Study on the Isolation of Fungi from the Soil Samples and Their Antimicrobial Activities

Swe Zin Win[*], Khin Thida Swe[**], Hla Myo Thein[***]

Abstract

In the study, fifteen different fungi were isolated from different soil samples. It was collected from five different places in BYE DA YAW village in Sagaing Township, in June, 2020. The macroscopic and microscopic characters of soil fungi have been undertaken. Isolated soil fungi were tested by using six test organisms. Fifteen fungal strains showed antimicrobial activities against the six test organisms during the preliminary screening. Among them soil fungi SF-1, SF-3, SF-5 and SF-15 were shown distinct activities against six test organisms during the confirmatory screening. The selected soil fungus SF-15 was tested by using paper disc diffusion method (NITE, 2004). In the screening program, soil fungus SF-15 exhibited the highest antibacterial activities on *Escherichia coli*. Therefore, this fungus SF-15 was selected for further investigation and will produce primary and secondary metabolities. In order to discover novel antibiotics produced by soil microbes.

Keywords: Soil fungi, antimicrobial activities

Introduction

Life on earth would have been impossible without microorganisms in nature. Man has taken advantages activities of microorganisms to his benefit (Ando, 2004). Soil is the most suitable environment for microbial growth (Cavalcanti *et al.*, 2006). Soil sample is the primary source of microorganisms. Soil bacteria and fungi have played a significant and a key role in many essential processes such as antibiotic activities. Soil fungi are the main source for the discovery of novel antibiotics. The antimicrobial activities processed by selected fungal strain isolated from preliminary screening. Secondary screening isolates were shown the ability to inhibit the growth of test bacteria (Aneja, 2005).

Antibiotic is one of the most important things commercially exploited secondary metabolites produced by soil micro fungi and employed in a wide range. Most of the antibiotics are used today from the soil microbe (Alexander *et al.,* 1977). The secondary metabolites are obtained by optimal fermentation processes (Omura, 1985 and crueger, 1989).

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The present study focuses on the isolation of microbial strains having antimicrobial activities from different soil samples. These samples were collected from Bye Da Yaw village in Sagaing Township area for the production of antibacterial metabolites against *Escheriachia coli*.

Materials and Methods

Collection and Preparation of Soil Samples

In systematic screening program for isolation of fungi, five different soil samples were collected at Bye Da Yaw village in Sagaing Township. Soil samples (approximately 5 g) were collected, using some clean dry and sterile polythene bag along with sterile spatula. The samples were brought to the laboratory. The soil texture, soil pH and soil moisture % were measured at Department of Agriculture (LAND USE). The interpretation of results in Mandalay Township is described in Table 1. The two-gram soil samples were dissolved in 4 mL of water to make soil suspension.

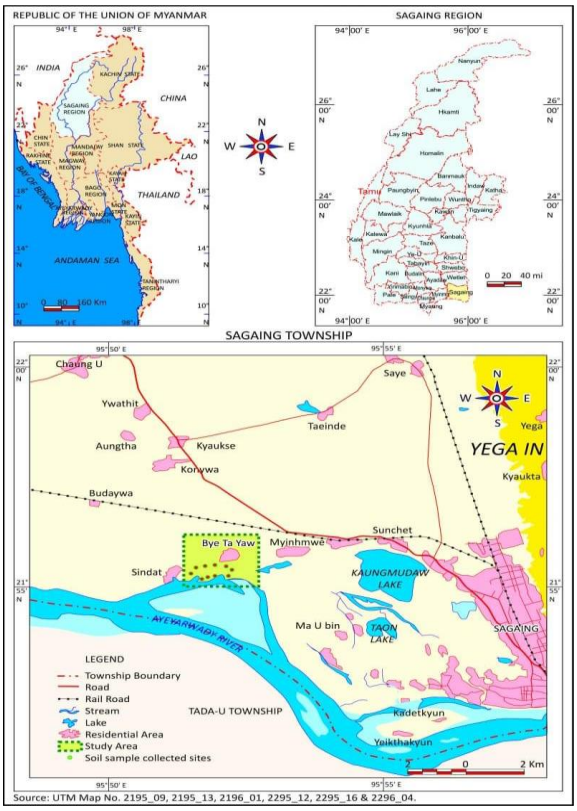


Figure 1. Map of soil collection (Bye Da Yaw village)

