciens, Candida albicans, Staphylococcus aureus* were used in this experiment (Ando, 2004).

Screening of antimicrobial activity of YTF 8 by agar well diffusion method

According the preliminary test, among 18 fungi, only YTF 8 occurred to show the antimicrobial activity against *C. albicans*.1 day old culture test broth (0.01 mL) was added to 25 mL of assay medium and thoroughly mixed and poured into plate. After solidification, cork borer was used to make the wells (wells - 8 mm). The fermented broth (20 µL) was carefully added into the wells and incubated at room temperature for 24-48 h. The diameter of the zones of inhibition around each well was measured and recorded after 24-48 h incubation (Collins, 1965).

Fermentation medium with various carbon sources

The initial basal medium contained yeast extract (0.5g), K_2HPO_4 (0.001g), $MgSO_4$ (0.001g), $CaCO_3$ (0.1 g), DW (100 mL), pH 7. After that glucose (1 g, 1.5, 2, 2.5 and 3 g) was added to the initial basal medium. The medium constituents were sterilized by autoclaving at 121˚C for 30 min, and were cooled thoroughly before inoculation. And then, the strain was cultured. Fermentation was carried out for 5 days and antimicrobial activity was tested by agar well diffusion method.

Fermentation medium with various nitrogen sources

The initial basal medium used contained glucose (1 g), soluble starch (0.5 g), K_2HPO_4 (0.001 g), $MgSO_4$ (0.001 g), $CaCO_3$ (0.1 g), DW (100 mL), pH 7. After that peptone (1 g, 1.5, 2, 2.5 and 3 g) was added to the initial basal medium. The medium constituents were sterilized by autoclaving at 121°C for 30 min, and were cooled thoroughly before inoculation. And then, the strain was cultured. Fermentation was carried out for 5 days and antimicrobial activity was tested by using agar well diffusion method.

The effect of pH on fermentation conditions

The pH sensitivity of the culture supernatant recovered during stationary growth phase of the isolates, pH values were adjusted ranging from 4 – 10 by using 0.1 M NaOH or 0.1 M HCl. The medium constituents were sterilized by autoclaving at 121°C for 30 min, and were cooled thoroughly before inoculation. And then, the strain was cultured. Fermentation was carried out for 5 days and antimicrobial activity was tested by agar well diffusion method (Hernadez *et al*., 2005).

The effect of temperature on fermentation

The medium constituents were sterilized by autoclaving at 121°C for 30 min, and were cooled thoroughly before inoculation. And then, the strain was cultured. The selected fungus were incubated at 20 °C, 25 °C, 30 °C, 35 °C , 40 °C and 45 °C respectively. Fermentation was carried out for 5 days and antimicrobial activity was tested by using agar well diffusion method (Hernadez *et al*., 2005)

The static and shaker of fermentation conditions

Using all optimized medium components, the shake-flasks was done using (1.5 g) of glucose as a carbon sources and (1 g) of peptone as nitrogen source. The flasks were placed in TS-2000A VDRL shaker. After 5 days, the fermented broth was tested on agar well diffusion method. Precisely, the static fermented broth was tested on agar well diffusion method.

Screening of Bioactive Compounds in the Fermented Broth (YTF 8) by Paper chromatography

The purpose of paper chromatography is to know how to extract the bioactive compound from fermentation broth by using which solvent system. The filter paper and four solvents; 20 % NH_4Cl, n-butanol saturated with water, n-butanol-acetic acid-water (3:1:1), and ethyl acetate saturated with water were used for preliminary characterization of antibiotics. The fermented broth samples were applied on the paper and allowed to dry. The papers were chromatographed in each solvent. Then, bioautography was done to check the antifungal activity of each. Each paper was placed on assay agar plates, in the same method as paper disc assay, except that after one hour the paper was taken out, then the plates were incubated for 24-36 h. The inhibitory zone was measured yielding R_f value for the corresponding bioactive compound (Tomita, 1988).

