A PRELIMINARY STUDY ON THE ANTIMICROBIAL ACTIVITIES OF ISOLATED SOIL FUNGI FROM THA-BEIK-KYIN AREA

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Abstract

In this present study, seven different soil samples were collected in the Tha-Beik-Kyin area of Mandalay region for the isolation of soil microorganisms. After that soil fungi were isolated from different soil samples at Biological Resource and Biotechnology Development Center (BRBDC) of Botany Department in Pathein University. Eleven fungi were isolated from these seven different soil samples by using physical treatment dilution method on low carbon medium. Then, the isolated fungi were photographed and the studies were conducted on their respective morphological characters. Following that, antimicrobial activities of the fungi were tested by the paper disc diffusion assay method. In the study of antimicrobial activities, 8 different test organisms were utilized. According to the results, three isolated strains (MAI 01, MAI 06, and MAI 07) showed activity against the test organisms. Among them, strain MAI-07 showed the highest antibacterial activity against *Bacillus subtilis* (25.70 mm). In the fermentation studies, 84 hr age and 15% size of inoculums were optimized for the fermentation. Carbon sources (glucose, glycerol) and nitrogen sources (yeast extract, polypeptone) showed the best activities on the fermentation.

Keywords: *Bacillus subtilis*, antimicrobial activities

Introduction

Microorganisms have been traditionally used to produce a variety of important substances for the pharmaceutical and food industries (Demain, 2000). Antibiotics are microbial products or their derivatives that can kill susceptible microorganisms or inhabit their growth. The fungi have been widely studied for their bioactive metabolites and have proven to be a rich and promising source of noble anticancer, antibacterial, antiflammatory and anti-viral agents (Rajalakshmi and Mahesh, 2014).

Microbial secondary metabolites continue to be a chemically diverse source for the discovery and development of pharmaceutical agents and also biochemical probes to study human disease process (Tamotsu *et al.,* 2002). The main basic for the therapy of microbial (bacterial and fungal) infection is provided by antibiotics (Khan, 2004). Microorganisms have been traditionally used to produce a variety of important substances for the pharmaceutical and food industries (Demain, 2000). The aim and objectives are to contribute the valuable knowledge for applied microbiology, to isolate useful soil fungi, to study their antimicrobial potentials and to optimize the fermentation of selected fungus for further study.

Materials and Methods

Samples collection and isolation of soil fungi

Seven different soil samples were collected from Thabeik-kyin Area, Mandalay Region (Figure 1). The collected soil samples were air dried at room temperature. Then the isolation of fungi was carried out by physical treatment dilution method as shown in Figure 2.

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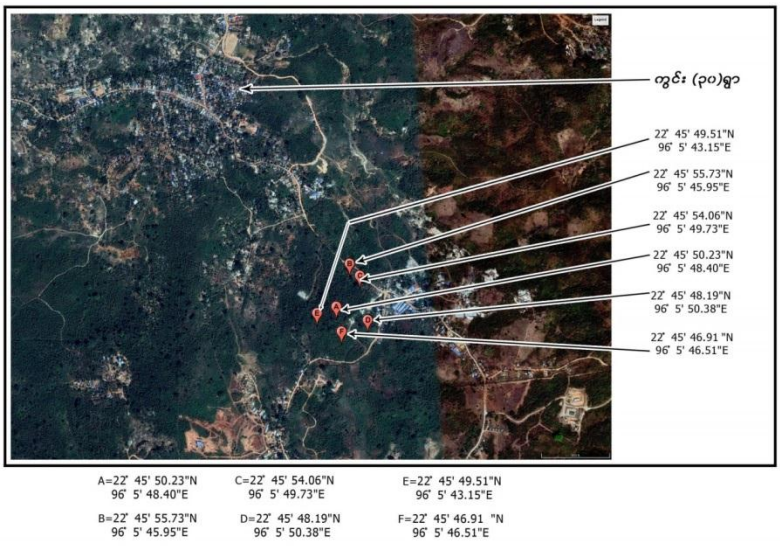