Table 1. Location of Five Different Soil Samples in Collected Area

Soil sample	Collected places	Soil type	pH	Location	Collected date
1	Bye Da yaw village	Loam	6.5	N 21º56.817' E 95º52.346'	15. 6 .2020
2	Bye Da yaw village	Loam	6. 9	N 21º55.973' E 95º52.346'	25.6. 2020
3	Bye Da yaw village	Slity Loam	6.7	N 21º 55.717' E 95º 52.412'	27.6.2020
4	Bye Da yaw village	Slity Loam	6.5	N 21º 55.719' E 95º 52.393'	28.6.2020
5	Bye Da yaw village	Loam	7.9	N 21º 55.634' E 95º 52.524'	29.6.2020

Isolation of Soil Fungi from five Different Soil Samples

Soil fungi were isolated from five different places. After three days, the isolation of soil fungi was done by chemical treatment dilution Methods (Hayakawa and Kobayashi, 2005).

Chemical Treatment Dilution Method (Hayakawa and Kobayashi, 2005)

The collected soil was air–dried at room temperature. The soil sample was grounded and sieved. Two gram of the sieved soil was put into test tube and then 4 mL of sterilized water was also put into the tube containing soil samples. 14 mL of 70% of methanol solution was then added into the tube containing soil suspension and was shaken for 1 minute and diluted with sterile water (Figure. 2). The dilution series were cultured on low carbon source medium such as glucose yeast extract agar medium (GYA).

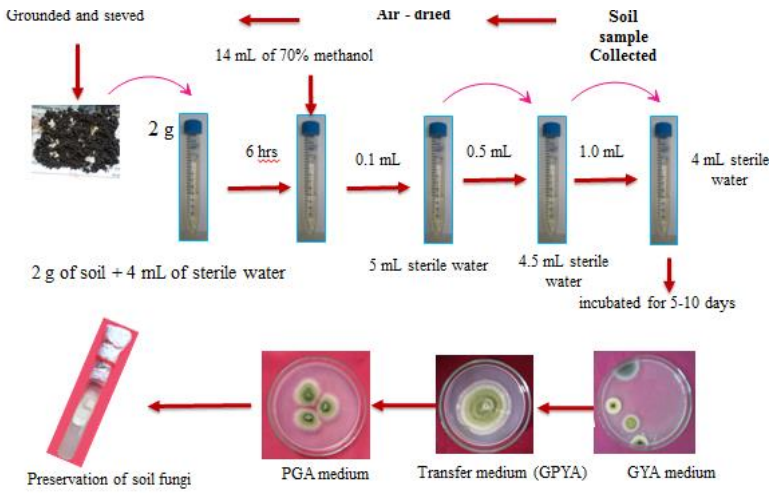


Figure 2. Chemical treatment dilution method used in the isolation of soil fungi

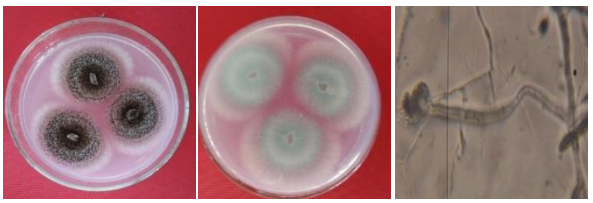
Table 3. Isolated soil fungi obtained by chemical treatment dilution method