R_f value was calculated by the following equation.

$$R_f \text{ value} = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by the solvent}}$$

Identification of the Selected Fungus YTF 8

The selected fungi were cultured on seven differential media such as Blakeslee's Malt Extract Agar (BMEA), Malt Extract Agar (MEA), Czapek Dox Agar (CZA), Dichloran-Rose Bengal-Chloramphenicol Agar (DRBC), Glucose Ammonium Nitrate Agar (GAN), Potato Glucose Agar (PGA) and Low Carbon Agar (LCA). After seven days of incubation, the selected fungus culture were observed for macroscopic characteristics such as colony diameter, colony colour and microscopic characteristics including

conidiophore, vesicle, phialides and conidia. For microscopic characteristics, slides were stained with cotton blue and mounted in lactophenol (Nyongesa *et al*., 2015)

Microscopic examination with lactophenol cotton blue

The drop of lactophenol cotton blue was placed on a clean glass slide. With a bent dissecting needle, a small portion of the colony was removed from the agar surface and it was placed in the drop of LPCB. With two dissecting needles, apart the mycelial mass of the colony gently teased on the slide, with a coverslip was covered, and under the light microscope was observed with low power (x 40) magnification

Results and Discussion

Isolation of Fungi from Soil Samples

In the investigation, 18 fungi were isolated from the five different soil samples of Kan Gyi Daung Township. Isolated fungi YTF 1 to YTF 4 were collected from Kan Gyi Daung, YTF 5 to YTF 6 were collected from Ta Kone Gyi, YTF 7 to YTF 8 were collected from Kyon Pa Doke, YTF 9 to YTF 13 were collected from Nyaung Gyaung and YTF 14 to YTF 18 were collected from Kyiak Lat. These results are shown in Table 1.

The isolated fungi were designated as YTF 1 to YTF 18. The surface colour of isolated fungi YTF 1, 6, 13 and 15 were white and their reverse colours were yellow, pink and white respectively. The surface colour of isolated fungi YTF 2, 10, 12 and 18 were green and their reverse colours were yellow, greenish white, white and green respectively. The surface colour of isolated fungus YTF 3 was black green and its reverse colour was white. The surface colour of isolated fungi YTF 4, 14, 16 and 17 were blue and their reverse colour were blue, pink, white and greenish white. The surface colour of isolated fungus YTF 5 was yellow, YTF 8 was pale yellow and their reverse colours were yellow. The surface colour of isolated fungus YTF 7 was pale green and its reverse colour was pale yellow. The surface colour of isolated fungus YTF 9 was black and its reverse colour was greenish white (Figure 1 and 2).

Table 2: The Isolated Fungi from the Collected Soil Samples

Soil Samples	Isolated Fungi
S -I	YTF 1, YTF 2,YTF 3, YTF 4
S -II	YTF 5, YTF 6
S -III	YTF 7, YTF 8
S -IV	YTF 9, YTF 10, YTF 11, YTF 12, YTF 13
S -V	YTF 14, YTF 15, YTF 16, YTF 17, YTF 18