Figure 1 Soil samples collected at different places of Tha-beik-kyin

Map Source: Google Earth

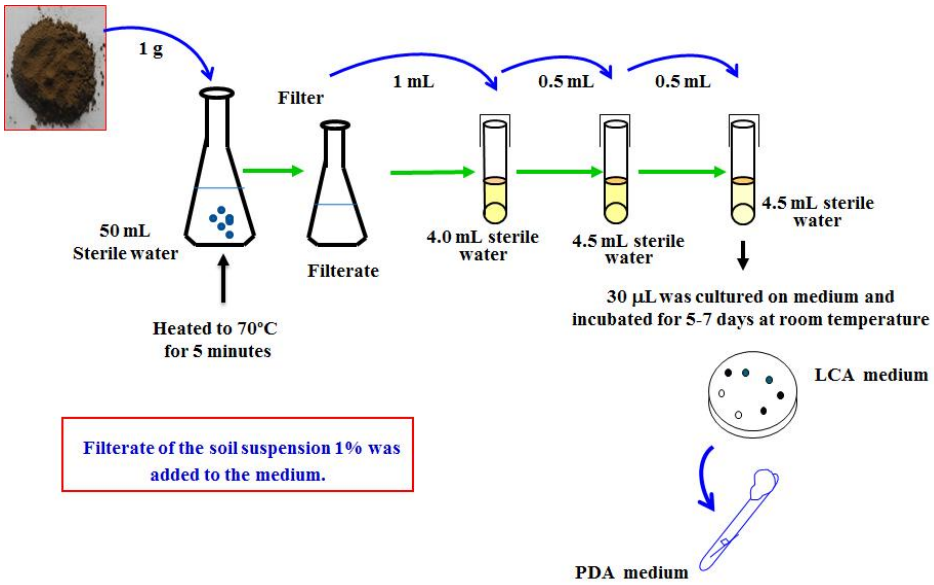

Figure 2 Physical treatment dilution method (Hayakawa and Kobayashi, 2005)

Preliminary study on antimicrobial activities of isolated fungi

Preliminary study for antimicrobial activities was carried out by paper disc diffusion assay (Tomita, 1988). The isolated fungi were grown on medium for 7 days at room temperature for sporulation. Then that fungal stain was inoculated into seed medium (Glucose 2 g, Sucrose 0.3 g, Yeast Extract 0.3 g, KNO_3 0.1 g, K_2HPO_4 0.01 g, DW 100 mL at pH 6.5) for 3 days and incubated for 3 days at room temperature. Fermentation (Glucose 2.5 g, Yeast Extract 1g, NZ Amine Type A 0.5 g, K_2HPO_4 0.001 g, $MgSO_4$ 0.001 g, $CaCO_3$ 0.1 g, DW 100 mL at pH 6.5) was undertaken at room temperature for 10 days. Twenty µL of fermented broth was put onto the paper disc (8 mm) and placed on assay plates containing test organism and incubated for 24 to 36 hrs (Figure 3). Test organism utilized in the study of antimicrobial activities were *Aspergillus*

flavus IFO 3290, *Bacillus subtilis* KY-327, *Candida albicans* NITE 09542, *E. coli* AHU 5436, *Micrococcus luteus* NITE 83297, *Pseudomonas fluorescens* NITE 52847 and *Salmonella typhi* AHU 7943. These test organisms were supported by NITE (National Institute of Technology and Evaluation), Japan, 2005.

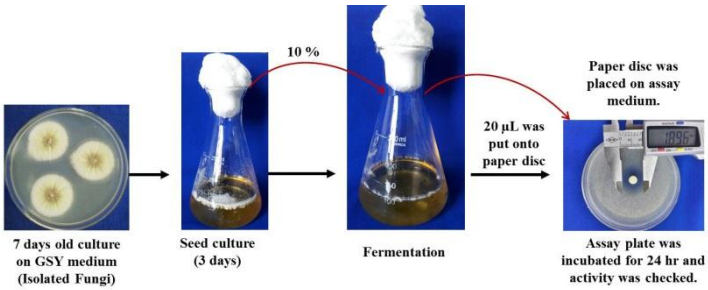

Figure 3 Preliminary study for antimicrobial activity against test organisms

Microbial growth kinetics of MAI-07

The strain MAI-07 was inoculated into the medium and incubated for 144 hrs. The culture sample was checked in 12 hrs interval for the growth at 2000 rpm for 30 minutes and packed cell volume% (PCV%) was calculated (Omura, 1985 and Crueger and Crueger, 1989).

