

## ISOLATION, IDENTIFICATION AND ANTIMICROBIAL ACTIVITY OF SOIL FUNGI

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### Abstract

In this study, three different soil samples were collected from three Townships (North Oakkala, Mayangone and Insein) in Yangon Region. The soil type from North Oakkala and Insein was clay loam whereas that from Mayangone was sandy clay. Twenty-four soil fungi were isolated from three different soil samples by the serial dilution method. The forms of fungal colonies were circular, irregular, filamentous and rhizoid. The elevation of colonies was raised, convex, flat, umbonate while the margins of colonies of isolated fungi were entire, undulate, filiform, curled and lobate. Antimicrobial activity of isolated fungal strains was evaluated by the agar well diffusion method with eight test organisms: *Agrobacterium tumefaciens*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus pumilus*, *Malassezia furfur* and *Candida albicans*. Among 24 isolates, seventeen strains showed different levels of antimicrobial activities. Among seventeen active strains, isolated fungal strains WT-14, 17 and 24 were moderately antimicrobial activity while seven strains WT-1, 2, 4, 7, 9, 13 and 15 showed highly antimicrobial activity against all test organisms. According to the morphological and microscopical characters of bioactive seven strains, six of them (strains WT-1, 2, 4, 9, 13 and 15) were identified as *Aspergillus* sp. while strain WT-7 was *Rhizoctonia* sp.

**Keywords:** Antimicrobial activity, Identification, Morphological characters, Soil fungi

### Introduction

Soil provides ecosystem services critical for life: soil acts as a water filter and a growing medium; provides habitat for billion of organisms, and supplies most of the antibiotics used to fight diseases (Soil Science Society of America). Biodiversity loss also occurs in soil in which there is great diversity of living organisms that depends on the vegetation, as well as on the quantity of organic material produced (Fierer and Jackson, 2006).

The microorganisms plays significant role in soil ecosystem. Estimate point to 1.5 million fungal species worldwide of which only about 99,000 has been described using classical taxonomic approaches (Hawksworth, 1991; 2001). Fungi are very vital for the soil ecosystem since they play a key role in different essential processes including organic matter decomposition (Christensen, 1998). The novel discoveries of microorganisms can produce as a potential source of new antibiotics in the recent decades (Ullah *et al.*, 2017). Fungi produce many antibiotics, having antibiotics and antifungal activity, which are widely used as drugs over the world especially the penicillin, cephalosporin and fusidic acid (Dobashi *et al.*, 1998).

The objectives of this paper were to isolate the soil fungi on four different media Blakeslee's Malt Extract Agar, Czapek Dox Agar, Potato Dextrose Agar and Low Carbon Agar, to study the colony morphology and the cultural characteristics of isolated soil strains, to observe the antimicrobial activity on eight test organisms and to identify possible genus of active soil fungi.

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## Materials and Methods

### Collection of soil samples

The soil samples (15 cm depth) were collected from three different Townships in Yangon Region, during July 2019, and put sterilized polythene bags after removing the surface soil for the isolation of fungi. Soil colour, soil type and their location were shown in Table 1.

**Table 1 Collected soil samples from three different townships of Yangon Region**

| Sample | Place         | Soil Color | Soil Type  | pH   | Location                    |
|--------|---------------|------------|------------|------|-----------------------------|
| 1      | North Oakkala | Red        | Clay Loam  | 7.02 | N16°53.665"<br>E 96°08.257" |
| 2      | Mayangone     | Brown      | Sandy Clay | 6.27 | N16°52.307"<br>E 96°08.412" |
| 3      | Insein        | Red        | Clay Loam  | 6.40 | N16°53.080"<br>E 96°06.608" |

### Isolation of soil fungi by serial dilution method (Dubey, 2002)

Each soil sample (1.0 g) was introduced into a conical flask containing 99 mL of distilled water. The flask was then shaken 30 minutes in order to make the soil particles free from each other. This solution was serially diluted from  $10^{-3}$  to  $10^{-7}$  dilution in separate test tubes and 0.5 mL each of the above dilution was separately transferred into sterile petri dishes under aseptic condition. The sterilized medium in conical flask was cooled down to about 45°C and separately poured into each of the petri-dish containing the respective solid dilutions.

The inoculated plates were shaken clock-wise and anticlock-wise direction for about 5 minutes so as to make uniform distribution of the fungi inoculums. When the agar was solidified, the inoculated plates were inverted and incubated at 27°-30° C for 3-6 days at the Laboratory of Biological Development Center of Patheingyi University.

### Four different culture media for the isolation of fungi (Ando, 2004)

#### 1. Blankeslee's Malt Extract Agar (BMEA medium)

Malt extract 20.0 g, Peptone 1.0 g, Glucose 20.0 g, Agar 18.0 g, DW 1 L, pH 6.5

#### 2. Czapek-Dox Agar (CzA medium)

Sucrose 30.0 g, NaNO<sub>3</sub> 2.0 g, K<sub>2</sub>HPO<sub>4</sub> 1.0 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g, KCl 0.5 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01g, Agar 18.0 g, Distilled Water 1L, pH 7.0

#### 3. Potato Dextrose Agar (PDA Medium)

Sucrose 30.0 g, NaNO<sub>3</sub> 2.0 g, K<sub>2</sub>HPO<sub>4</sub> 1.0 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g, KCl 0.5 g, FeSO<sub>4</sub>·7H<sub>2</sub>O 0.01g, Agar 18.0 g, Distilled Water 1 L, pH 7

#### 4. Low Carbon Agar (LCA) medium for first culture

Glucose 2.0 g, Sucrose 2.0 g, K<sub>2</sub>HPO<sub>4</sub> 1.0 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.5 g, KNO<sub>3</sub> 1.0 g, KCl 0.5 g, Agar 18.0 g, DW 1 L, pH6.