Figure 1: Morphological Character of Isolated Fungi YTF 1 to YTF 4

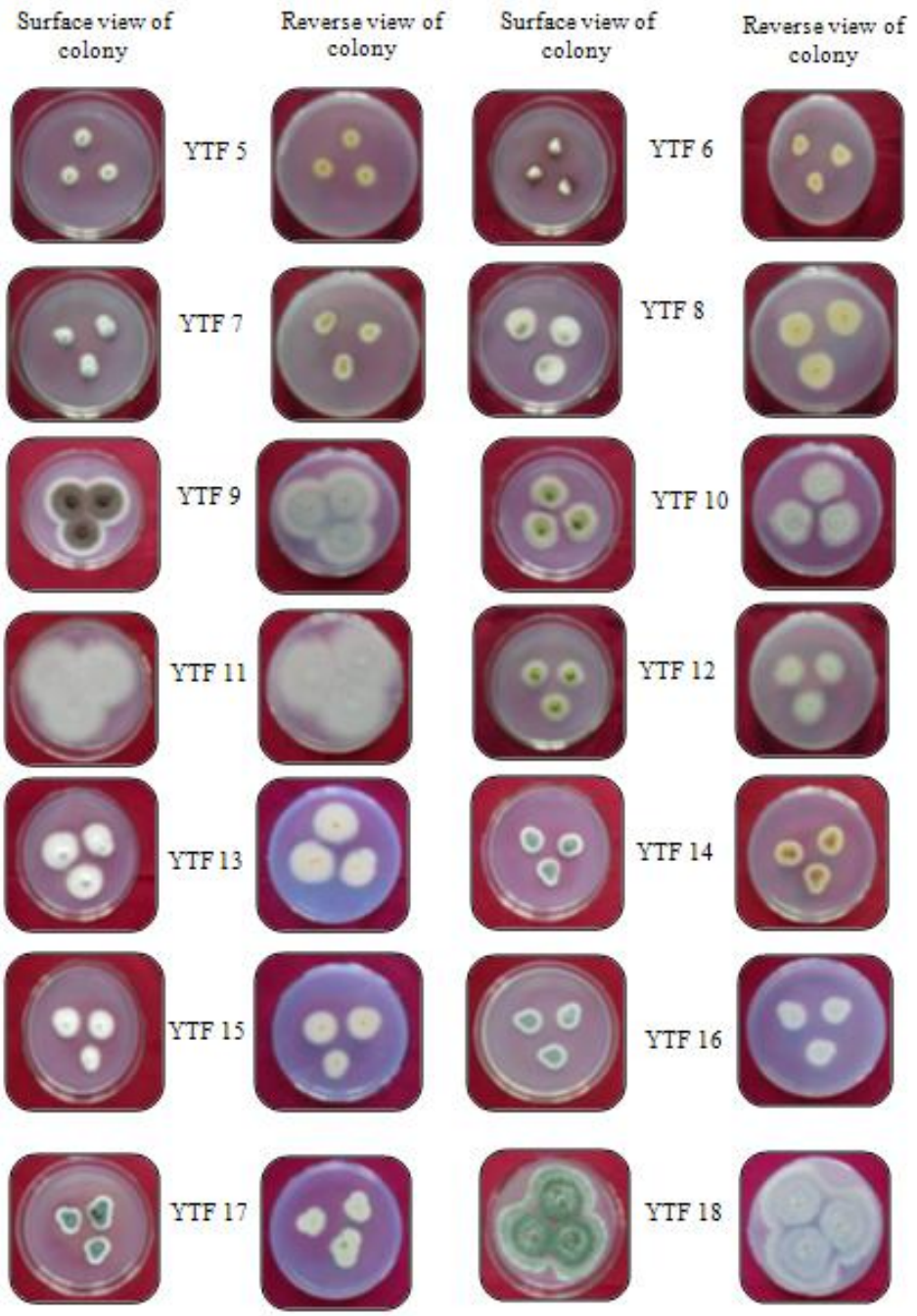

Figure 2: Morphological Character of Isolated Fungi YTF 5 to YTF 18

Isolated Fungi and their Antimicrobial Activity

In this study, thirteen fungi strains were tested with three test organisms by agar well diffusion method. There are *Agrobaterium tumefaciens, Candida albicans, Staphylococcus aureus.* YTF 8 gave the best activities on *Candida albicans* (Table 3 and Figure 3)

Table 3: The Isolated Fungi and their Antimicrobial Activity

No	Isolated fungi	Activitty (clear zone diameter, mm)		
		A.tumefaciens	***C.albicans***	***S.aureus***
1	YTF 1	20.07	34.07	18.25
2	YTF 2	-	34.30	17.95
3	YTF 5	-	-	-
4	YTF 6	-	-	-
5	YTF 7	24.71	35.26	-
6	YTF 8	28.37	37.39	19.84
7	YTF 9	-	-	-
8	YTF 11	13.71	-	18.95
9	YTF 12	-	-	20.12
10	YTF 15	18.25	33.49	24.22
11	YTF 16	17.95	33.85	-
12	YTF 17	-	-	-
13	YTF 18	-	-	-