Effects of ages and size of inoculums on the fermentation

According to the results of microbial growth kinetics of MAI-07 (66, 72, 78, 84, 90 and 96 hrs) seed culture were utilized for the fermentation (Figure 4). Based on the results of the ages of inoculums of MAI-07 (5%, 10%, 15%, 20%, 25% and 30%) of 84 hr seed cultures were employed for the fermentation. Fermentation was carried out 6 days and antibacterial activity was tested by paper disc diffusion assay method (Figure 5).

Effects of carbon and nitrogen sources on the fermentation

In this study, carbon sources such as glucose, glycerol, sucrose, soluble starch and potato powder and nitrogen sources such as yeast extract, polypeptone, KNO_3, rice ban and meat extract were employed. Fermentations were incubated at room temperature for 6 days. Each 100 mL fermentation medium was prepared for different sources test. The antibacterial activities were checked by paper disc diffusion assay method. Carbon source (2) g and nitrogen source (0.5) g was added to 100 mL of their basal fermentation medium (Yeast Extract 0.5 g, NZ Amine Type A 0.5 g, K_2HPO_4 0.001 g, $MgSO_4$ 0.001 g, $CaCO_3$ 0.01 g) and (Glucose 2 g, Soluble starch 0.5 g, K_2HPO_4 0.001 g, $MgSO_4.7H_2O$ 0.001 g, $CaCO_3$ 0.01 g) at pH 6.5 respectively.

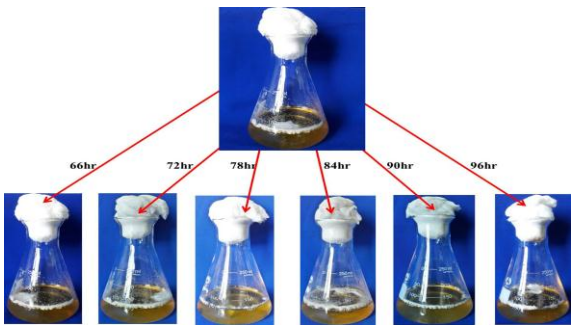

Figure 4 Effects of ages of inoculum of MAI-07 on the fermentationb

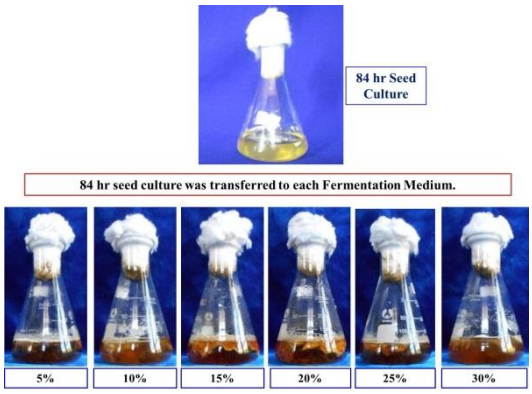

Figure 5 Effects of sizes of inoculum of MAI-07 on the fermentation

Results

Isolation of fungi from soil samples

In the isolation of soil fungi, eleven different fungi were isolated from seven different kinds of soil samples collected at Tha-Beik-kyin Area, Mandalay Region. Colony morphologies of isolated soil fungi were shown in Figures 6.