Soil sample	Isolated Fungi	Totals of soil fungi
Soil sample 1	SF-1, SF-2, SF-3, SF-4 and SF-5	5
Soil sample 2	SF-6, SF-7 and SF-8	3
Soil sample 3	SF-9, SF-10 and SF-11	3
Soil sample 4	SF-12 and SF-13	2
Soil sample 5	SF-14 and SF-15	2

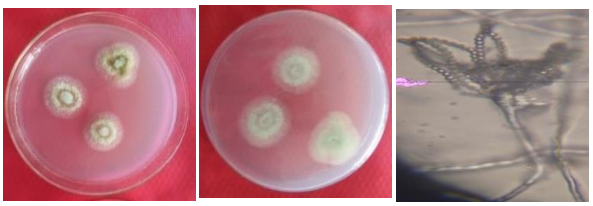
Morphology and Microphotograph of Isolated Soil Fungi

Front View of Colony	Reverse View of Colony	Microphotograph (x40)	Front View of Colony	Reverse View of Colony	Microphotograph (x40)

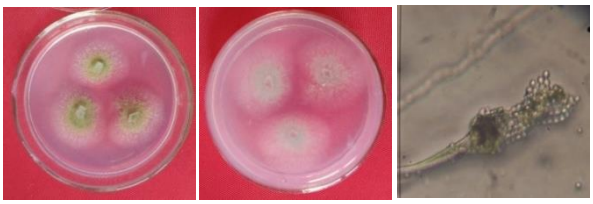
SF-1



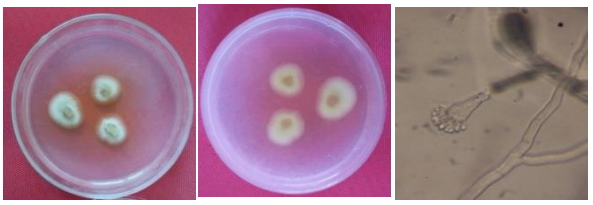
SF-2



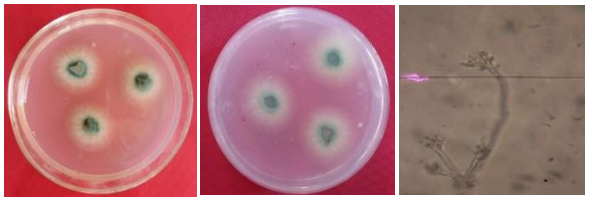
SF-3



SF-4



SF-5



SF-6

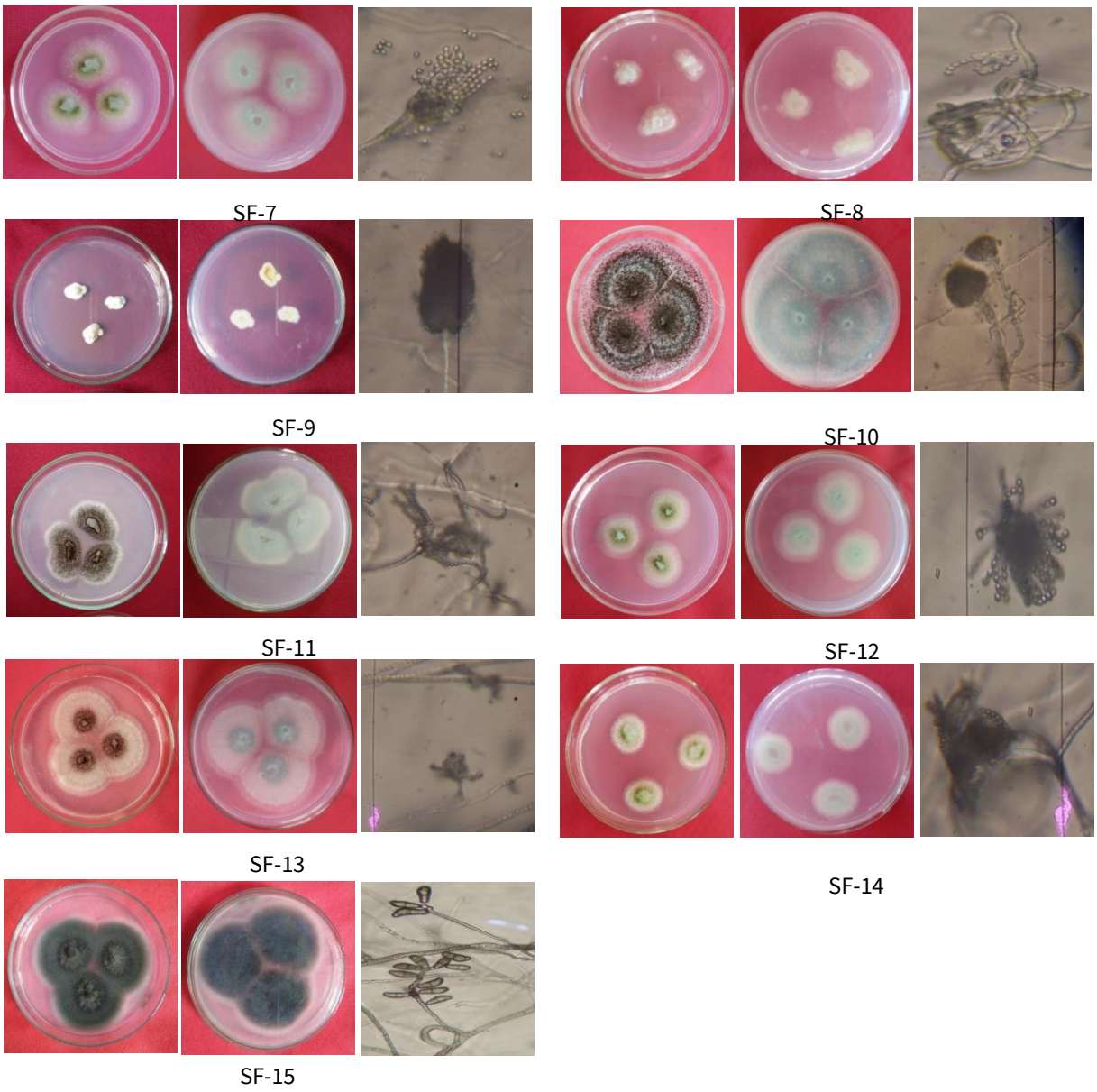


Figure 4. Morphology and Microphotograph of isolated soil fungi (5 days old cultured on PGA medium)

Preliminary Study for Antimicrobial Activities by Paper Disc Diffusion assay (NITE,2004)

In the present research, fifteen fungi were isolated from five different soil samples collected from Bye Da Yaw village in Sagaing Township. In the preliminary screening, the antimicrobial activities were carried out by using these fungi SF-6, SF-14 and SF-15 which showed the antimicrobial activities against *Agrobacterium tumefaciens.* SF-1, SF-9, SF-13, SF-14 and SF- 15 showed the antimicrobial activities against *Aspergillus paraciticus.* SF-2, SF-3, SF-5, SF-9, SF-10, SF-13, SF-14 and SF-15 Showed the antimicrobial activities against *Escherichia coli.* SF-2, SF-3, SF-5, SF-9 and SF-10 showed the antimicrobial activities against *Saccharomyces cerevisiae.* SF-1, SF-13, and SF-14 showed the antimicrobial activities against *Pseudomonas fluorescens.*SF-3, SF-7 and SF-10 showed the antimicrobial activities against *Staphylococcus aureus.* Among them

SF-1, SF-3, 5 and SF-15 showed highly antimicrobial activities respectively. SF-4, SF-8, SF-11 and SF-12 did not show antimicrobial activities against six test organisms (Table. 4).