After autoclaving (121°C for 30mins), Chloranphenicol was added into the medium.

### Morphological characters of isolated fungi (Ando, 2004)

After incubating fungi on the plate cultures for 5 days, colony morphological characters such as the surface color and the reverse color, the elevations of colonies and the margins of colonies of all isolated fungi were photographed and measured.

### Antimicrobial activities by agar well method (Collin, 1965)

Isolated stains were tested by agar well method for their preliminary antimicrobial activities. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Wells impregnated with 3-6 days old culture fermented broth (20 $\mu$ L) were incubated at room temperature for 24-28 hours. After 24-28 hours of incubations, the clear zones (inhibitory zones) were measured. Therefore, the diameter of clear zones had been observed as potent activity as shown by respective strain. Clear zones surrounding the wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively (Table 2).

**Table 2 Eight kinds of test organisms used for antimicrobial activity**

| Test Organisms                   | Code Numbers | Disease                   |
|----------------------------------|--------------|---------------------------|
| <i>Agrobacterium tumefaciens</i> | NITE 09678   | Crown gall disease        |
| <i>Escherichia coli</i>          | AHU 5436     | Diarrhoea, abdominal pain |
| <i>Staphylococcus aureus</i>     | AHU 8456     | Boil and food poisoning   |
| <i>Pseudomonas fluorescens</i>   | IFO 94307    | Septicemia                |
| <i>Bacillus subtilis</i>         | IFO 90571    | Fever                     |
| <i>Bacillus pumilus</i>          | IFO 90571    | Fever                     |
| <i>Malassezia furfur</i>         | AUS 01020    | Dandruff, Dermatitis      |
| <i>Candida albicans</i>          | NITE 09542   | Candidiasis, Skin disease |

NITE = National Institute of Technology Evaluation

## Results

### Isolation of fungi from soil samples

In present research work, the soil samples were collected from three different places: North Oakkala, Mayangone and Insein Townships in Yangon Region. The soil type of North Oakkala was clay loam and pH value of 7.02. Mayangone Townships was sandy clay and pH value of 6.27, and Insein Townships was clay loam and pH value of 6.40 respectively.

All isolated fungal strains were temporarily named as WT-1 to WT-24. These total of 24 fungal isolates were obtained; five strains from soil sample 1, seven strains from soil sample 2 and twelve strains from soil sample 3. Soil fungal strains were isolated on four different media: 11 strains were isolated on BMEA Medium, 8 strains on PDA Medium, 4 strains on LCA Medium and 1 strain on CzA.

### Morphological characters of isolated fungi

The surface color of strains WT-1, 11, 17, 18, 19, 22 and 23 was white. The surface colors of other strains were green, greenish white, dark green, pale green, black, white black and yellow. The reverse color of WT-1 was white and the other strains were white black, green, greenish white, pale green, black and cream. The forms of colonies were circular, irregular, filamentous and rhizoid. The elevations of fungal colonies were raised, convex, flat, and umbonate. The margins of

fungus colonies were entire, undulate, filiform, curled and lobate. These results were shown in Table 3 and Figures 1 to 12.

**Table 3 Morphological character of isolated fungi**

| Strain No. | Surface color  | Reverse color   | Form        | Elevation   | Margin   | Size   |
|------------|----------------|-----------------|-------------|-------------|----------|--------|
| WT-1       | White          | Yellowish White | Circular    | Raise       | Undulate | Large  |
| WT-2       | White          | White           | Circular    | Flat        | Entire   | Small  |
| WT-3       | White          | Cream           | Circular    | Raised      | Entire   | Small  |
| WT-4       | Black White    | Black White     | Irregular   | Convex      | Entire   | Medium |
| WT-5       | Black White    | Greenish White  | Filamentous | Flat        | Undulate | Medium |
| WT-6       | Greenish White | Greenish White  | Circular    | Flat        | Entire   | Medium |
| WT-7       | Black White    | Greenish White  | Circular    | Raised      | Entire   | Medium |
| WT-8       | White          | Yellowish White | Circular    | Flat        | Undulate | Small  |
| WT-9       | White          | Yellowish White | Circular    | Raised      | Undulate | Large  |
| WT-10      | Black White    | Yellowish White | Circular    | Flat        | Entire   | Large  |
| WT-11      | Black White    | White           | Circular    | Raised      | Entire   | Small  |
| WT-12      | Pale Green     | Pale Green      | Irregular   | Convex      | Entire   | Large  |
| WT-13      | White          | Cream           | Filamentous | Filamentous | Undulate | Medium |
| WT-14      | Dark Green     | Dark Green      | Circular    | Flat        | Entire   | Medium |
| WT-15      | White          | White           | Circular    | Raised      | Entire   | Large  |
| WT-16      | Greenish White | White           | Circular    | Flat        | Undulate | Small  |
| WT-17      | Green          | White           | Circular    | Raise       | Undulate | Large  |
| WT-18      | Black          | White           | Circular    | Flat        | Entire   | Small  |
| WT-19      | Black          | White Black     | Circular    | Raised      | Entire   | Small  |
| WT-20      | Pale Green     | White           | Irregular   | Convex      | Entire   | Medium |
| WT-21      | Greenish White | White           | Filamentous | Flat        | Undulate | Medium |
| WT-22      | Dark Green     | Cream           | Circular    | Flat        | Entire   | Medium |
| WT-23      | Black White    | Cream           | Circular    | Raised      | Entire   | Medium |
| WT-24      | Cream          | Pale Yellow     | Circular    | Flat        | Undulate | Small  |