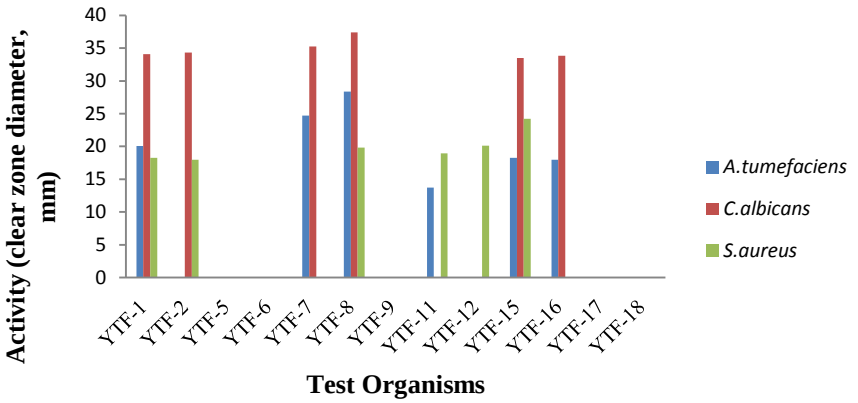

Figure 3: The isolated fungi and their antimicrobial activity

The effects of ages of inoculum on the fermentation

YTF 8 reached the highest activities (35.26 mm) in 5 days age of inoculum on *Candida albicans* (Table 4 and Figure 4).

Table 4: The Effects of Ages of Inoculum on the Fermentation for YTF 8

No	Fermentation Period (days)	Activity (clear zone diameter, mm)
1	3	31.15
2	4	34.07
3	5	35.26
4	6	31.15

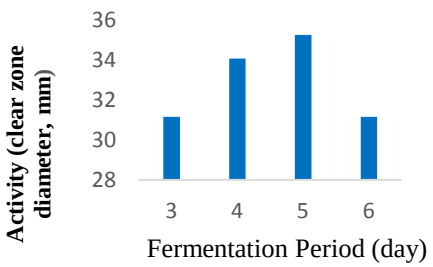

Figure 4 : The effects of ages of inoculum on the fermentation for YTF 8

The effects of sizes of inoculum on the fermentation

In the proper size of inoculum, 20 % was the most suitable activity 35.26 mm in YTF 8 followed by the high activities of 5 % and 15 % respectively (Table 5 and Figure 5)

Table 5: The Effects of Sizes of Inoculums on the Fermentation for YTF 8

Sr. No	Sizes of inoculum at 5 days (%)	Activity (clear zone diameter, mm)
1	5	34.07
2	15	33.49
3	20	35.26

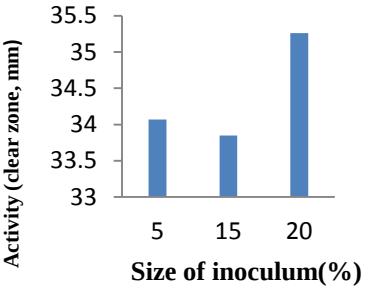

Figure 5: The effects of sizes of inoculums on the fermentation for YTF8

The effects of carbon and nitrogen sources

Moderate growth and the antifungal substance production in YTF 8 was influenced by addition of potato reaching the highest activity 33.96 mm and maximum production of antifungal metabolite YTF 8 was observed on peptone 32.90 mm (Table 6, 7 and Figure 6, 7).

Table 6: The Effects of Carbon Sources

Sr. No	Carbon sources	Activity (clear zones diameter, mm)
1	Glucose	30.37
2	Sucrose	30.18
3	Soluble starch	30.10
4	Oat	30.45
5	Glycerol	29.96
6	Potato	33.96