Table 4. Preliminary studies of antimicrobial activities (Fermentation Period 7days)

Soil Fungi	*A. tumefaciens*	*A. paraciticus*	*E coli*	*S. cerevisiae*	*P. fluorescens*	*S. aureus*
SF-1	-	27.74	-	-	26.57	-
SF-2	-	-	16.94	18.15	-	-
SF-3	-	-	16.94	16.15	-	19.33
SF-4	-	-	-	-	-	-
SF-5	-	-	19.43	22.32	-	-
SF-6	16.50	-	-	-	-	-
SF-7	-	-	-	-	-	17.32
SF-8	-	-	-	-	-	-
SF-9	-	17.88	15.92	17.48	-	-
SF-10	-	-	12.9	18.34	-	12.33
SF-11	-	-	-	-	-	-
SF-12	-	-	-	-	-	-
SF-13		16.53	19.38	-	19.11	-
SF-14	18.90	17.34	16.00	-	18.74	-
SF-15	19.08	20.75	29.95	-	-	-

Antimicrobial activities of selected fungus by Paper Disc Diffusion assay (NITE, 2004)

In this study, fifteen soil fungi were tested by using six kinds of test organisms. Among them SF-1, SF-3, SF-5 and SF-15 were selected and confirmed their antimicrobial activities. In the present investigation, fungus SF-15 inhibited on *Agrobacterium tumefaciens* (23.08mm), *Aspergillus paraciticus* (17.73mm), *Pseudomonas fluorescens* (17.31mm), *Saccharomyces cerevisiae* (21.89mm), *Staphylococcus aureus* (23.65 mm) and *Escherichia coli* (25.94mm) respectively. Therefore, SF-15 was selected for further investigation due to its more highly activities against *Escherichia coli* and prominent fungi.

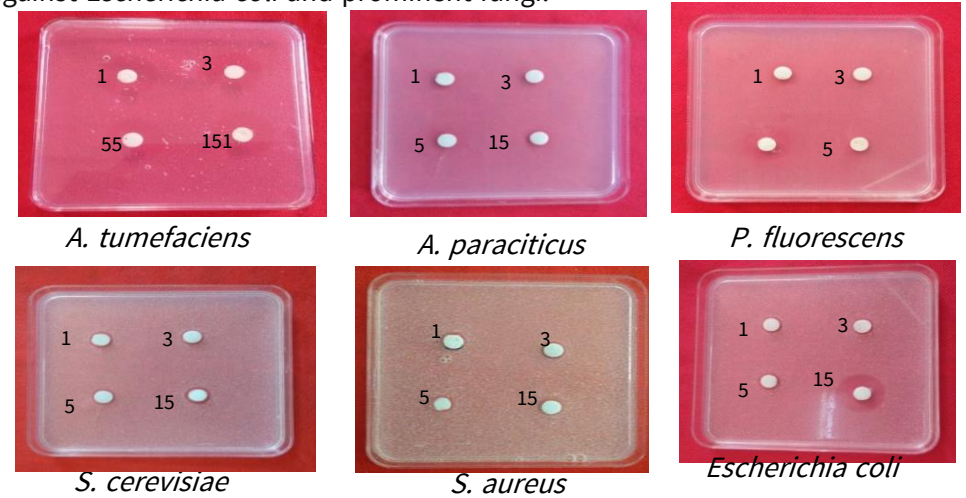


Figure 6. Antimicrobial activities of isolated fungi SF-1, SF-3, SF-5 and SF-15 against six test organisms