Front view (WT-1) Reverse view

Front view (WT-2) Reverse view

**Figure 1** Colony morphology of isolated soil fungi WT-1 and WT-2



Front view (WT-3) Reverse view

Front view (WT-4) Reverse view

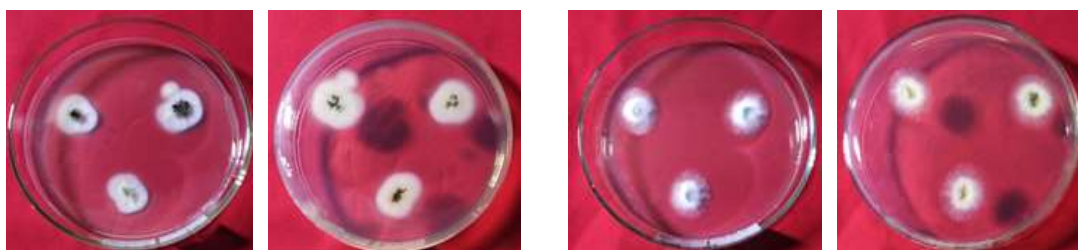
**Figure 2** Colony morphology of isolated soil fungi WT-3 and WT-4



Front view (WT-5) Reverse view

Front view (WT-6) Reverse view

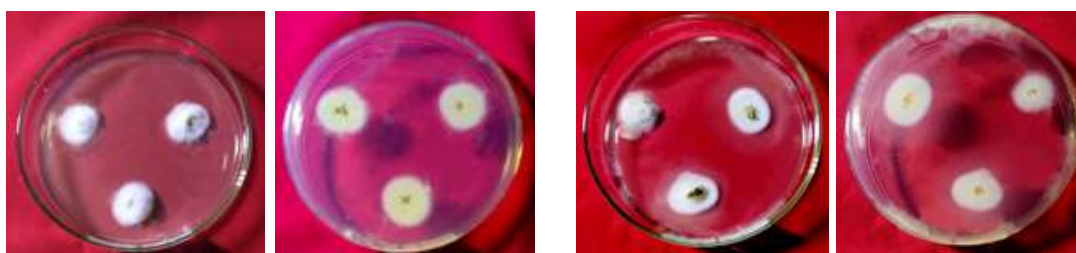
**Figure 3** Colony morphology of isolated soil fungi WT-5 and WT-6



Front view (WT-7) Reverse view

Front view (WT-8) Reverse view

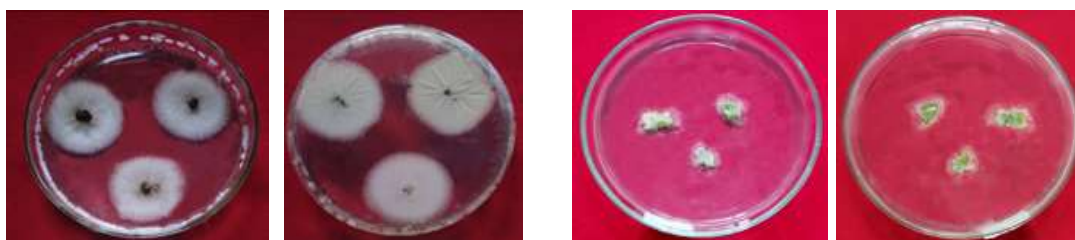
**Figure 4** Colony morphology of isolated soil fungi WT-7 and WT-8



Front view (WT-9) Reverse view

Front view (WT-10) Reverse view

**Figure 5** Colony morphology of isolated soil fungi WT-9 and WT-10



Front view (WT-11) Reverse view

Front view (WT-12) Reverse view

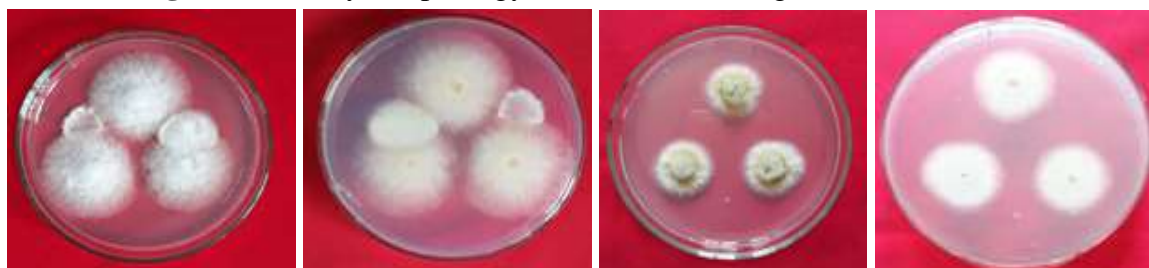
**Figure 6** Colony morphology of isolated soil fungi WT-11 and WT-12