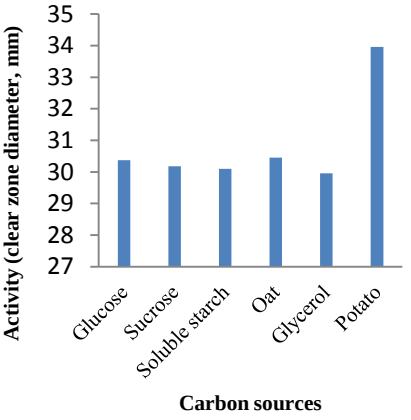

Figure 6: The effects of carbon sources

Table 7 : The Effects of Nitrogen Sources

Sr . No	Nitrogen sources	Activity (clear zones diameter, mm)
1	NH_4Cl	30.67
2	Soy bean	31.12
3	Yeast extract	29.99
4	Poly peptone	26.85
5	Malt extract	32.28
6	Fish cake	28.46
7	Gelatin	30.52
8	Peptone	32.90

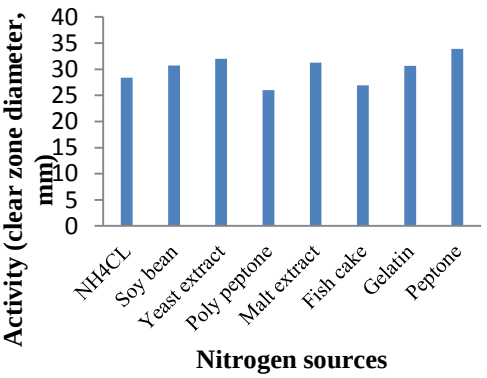

Figure 7: The effects of nitrogen sources

The effects of carbon and nitrogen sources on various fermentation media of YTF 8

In this study, 1.5 g of glucose, FM 6 (39.62 mm) as a carbon source and 1g of peptone, FM 14, (30.83 mm) as a nitrogen source showed the maximum antifungal activity against *Candida albicans* (Table 8, 9 and Figure 8, 9).

Table 8: Antifungal Activity of YTF 8 on the Fermentation Medium with the Various Carbon Concentration

Sr. No	Fermentation media	Antifungal activity diameter,(mm)
1	FM-1	28.90
2	FM-2	25.87
3	FM-3	30.45
4	FM-4	16.80
5	FM-5	31.52
6	FM-6	39.62
7	FM-7	31.51
8	FM-8	14.11
9	FM-9	13.92

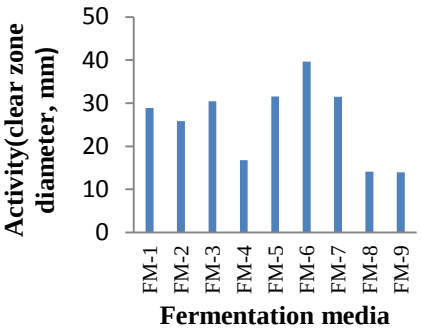

Figure 8 : Antifungal activity of YTF 8 on the fermentation medium with the various carbon concentration

Table 9: Antifungal Activity of YTF 8 on the Fermentation Medium with the Various Nitrogen Concentration

Sr. No	Fermentation media	Antifungal activity (clear zone diameter, mm)
1	FM-10	20.75
2	FM-11	19.05
3	FM-12	18.57
4	FM-13	17.15
5	FM-14	30.83
6	FM-15	26.57
7	FM-16	21.90
8	FM-17	13.17
9	FM-18	13.08

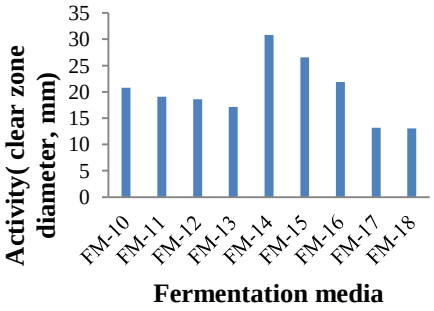

Figure 9: Antifungal activity of YTF 8 on the fermentation medium with the various nitrogen concentration

The effect of pH on the fermentation conditions

In this study, the highest antifungal activity was obtained at pH 6 (35.38 mm) against *Candida albicans* (Table 10 and Figure 10).