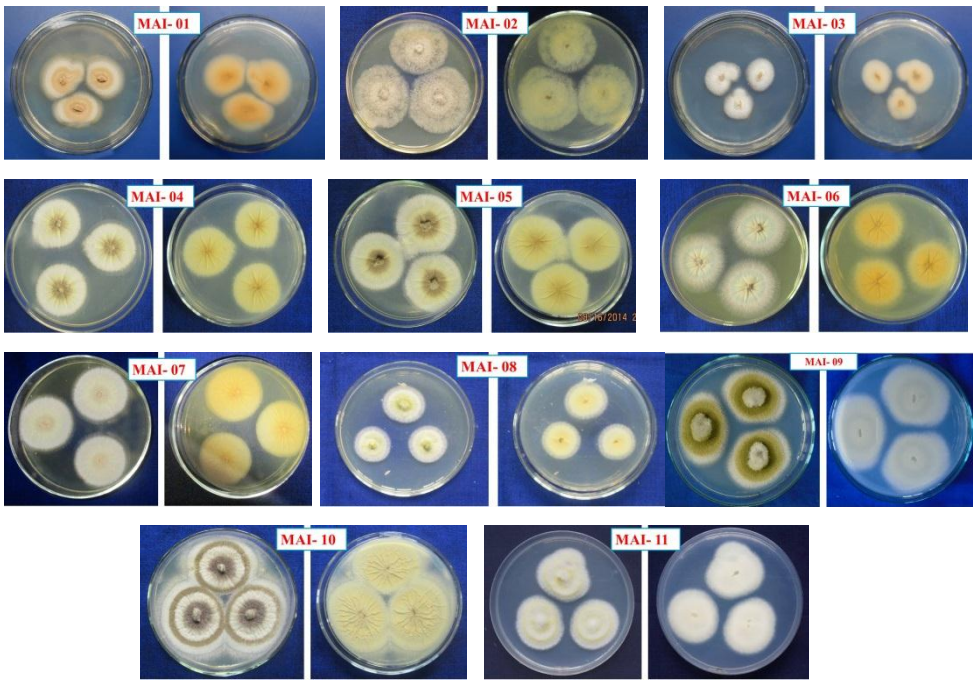

Figure 6 Colony morphologies of isolated fungi

Preliminary study on antimicrobial activities

Preliminary study on antimicrobial activities of isolated fungi was carried out by paper disc diffusion assay method. In this investigation, three strains (MAI-01, MAI-06 and MAI-07) showed activity against test organisms. MAI-01 against *E. coli* (17.54 mm), MAI-06 against *Salmonella typhi* (13.35 mm), MAI-07 against *Bacillus subtilis* (25.70 mm). Strain MAI-07 was selected for further studies because it showed the highest antibacterial activity against *Bacillus*

subtilis KY-327 (25.70 mm). Antimicrobial activities of isolated fungi were shown in Table 1 and Figure 7.

Table 1 Antibacterial activity of isolated fungi against test organisms

Soil No.	Isolated Fungi	Antibacterial activity
1	**MAI-01**	***E. coli* (17.54 mm)**
	MAI-02	No Activity
2	MAI-03	No Activity
	MAI-04	No Activity
3	MAI-05	No Activity
4	**MAI-06**	***Salmonella typhi* (13.35 mm)**
5	**MAI-07**	***Bacillus subtilis* (25.70 mm)**
	MAI-08	No Activity
6	MAI-09	No Activity
7	MAI-10	No Activity
	MAI-11	No Activity

(A) (B) (C)

Figure 7 Antibacterial activities of isolated fungi against test organisms

(A). Antibacterial activity of MAI-01 against *Escherichia coli* AHU5436

(B). Antibacterial activity of MAI-06 against *Salmonella typhi* AHU7943

(C). Antibacterial activity of MAI-07 against *Bacillus subtilis* KY-327

Microbial growth kinetics of MAI-07

In the growth kinetics of fungus MAI-07, it was found that growth phase was between 48 hr and 96 hr. According to Crueger and Crueger (1989), it was considered that ages of inoculum (66, 72, 78, 84, 90 and 96 hr) were suitable for the optimization of fermentation (Table 2 and Figure 8).