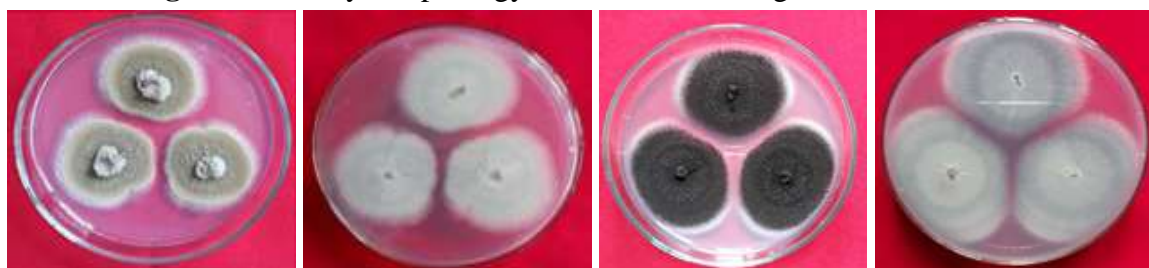
Front view (WT-13) Reverse view

Front view (WT-14) Reverse view

**Figure 7** Colony morphology of isolated soil fungi WT-13 and WT-14

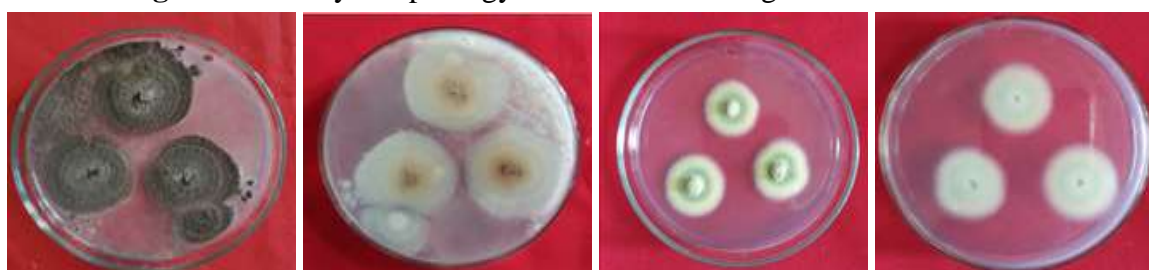
Front view (WT-15) Reverse view

Front view (WT-16) Reverse view

**Figure 8** Colony morphology of isolated soil fungi WT-15 and WT-16

Front view (WT-17) Reverse view

Front view (WT-18) Reverse view

**Figure 9** Colony morphology of isolated soil fungi WT-17 and WT-18

Front view (WT-19) Reverse view

Front view (WT-20) Reverse view

**Figure 10** Colony morphology of isolated soil fungi WT-19 and WT-20

Front view (WT-21) Reverse view

Front view (WT-22) Reverse view

**Figure 11** Colony morphology of isolated soil fungi WT-21 and WT-22



Front view (WT-23) Reverse view Front view (WT-24) Reverse view

**Figure 12** Colony morphology of isolated soil fungi WT-23 and WT-24

### Antimicrobial activity of all isolated fungal strains

All fungal strains were tested their antimicrobial activity by using eight test organisms. Among 24 isolates, seventeen strains showed different levels of antimicrobial activity. Among seventeen strains, seven isolated fungi (WT-1, 2, 4, 7, 9, 13 and 15) showed highly antimicrobial activity against all test organisms. Fungal strain WT-4 showed the highest antimicrobial activity (24.8 mm and 26.4 mm) on *Bacillus pumilus* and *Bacillus subtilis* respectively. Strains WT-9 and WT-13 also exhibited highly antimicrobial activity (25.1 mm) and WT-7 showed (23.6 mm) on *Bacillus subtilis*. Strain WT-15 also exhibited high antimicrobial activity (22.3 mm) against *Agrobacterium tumefaciens* respectively. Moreover, strains WT-1 and WT-2 showed highly antifungal activity (19.2 mm) and (21.9 mm) against *Candida albicans*.

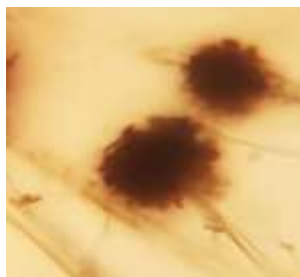
### Identification of Active Fungal Strains

#### Microscopic characters of strains WT-1, 2, 4, 9, 13 and 15

Conidiophores were upright, simple, terminating in a globose or clavate swelling, bearing phialides at the apex. Conidia 1 celled, globose, often variously colored in mass. Therefore, these strains WT-1, 2, 4, 9, 13 and 15 were identified as *Aspergillus* sp. (Figures 13–18).



**Figure 13** Microscopic characters of WT-1



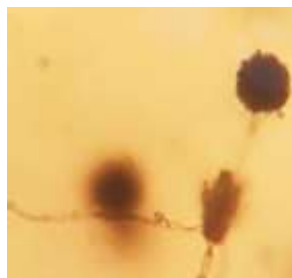
**Figure 14** Microscopic characters of WT-2



**Figure 15** Microscopic characters of WT-4



**Figure 16** Microscopic characters of WT-9



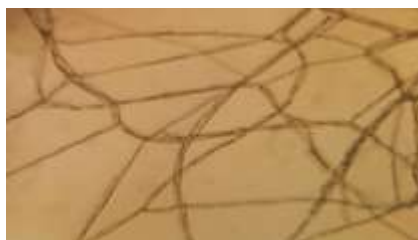
**Figure 17** Microscopic characters of WT-13



**Figure 18** Microscopic characters of WT-15

### Microscopical characters of strain WT-7

Mycelium hyaline was white. Cells of mycelium usually long, septa of branches and set off from the main hyphae. Asexual fruit bodies, conidia absent, chlamydospore-like cells in chains. This fungus WT-7 was identified as *Rhizoctonia* sp. (Figure 19).