Table 10: The Effects of pH on the Fermentation Conditions of YTF 8

Sr. No	pH	Antifungal activity (clear zone diameter, mm)
1	4	31.38
2	5	34.70
3	6	35.38
4	7	34.50
5	8	34.18
6	9	32.68
7	10	26.59

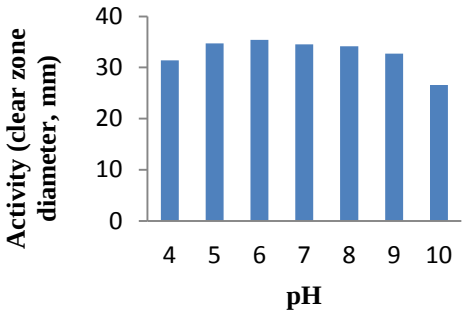

Figure 10: The effects of pH on the fermentation conditions of YTF 8

The effect of temperature on the fermentation condition

In this investigation, temperature 30˚C showed the highest antifungal activity (33.44 mm) against on *Candida albicans* (Table 11 and Figure 11).

Table 11: The Effects of Temperature on the Fermentation Conditions of YTF 8

Sr. No	Temperature (˚C)	Antifungal activity (clear zone diameter, mm)
1	20	20.13
2	25	33.24
3	30	33.44
4	35	31.97
5	40	26.46
6	45	23.99

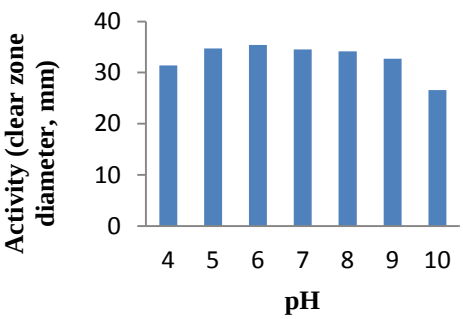

Figure 11: The effects of temperature on the fermentation conditions of YTF 8

The antifungal effects on static and shaking culture of YTF 8 with the carbon and nitrogen sources

In the comparison of static and shaker fermentation conditions, the static fermentation showed the highest antifungal activity (42.10 mm) on glucose (1.5 g) and (31.51 mm) on peptone (1 g) against *Candida albicans* (Table 12,13 and Figure 12,13).

Table 12: The Antifungal Effects on Static and Shaking Culture of YTF 8 with the Carbon Sources

Sr. No	Fermentation condition	Antifungal activity (clear zone diameter , mm)
1	Static	42.10
2	Shaker	23.88

Static Shaker

Figure 12: The antifungal effects on static and shaking culture of YTF 8 with the carbon sources

Table 13: The Antifungal Effects on Static and Shaking Culture of YTF 8 with the Nitrogen Sources

Sr. No	Fermentation condition	Antifungal activity (clear zone diameter, mm)
1	Static	31.51
2	Shaker	17.24

Static Shaker

Figure 13: The antifungal effects on static and shaking culture of YTF 8 with the nitrogen sources

Suitable Synthetic Fermentation Medium

This medium consisted of: peptone - 1 g, glucose - 1.5 g, soluble starch - 0.5 g, $CaCO_3$ - 0.1 g, KH_2PO_4 - 0.001 g, $MgSO_4$ - 0.001 g, pH - 6, temperature - 30˚C and the highest antifungal activity (41.50 mm) was obtained by using this medium (Table 14 and Figure 14).

Table 14: Antifungal Activity on the Suitable Synthetic Fermentation Medium Against *Candida albicans*

Sr. No	Fermentation Period, (days)	Antifungal activity (clear zone diameter, mm)
1	3	25.87
2	4	28.90
3	5	31.51
4	6	41.50
5	7	30.83
6	8	30.45
7	9	28.63

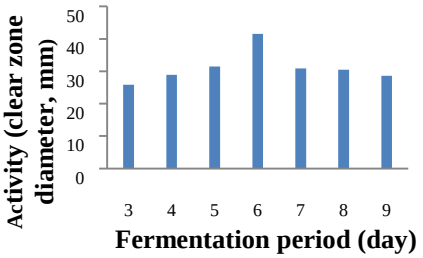

Figure 14: Antifungal activity on the suitable synthetic fermentation medium against *Candida albicans*

Screening of Bioactive Compounds in the Fermented Broth (YTF 8) by Paper Chromatography

In paper chromatography, the antifungal efficacy of the selected fungus was tested by using four different solvent- NH_4Cl, n-butanol saturated with water, n-butanol-acetic acid-water (3:1:1) and ethyl acetate saturated with water. Ethyl acetate extract produced the maximum inhibitory zones and R_f value had 0.8 (Figure 15).