Table 2 Microbial growth kinetics of MAI-07

Culture Time (hr)	PCV of 5 mL	PCV %
24	0.2	4
36	0.25	5
48	0.35	7
60	0.55	11
72	0.85	17
84	1.1	22
96	**1.25**	**25**
108	1.25	25
120	1.2	24
132	1.1	22
144	1.0	20

*Packed Cell Volume (PCV)

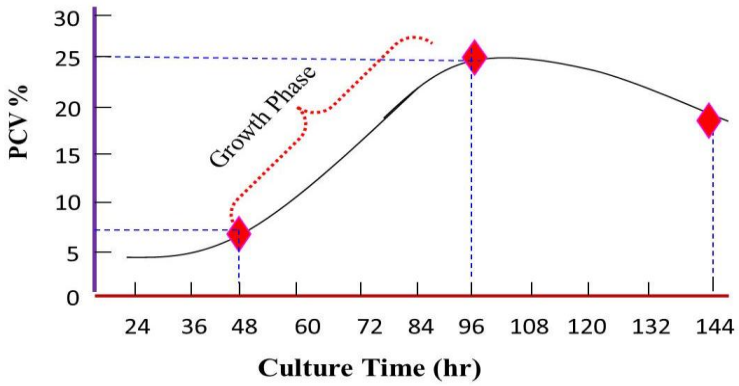

Figure 8 Microbial growth kinetics of MAI-07

Effects of ages of inoculum on the fermentation

According to the results of microbial growth kinetics of MAI-07, (66, 72, 78, 84, 90 and 96 hr) seed culture were utilized for the fermentation. It was considered that 84 hr seed culture showed the best activity on *B. subtilis* than others seed culture (Table 3 and Figure 9).

Table 3 The effects of ages of inoculum on the fermentation

Ages of culture (hr)	Activity (Inhibitory Zone, mm)
66	15.68
72	18.73
78	27.56
84	**29.85**
90	28.29
96	20.12

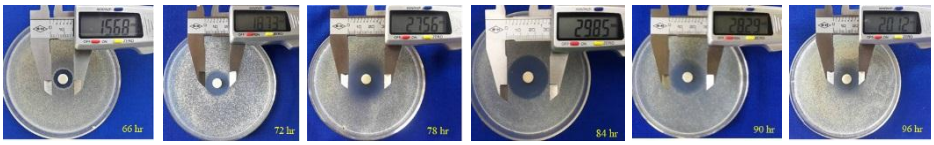

Figure 9 The effects of ages of inoculum on the fermentation

Effects of sizes of inoculum on the fermentation

Based on the results of the ages of inoculum of MAI-07, (5%, 10%, 15%, 20%, 25% and 30%) of 84 hr seed cultures were utilized for the fermentation. It was considered that 15% size of inoculum showed the best activity on *B. subtilis* (Table 4 and Figure 10).

Table 4 Effects of sizes of inoculum on the fermentation

Sizes of culture (%)	Activity (Inhibitory Zone, mm)
5	14.95
10	15.70
15	**30.31**
20	19.11
25	18.96
30	16.51

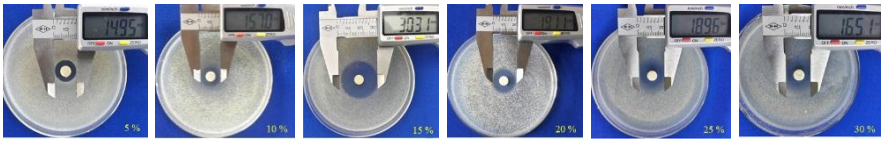

Figure 10 Effects of sizes of inoculum on the fermentation

Effects of different carbon sources utilization on the fermentation

In the studies of different carbon sources utilization on the fermentation of MAI-07, glucose and glycerol showed the best activities on *B. subtilis*(Table 5 and Figure 11).