**Figure 19** Microscopic characters strain WT-7

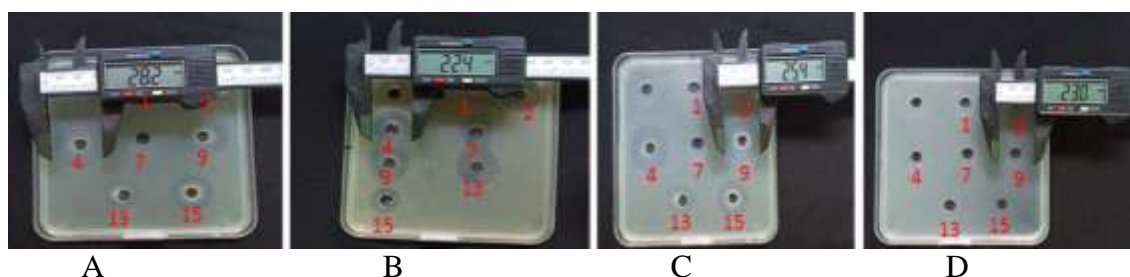
### Antimicrobial activity of seven fungal strains

Strain WT-1 showed moderate activity at 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> day fermentation. Strain WT-2 showed high activity at 3<sup>rd</sup>, moderate activity at 4<sup>th</sup> and 5<sup>th</sup> day fermentation. Strain WT-4 showed high activity at 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> day fermentation. Strain WT-7 showed moderate activity at 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day fermentation. Strain WT-9 showed moderate activity at 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day, and high activity at 6<sup>th</sup> and 7<sup>th</sup> day fermentation. Strain WT-13 showed moderate activity at 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> day and high activity at 3<sup>rd</sup> day and 4<sup>th</sup> day. Strain WT-15 showed moderate activity at 3<sup>rd</sup> day to 7<sup>th</sup> day on *Agrobacterium tumefaciens* (Table 4 & Figure 20). All seven active strains showed antimicrobial activities on other test organisms: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus pumilus*, *Malassezia furfur* and *Candida albicans* (Tables 5-11 and Figures 21-27).

**Table 4** Antimicrobial Activity of isolated fungal strains against *Agrobac. tumefaciens*

| No | Isolated Fungi | Fermentation period (Day) and Inhibitory Zone(mm) |                      |                      |                      |                      |
|----|----------------|---|----------------------|----------------------|----------------------|----------------------|
|    |                | 3 <sup>rd</sup> -day                              | 4 <sup>th</sup> -day | 5 <sup>th</sup> -day | 6 <sup>th</sup> -day | 7 <sup>th</sup> -day |
| 1  | WT-1           | 10.0  | 13.4                 | 16.6                 | 16.0                 | 15.1                 |
| 2  | WT-2           | 20.7  | 17.7                 | 17.6                 | 13.0                 | 12.0                 |
| 3  | WT-4           | 17.2  | 19.6                 | 21.6                 | 28.2                 | 22.4                 |
| 4  | WT-7           | 16.9  | 16.4                 | 16.1                 | 14.0                 | 12.5                 |
| 5  | WT-9           | 18.0  | 18.2                 | 18.2                 | 21.4                 | 23.0                 |
| 6  | WT-13          | 19.4  | 19.2                 | 17.2                 | 16.5                 | 16.2                 |
| 7  | WT-15          | 14.5  | 17.0                 | 16.9                 | 16.9                 | 15.5                 |

12-14 mm = weak activity, 15-19 mm = moderate activity, > 20 mm = high activity



A: WT-4 against *A. tumefaciens* at 6<sup>th</sup> day of fermentation period  
 B: WT-4 against *A. tumefaciens* at 7<sup>th</sup> day of fermentation period  
 C: WT-9 against *A. tumefaciens* at 6<sup>th</sup> day of fermentation period  
 D: WT-9 against *A. tumefaciens* at 7<sup>th</sup> day of fermentation period

**Figure 20** Antimicrobial activities of selected fungi against *Agrobacterium tumefaciens*



**Table 5** Antimicrobial activity of isolated fungal strains against *Escherichia coli*

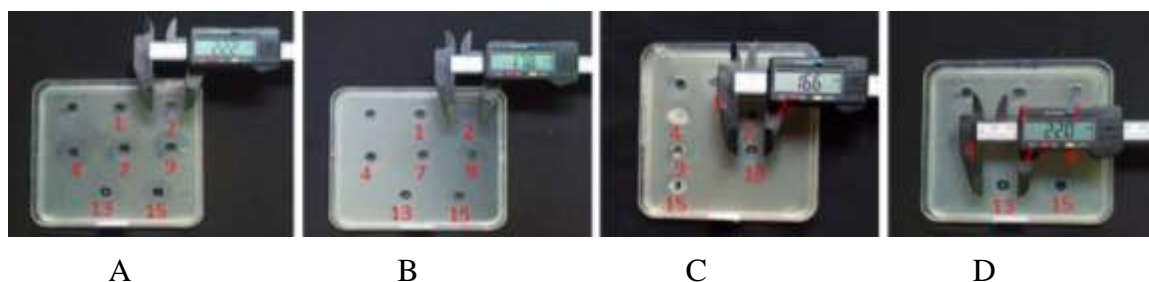
| No. | Isolated Fungi | Fermentation period (Day) and Inhibitory zone (mm) |                      |                      |                      |                      |
|-----|----------------|--|----------------------|----------------------|----------------------|----------------------|
|     |                | 3 <sup>rd</sup> -day                               | 4 <sup>th</sup> -day | 5 <sup>th</sup> -day | 6 <sup>th</sup> -day | 7 <sup>th</sup> -day |
| 1   | WT-1           | -  | 21.6                 | 18.0                 | 16.1                 | 14.4                 |
| 2   | WT-2           | -  | 16.0                 | 16.0                 | 17.2                 | 14.6                 |
| 3   | WT-4           | 12.9   | 16.6                 | 18.3                 | 15.7                 | 13.7                 |
| 4   | WT-7           | 9.6  | 15.1                 | 15.0                 | 14.1                 | 13.7                 |
| 5   | WT-9           | 14.0   | 16.7                 | 14.2                 | 13.1                 | 13.0                 |
| 6   | WT-13          | 11.1   | 14.2                 | 14.0                 | 13.2                 | 13.6                 |
| 7   | WT-15          | 12.7   | 15.7                 | 16.0                 | 16.2                 | 15.8                 |