A = NH_4Cl
B = n-butanol saturated with water
C = n-butanol-acetic acid-water (3:1:1)
D = ethyl acetate saturated with water

Figure 15 : Bioautography of paper chromatography with four solvent system

Effect of extract ratio on fermentation conditions against *Candida albicans*

In the extract ratio of fermentation conditions, ethyl acetate extract (1:1) gave the maximum inhibitory zones (30.45 mm) against *Candida albicans* (Table 15 and Figure 16).

Table 15: Effect of Extract Ratio on Fermentation Conditions Against *Candida albicans*

Sr. No	Extract Ratio (EtOAc: fermented broth)	Antifungal activity (clear zone diameter, mm)
1	1:1	30.45
2	2:1	28.90
3	3:1	20.37

1:1 2:1 3:1

Figure 16: Antifungal activity of the extracts in the different ratio with fermentation medium against *Candida albicans*

Effect of pH of extract on fermentation conditions against *Candida albicans*

In the extract pH of fermentation conditions, extract pH 6 showed the best antifungal activity (29.55mm) against *Candida albicans* (Table 16 and Figure 17).

Table 16: Effect of pH of Extract on Fermentation Condition Against *Candida albicans*

Sr. No	pH	Antifungal activity (clear zone diameter, mm)
1	4	18.57
2	5	19.46
3	6	29.55
4	7	29.07
5	8	23.88
6	9	22.81
7	10	20.37

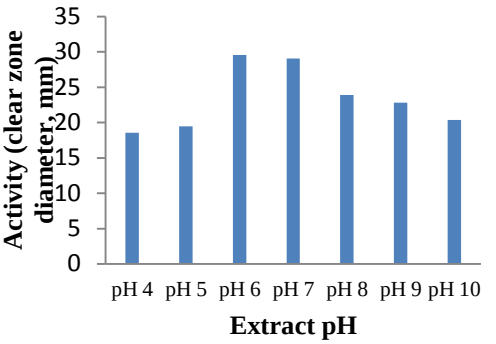

Figure 17: Effect of pH of extract on fermentation condition against *Candida albicans*

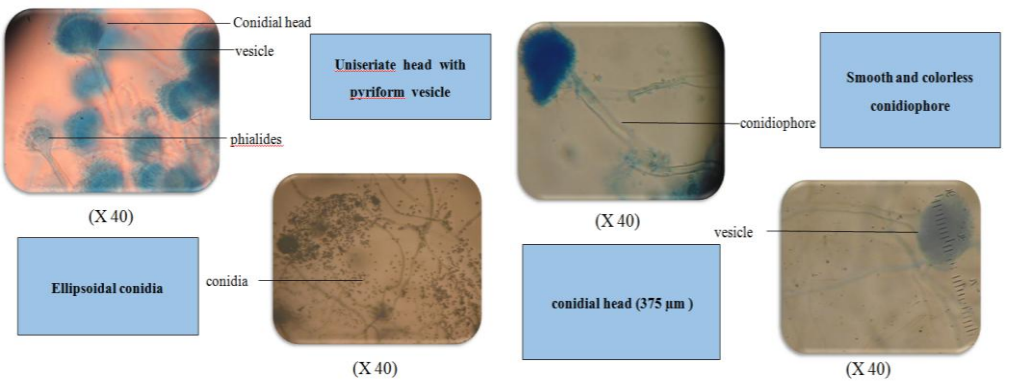

Figure 18 : Microscopic character of isolated fungus YTF 8

Identification key for *Aspergillus clavatus*

1. Isolates were predominantly Uniseriate----------2
2. Condia head were clavate----------3
3. Colonies had regular edges on MEA, with moderate length and width of the stipe and ellipsoidal conidia that was blue grey---------- 4
4. The colonies had white edges of mycelium, with blue-grey appearance, formed radial furrows and sporulation rings on MEA, on CZ also formed sporulation rings and had two types of conidia heads ----------*A. clavatus*