Table 5 Effects of different carbon sources utilization on the fermentation

Carbon Source	Activity (Inhibitory Zone, mm)
Glucose	**23.5**
Sucrose	18.6
Soluble starch	20.4
Potato powder	20.6
Glycerol	**21.7**

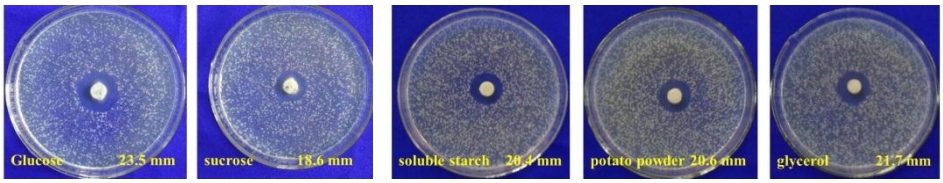

Figure 11 Effects of different carbon sources utilization on the fermentation

Effects of different nitrogen sources utilization on the fermentation

In the studies of five different nitrogen sources utilization for the fermentation of MAI-07, yeast extract and polypeptone showed the best activities on *B. subtilis* (Table 6 and Figure 12).

Table 6 Effects of different nitrogen sources utilization on the fermentation

Nitrogen sources	Activity (Inhibitory Zone, mm)
Yeast extract	**22.5**
Meat extract	19.5
Rice bran	18.2
KNO_3	19.4
Polypeptone	**21.3**

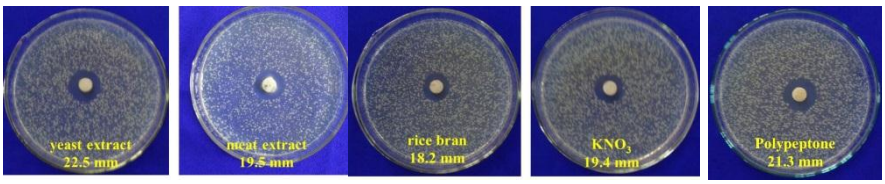

Figure 12 Effects of different nitrogen sources utilization on the fermentation

Discussion and Conclusion

In this study, soil was collected from seven different places of Tha-Beik-Kyin area of Mandalay region. After that the isolation and screening of effective soil fungi were investigated. In this investigation, a total of eleven fungi were isolated from seven different soil samples at Tha-Beik-kyin area. The isolation of soil fungi was carried out by physical treatment dilution method with low carbon agar medium. In this investigation, three strains MAI-01, MAI-06 and MAI-07 were showed the activity against test organisms. MAI-01 showed against *E. coli*, MAI-06 showed against *Salmonella typhi* and MAI-07 showed against *Bacillus subtilis.* Strain MAI-07 showed the highest antibacterial activity against *Bacillus subtilis* KY-327 (25.70 mm). Fungi are remarkable organisms that readily produce a wide range of natural products often called secondary metabolites. In many cases, the benefits of these compounds confer on the organisms is unknown. Secondary metabolite production usually commences late in the growth of the microbe, often upon entering the stationary or resting phase (Bu'lock, 1961).

Fermentation time is a very important factor, which affect the yield and quality of metabolites (Chen, 2003). Mansi and Charlie, 2003 reported that in search of favorable fermentation conditions, the types of fermentation used as well as its size, durations, and

nutrients profile will depend critically on the nature of the microbial products. Therefore fermentation optimization was undertaken to produce the antibacterial metabolite in high yield. The fermentation conditions were investigated for the production of antibacterial metabolite against *Bacillus subtilis.*

Based on the growth kinetics of MAI-07, 84 hrs age and 15% size of inoculums were optimized for the production of metabolite. In the studies of different carbon and nitrogen sources utilization on the fermentation, carbon sources (glucose, glycerol) and nitrogen sources (yeast extract, polypeptone) showed the best activities. According to Ronald, 1988, the composition of the fermentation medium must include the nutrients essential to support the growth of the microbial strain and the formation of the desired product. Essential nutrients for microbial growth include sources of carbon, nitrogen and phosphorous.

In conclusion, it was found that there is high potential to discover useful antibiotic producing from the site with some possibly novel strain for applied microbiology. Therefore more work can be conducted for further study.

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