A: WT-9 against *Escherichia coli* at 4<sup>th</sup> day of fermentation period  
 B: WT-9 against *Escherichia coli* at 5<sup>th</sup> day of fermentation period  
 C: WT-15 against *Escherichia coli* at 6<sup>th</sup> day of fermentation period  
 D: WT-15 against *Escherichia coli* at 7<sup>th</sup> day of fermentation period

**Figure 21** Antimicrobial activities of selected fungi against *Escherichia coli***Table 6** Antimicrobial Activity of isolated fungal strains against *Staphylococcus aureus*

| No. | Isolated Fungi | Fermentation period (Day) and Inhibitory Zone(mm) |                      |                      |                      |                      |
|-----|----------------|---|----------------------|----------------------|----------------------|----------------------|
|     |                | 3 <sup>rd</sup> -day                              | 4 <sup>th</sup> -day | 5 <sup>th</sup> -day | 6 <sup>th</sup> -day | 7 <sup>th</sup> -day |
| 1   | WT-1           | -   | 16.7                 | 12.4                 | -                    | -                    |
| 2   | WT-2           | -   | 14.1                 | 22.2                 | 17.8                 | -                    |
| 3   | WT-4           | 10.8  | 15.2                 | 22.5                 | 14.3                 | 13.3                 |
| 4   | WT-7           | -   | 13.7                 | 18.6                 | -                    | -                    |
| 5   | WT-9           | 13.3  | 15.2                 | 20.0                 | 19.0                 | 13.7                 |
| 6   | WT-13          | -   | 16.6                 | 22.0                 | -                    | -                    |
| 7   | WT-15          | -   | 12.2                 | 19.1                 | 16.4                 | 13.7                 |

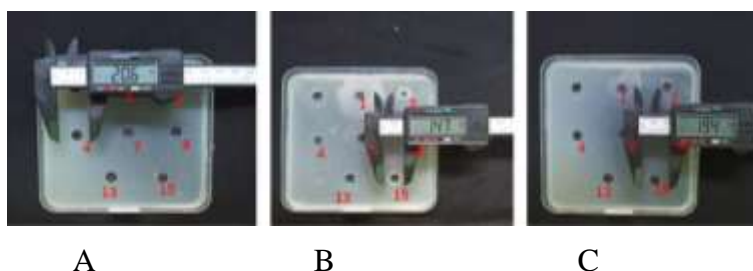


A: WT-2 against *Staphylococcus aureus* at 5<sup>th</sup> day of fermentation period  
 B: WT-2 against *Staphylococcus aureus* at 6<sup>th</sup> day of fermentation period  
 C: WT-13 against *Staphylococcus aureus* at 4<sup>th</sup> day of fermentation period  
 D: WT-13 against *Staphylococcus aureus* at 5<sup>th</sup> day of fermentation period

**Figure 22** Antimicrobial activities of selected fungi against *Staphylococcus aureus*

**Table 7** Antimicrobial Activity of isolated fungal strains against *Pseudomonas fluorescens*

| No. | Isolated Fungi | Fermentation period (Day) and Inhibitory Zone(mm) |                      |                      |                      |                      |
|-----|----------------|---|----------------------|----------------------|----------------------|----------------------|
|     |                | 3 <sup>rd</sup> -day                              | 4 <sup>th</sup> -day | 5 <sup>th</sup> -day | 6 <sup>th</sup> -day | 7 <sup>th</sup> -day |
| 1   | WT-1           | 14.9  | 22.1                 | 18.0                 | 16.2                 | 14.0                 |
| 2   | WT-2           | 21.3  | 22.1                 | 16.1                 | 14.5                 | 12.3                 |
| 3   | WT-4           | 27.1  | 26.4                 | 18.2                 | 20.6                 | 14.7                 |
| 4   | WT-7           | 23.6  | 16.2                 | 16.0                 | 15.1                 | 14.6                 |
| 5   | WT-9           | 25.1  | 23.0                 | 18.3                 | 18.8                 | 16.4                 |
| 6   | WT-13          | 22.5  | 25.1                 | 20.0                 | 17.2                 | 15.7                 |
| 7   | WT-15          | 21.4  | 18.7                 | 18.1                 | 19.4                 | 15.2                 |



A: WT-4 against *Pseudomonas fluorescens* at 6<sup>th</sup> day of fermentation period  
 B: WT-4 against *Pseudomonas fluorescens* at 7<sup>th</sup> day of fermentation period  
 C: WT-15 against *Pseudomonas fluorescens* at 6<sup>th</sup> day of fermentation period