Conclusion

Soil samples were collected from five different places of Kan Gyi Daung Township, Ayeyarwady Region. 18 fungi were isolated from five different soil. The isolated fungi were designated as YTF 1 to YTF 18. Especially YTF8 gave the best activity on *Candida albicans.* YTF 8 reached the highest activities 32.56 mm in 5 days age of inoculums on *Candida albicans*. In the proper size of inoculum, 20 % was the maximum activities 35.26 mm in YTF 8 followed by the high activities of 5 % and 15 % respectively. The nature of the nitrogen source has notable effect on the production of antifungal metabolite in YTF 8. Especially YTF 8 showed the moderate growth on almost all nitrogen sources. Maximum production of antifungal metabolite of YTF 8 was observed on peptone (32.90 mm) as nitrogen source.

Thus, the results of the optimum fermentation tests indicated that antimicrobial metabolite obtained from YTF 8 may be produced optimally in the presence of potato and peptone, 5 days age of inoculum and 20 % inoculum size. The antifungal substance production in YTF 8 was influenced by addition of FM 6 (glucose 1.5 g) reaching the highest antifungal activity (39.62 mm) followed by using (glucose 1 g) in FM 5 (31.52 mm) and (glucose 2 g) in FM 7 (31.51 mm). Maximum production of antifungal metabolite YTF 8 was observed on FM 14 (peptone 1 g) exhibited the highest antifungal activity (30.83 mm). And then, the effect of pH was studied by varying of pH 4, 5, 6, 7, 8, 9 and pH 10 tested on antifungal activity using agar well diffusion method. While, the optimum antifungal activity observed on pH 6 (35.38mm). In this study, the effect of temperature, temperature 20 ˚C, 25, 30, 35, 40 and 45˚C tested on antifungal activity by using agar well diffusion method. Among them, the maximal antifungal activity was achieved at temperature 30˚C after 5 days of the fermentation against *Candida albicans.* In the comparison of static and shaker fermentation conditions, the static fermentation showed the highest antifungal

activity (42.10 mm) on glucose (1.5 g) and (31.51 mm) on peptone (1 g) against on *Candida albicans*.

And then, the suitable synthetic fermentation medium consisted of: peptone-1 g, glucose -1.5 g, soluble starch-0.5 g, $CaCO_3$ - 0.1 g, KH_2PO_4 - 0.001 g, $MgSO_4$ - 0.001 g, pH-6, temperature - 30˚C and the highest antifungal activity (41.50 mm) was obtained by using this medium. In this investigation, four solvents 20% NH_4Cl, n-butanol saturated with water, butanol-acetic acid-water (3:1:1) and ethyl acetate saturated with water were used for proper chromatography. According to the R_f values, it was considered that n-butanol, n-butanol-acetic acid-water and ethyl acetate were suitable for the extraction of antimicrobial metabolite. However, ethyl acetate is the most suitable to extract the compound from fermented broth. In the identification of selected fungus YTF 8, conidia heads were conspicuous and attached to long hyphal threads. Mycelia were white with velvet appearance and produced colorless. They were uniseriate with radiate conidia heads. The small vesicle was pyriform measuring 325 µm. The conidiophores were expanding toward the tip and measured 875 µm long and 37 µm wide, smooth and colorless. The conidia were ellipsoidal, smooth and green measuring 17 µm in size. Conidial heads measuring 375 µm, with closely packed phialides. According to the results, the selected fungus YTF 8 was identified as *Aspergullus clavatus.* These results were in agreement with the descriptions of Ando (2016), Larone (1995), Barnette (1969) and Nyongesa (2015).

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