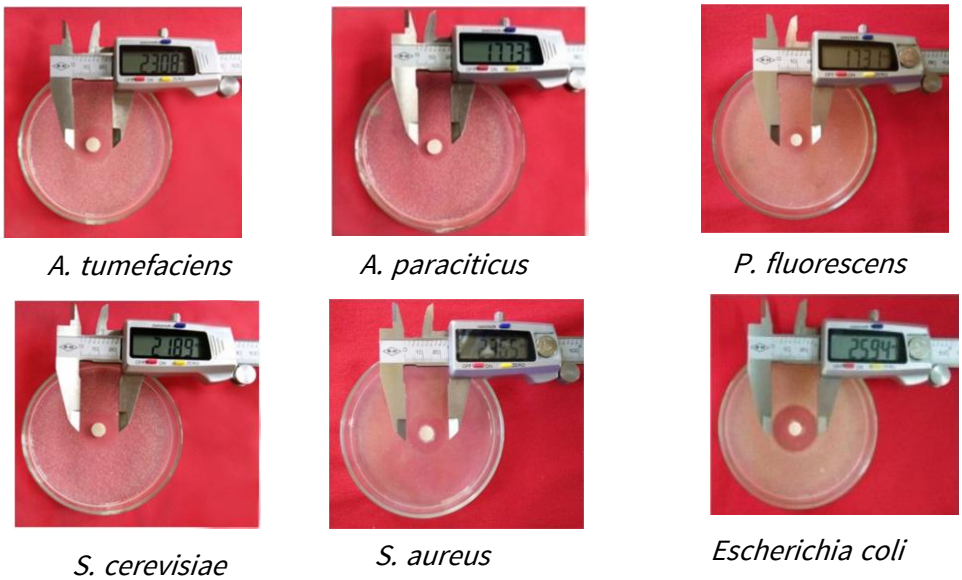


Figure 7. Antimicrobial activities of selected fungus SF-15 against six test organisms

Discussion and Conclusion

Natural products from microorganisms have been the most useful source that has been found in many applications in the fields of soil area. Fenical, 1993 reported that, Soil fungus is potentially rich source of unique bioactive materials. Fungi which are occured in the soil environment have various different ecological function. These abilities have exploited to isolate microbes from soil samples and specific medium that have been developed to select for certain groups of microorganisms.

In the present investigation, soil sample of five different fields were studied for observation and detection of fungal diversity. The results obtained clearly indicate that, fifteen fungi were isolated from five different soil samples. Five fungi were isolated from soil sample 1, three fungi were isolated from soil sample 2, three fungi were isolated from soil sample 3, two fungi were isolated from soil sample 4 and two fungi were isolated from soil sample 5, respectively. Fifteen fungi were cultured GYA medium and incubated at 27℃ for one to five days. Pure colonies were inoculated into slant culture containing in PGA medium. In this study, total of fifteen fungi strains were isolated. Then, surface colors of all fungi strains were white to bluish green (SF-1, 6), white to yellow green (SF-3, 4, 7, 12, 14), White to black (SF-2, 10, 11, 13), cream (SF-5, 9), white (SF-8), brown (SF-15) and reverse colors of all fungi strains were pale green, pale yellow, black, cream, white, pale brown respectively. Soil microorganisms have continually been screened for their useful antimicrobial activities. In this research, the samples of (SF-1 to SF-15) were tested by using six test organisms (Table 2).

Antimicrobial evaluation was carried out by paper disc diffusion method (NITE, 2004). Paper disc diffusion method is widely used to evaluate the antimicrobial activities of microorganism's extracts (Valgas *et al.,* 2011). Six kinds of test organisms were used in paper

disc diffusion assay method (Figure 3). In the present investigation, eleven kinds of fungi (SF-1, 2, 3, 5, 6, 7, 9, 10, 13, 14, 15) showed the antimicrobial activities and four kinds of fungi (SF-4, 8, 11, 12) did not show the antimicrobial activities on six test organisms (Table 4). Fungal strains SF-1, SF-3, SF-5 and SF-15 were shown in distinct clear zone against six kinds of test organisms (Figure 6). During confirmatory screening, SF-15 showed the best of inhibitory zone against all the test organisms (Figure 7). According to the results, it is concluded that this fungus SF-15 exhibited the highest activities against *Escherichia coli* (29.54 mm) (Figure 7). Therefore, this fungus SF-15 was the most promising and was selected for detail study which will produce secondary metabolites. Soil fungi produce secondary metabolite with the highest therapeutic value as antibiotics and insecticides.

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