**Figure 23** Antimicrobial activities of selected fungi against *Pseu. fluorescens*

**Table 8** Antimicrobial Activity of isolated fungal strains against *Bacillus subtilis*

| No. | Isolated Fungi | Fermentation period (Day) and Inhibitory Zone(mm) |                      |                      |                      |                      |
|-----|----------------|---|----------------------|----------------------|----------------------|----------------------|
|     |                | 3 <sup>rd</sup> -day                              | 4 <sup>th</sup> -day | 5 <sup>th</sup> -day | 6 <sup>th</sup> -day | 7 <sup>th</sup> -day |
| 1   | WT-1           | -   | -                    | -                    | -                    | -                    |
| 2   | WT-2           | 13.8  | 14.5                 | 15.1                 | 16.9                 | 12.3                 |
| 3   | WT-4           | -   | -                    | -                    | 20.6                 | 16.3                 |
| 4   | WT-7           | -   | -                    | -                    | -                    | -                    |
| 5   | WT-9           | 15.7  | 17.1                 | 18.5                 | 25.1                 | 16.4                 |
| 6   | WT-13          | -   | -                    | -                    | -                    | -                    |
| 7   | WT-15          | -   | -                    | 17.0                 | 19.4                 | 14.7                 |



A: WT-2 against *Bacillus subtilis* at 3<sup>rd</sup> day of fermentation period  
 B: WT-2 against *Bacillus subtilis* at 7<sup>th</sup> day of fermentation period  
 C: WT-9 against *Bacillus subtilis* at 6<sup>th</sup> day of fermentation period  
 D: WT-9 against *Bacillus subtilis* at 7<sup>th</sup> day of fermentation period

**Figure 24** Antimicrobial activities of selected fungi against *Bacillus subtilis*

**Table 9** Antimicrobial activity of isolated fungal strains against *Bacillus pumilus*

| No | Isolated Fungi | Fermentation period (Day) and Inhibitory Zone(mm) |                      |                      |                      |                      |
|----|----------------|---|----------------------|----------------------|----------------------|----------------------|
|    |                | 3 <sup>rd</sup> -day                              | 4 <sup>th</sup> -day | 5 <sup>th</sup> -day | 6 <sup>th</sup> -day | 7 <sup>th</sup> -day |
| 1  | WT-1           | 15.3  | 13.7                 | 17.2                 | 12.3                 | 12.7                 |
| 2  | WT-2           | 18.4  | 18.5                 | 22.0                 | 19.2                 | 15.0                 |
| 3  | WT-4           | 22.0  | 18.5                 | 28.4                 | 23.2                 | 24.7                 |
| 4  | WT-7           | 17.5  | 16.8                 | 15.0                 | 14.1                 | 11.8                 |
| 5  | WT-9           | 19.9  | 16.7                 | 22.8                 | 23.4                 | 30.3                 |
| 6  | WT-13          | 19.9  | 18.3                 | 22.8                 | 15.5                 | 18.1                 |
| 7  | WT-15          | 18.0  | 16.7                 | 21.4                 | 22.2                 | 20.8                 |



A: WT-2 against *Malassezia furfur* at 4<sup>th</sup> day of fermentation period  
 B: WT-2 against *Malassezia furfur* at 5<sup>th</sup> day of fermentation period  
 C: WT-9 against *Malassezia furfur* at 3<sup>rd</sup> day of fermentation period  
 D: WT-9 against *Malassezia furfur* at 4<sup>th</sup> day of fermentation period

**Figure 25** Antimicrobial activities of selected fungi against *Bacillus pumilus*

**Table 10** Antimicrobial Activity of isolated fungal strains against *Malassezia furfur*

| No | Isolated Fungi | Fermentation period (Day) and Inhibitory Zone(mm) |                      |                      |                      |                      |
|----|----------------|---|----------------------|----------------------|----------------------|----------------------|
|    |                | 3 <sup>th</sup> -day                              | 4 <sup>th</sup> -day | 5 <sup>th</sup> -day | 6 <sup>th</sup> -day | 7 <sup>th</sup> -day |
| 1  | WT-1           | 11.9  | 12.6                 | 16.7                 | 15.0                 | 13.9                 |
| 2  | WT-2           | 18.7  | 14.4                 | 22.7                 | 19.8                 | 19.0                 |
| 3  | WT-4           | 18.7  | 19.9                 | 19.4                 | 19.0                 | 18.0                 |
| 4  | WT-7           | 16.8  | 17.4                 | 14.7                 | 12.5                 | 10.5                 |
| 5  | WT-9           | 16.8  | 21.4                 | 19.7                 | 19.0                 | 18.1                 |
| 6  | WT-13          | 19.2  | 16.0                 | 20.9                 | 18.2                 | 17.0                 |
| 7  | WT-15          | 19.2  | 16.0                 | 20.9                 | 18.6                 | 18.1                 |



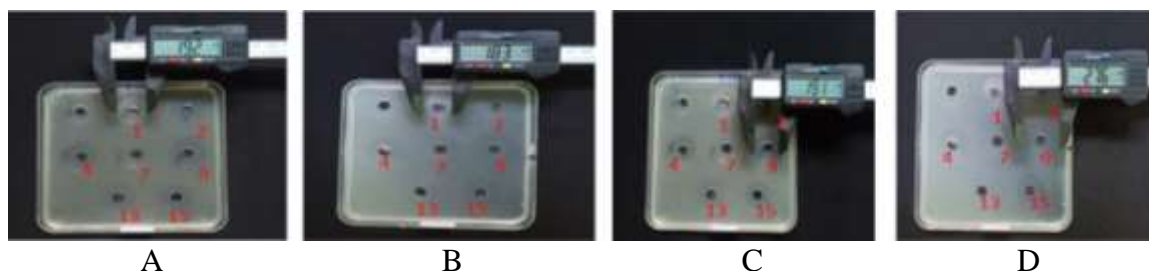
A  
A: WT-1 against *Malassezia furfur* at 4<sup>th</sup> day of fermentation period  
B: WT-1 against *Malassezia furfur* at 5<sup>th</sup> day of fermentation period  
C: WT-7 against *Malassezia furfur* at 3<sup>rd</sup> day of fermentation period  
D: WT-7 against *Malassezia furfur* at 4<sup>th</sup> day of fermentation period

**Figure 26** Antimicrobial activities of selected fungi against *Malassezia furfur*

**Table 11** Antimicrobial Activity of isolated fungal strain against *Candida albicans*

| No | Isolated Fungi | Fermentation period (Day) and Inhibitory Zone(mm) |                      |                      |                      |                      |
|----|----------------|---|----------------------|----------------------|----------------------|----------------------|
|    |                | 3 <sup>rd</sup> -day                              | 4 <sup>th</sup> -day | 5 <sup>th</sup> -day | 6 <sup>th</sup> -day | 7 <sup>th</sup> -day |
| 1  | WT-1           | 9.6   | 18.6                 | 19.2                 | 18.3                 | 13.3                 |
| 2  | WT-2           | 19.4  | 21.9                 | 21.8                 | 13.6                 | 13.0                 |
| 3  | WT-4           | 23.3  | 23.3                 | 20.6                 | 18.7                 | 14.2                 |
| 4  | WT-7           | 15.8  | 15.8                 | 15.9                 | 15.0                 | 14.0                 |
| 5  | WT-9           | 17.3  | 18.0                 | 19.1                 | 27.6                 | 16.6                 |
| 6  | WT-13          | 16.3  | 23.4                 | 21.5                 | 18.0                 | 16.3                 |
| 7  | WT-15          | 20.7  | 19.6                 | 19.6                 | 22.2                 | 14.7                 |

12-14 mm = weak activity, 15-19 mm = moderate activity, > 20 mm = high activity



A  
A: WT-1 against *Candida albicans* at 5<sup>th</sup> day of fermentation period  
B: WT-1 against *Candida albicans* at 6<sup>th</sup> day of fermentation period  
C: WT-9 against *Candida albicans* at 5<sup>th</sup> day of fermentation period  
D: WT-9 against *Candida albicans* at 6<sup>th</sup> day of fermentation period

**Figure 27** Antimicrobial activities of selected fungi against *Candida albicans*

## Discussion and Conclusion

In the present study, 24 fungal strains were isolated from three different Townships in Yangon Region. Fungal strains WT-1 to WT-5 were isolated from soil in South Oakkala, WT-6 to WT-12 from soil sample in Mayangone and WT-13 to WT-24 from soil sample in Insein. A total of 24 isolated fungi, 11 strains were isolated from BMEA Medium, 8 strains from PDA Medium, 4 strains from LCA Medium and 1 strain were isolated from CzA. Moreover, physicochemical properties of soil from different locations of Yangon Area were analyzed. The soil samples were pH slightly base (6.27–7.02). The temperature of the soil was high (30–45°C), with great variation



in moisture content (2.28 – 2.64), organic carbon (0.58- 4.07) and organic nitrogen (0.18 – 0.27), and texture.

Among 24 isolates, 7 strains showed highly antimicrobial activity. These six active strains were identified as *Aspergillus* sp. and the other one was *Rhizoctonia* sp. Morphological and microscope characters of isolated strains were investigated according to Barnett (1998) and other literatures. Seth *et al.*, (2016) isolated and identified soil fungi such as *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., and *Rhizoctonia* spp. from cultivated area in India. Moira (1961) isolated soil fungi and identified these strains to study their growth in pure culture. Gaddeya, *et al.*, (2010) isolated soil mycoflora in different crop fields for producing bioactive compounds. Farid and Nareen (2013) isolated and identified *Aspergillus* sp., and other fungi from soil and some medicinal plants. Ana *et al.*, (2010) isolated several soil borne microbes such as mycorrhizal fungi. Azaz (2003) also isolated *Aspergillus* sp., *Penicillium* sp., and *Rhizoctonia* sp., from the fields.

All isolated strains showed different antimicrobial activity on eight test organisms. According to the results of antimicrobial activity, bioactive strains WT-1, 2, 4, 7, 9, 13 and 15 showed highly antimicrobial activity on eight test organisms. Prabavathy and Nachiyar (2012) stated that the fungus *Aspergillus* sp., had antimicrobial activity. Many others researchers isolated soil fungi for producing bioactive agents. Devi & Joshi (2012) stated that most of soil fungi (e.g., *Aspergillus* sp. and *Rhizoctonia* sp.) had antimicrobial activity on some test organisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Candida krusei*). Srividya, *et al.* (2010) stated that the soil fungus showed antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Candida krusei*. Yee Yee Thu (2006), Kyawt Kyawt Aung (2014), Hnin Wit Mhone (2018) and Soe Soe Yu Hnin (2018) have also isolated many fungi including *Aspergillus* and *Rhizoctonia*, etc. from soil fungi and endophytic fungi.

It was concluded that the 24 soil fungi were isolated on four different media. Seven strains of them possessed highly antimicrobial activity on eight test organisms caused serious diseases and infections on man and plants. Therefore, these seven active strains will be chosen to continue fermentation studies for extraction and isolation of the bioactive compounds.

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