ISOLATION AND SCREENING OF SOIL FUNGI ISOLATED FROM SHIN MA TAUNG AND PONE TAUNG PONE NYAR AREAS

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Abstract

Soil sample collection was carried out on the 28th June, 2018 from the Shin Ma Taung and on the 29th June, 2018 from Pone Taung Pone Nyar Areas. Totally, 23 fungi (13 from Shin Ma Taung and 10 from Pone Taung Pone Nyar) were isolated from 15 different soil samples by Chemical Treatment Dilution Method. The specimens were cultured on Low Carbon Agar (LCA) and Glucose Soluble starch Yeast extract (GSY) plates and incubated at room temperature. Fungus MMH-11 possessed the highest antibacterial activity among the fungi of Shin Ma Taung Area. Among the fungi of Pone Taung Pone Nyar Area, fungus MMH-23 possessed the highest antibacterial activity. Fungus MMH-23 was selected for further investigations because it exhibited more high antibacterial activity on Methicillin Resistance *Staphylococcus aureus* (MRSA). The growth phase of the selected fungus MMH-23 was between 36 h and 72 h. Age of inoculum (42 h seed culture), size of inoculum (20 %) and initial fermentation pH 6.0 were suitable for the production of bioactive compound against MRSA.

Keywords: antibacterial activity, Low Carbon Agar, Glucose Soluble starch Yeast extract, Methicillin Resistance *Staphylococcus aureus*, inoculum, bioactive compound

Introduction

Modern and global healthcare today face the problem of multi-resistant bacteria. Many bacterial species have developed resistance mechanisms against several classes of antibiotics in a relatively short period of time after the clinical introduction of antibiotics (Stefan *etal.*, 2011). Shin Ma Taung Area have severe weather and environmental conditions. In this area, low rainfall, high temperature and frequent drought (Kaung, 2013). Pone Taung-Pone Nyar region has been protected under the 1998 law. The fossils in Pone Taung Pone Nyar region are believed to be 4 million years older than the Egyptian counterpart which were thought to be the oldest before (Shwe, 2019). Therefore, soil samples from Shin Ma Taung and Pone Taung Pone Nyar Areas are very interesting to isolate filamentous fungi. In the present work, the production of antimicrobial substances from the isolated fungi was investigated with antimicrobial activity against both susceptible and resistant strains of bacteria.

Materials and Methods

Soil Sample Collection and Isolation of Soil Fungi

Sample collection was carried out in Shin Ma Taung Area, Yesagyo Township, Pakokku District, Magway Region and Pone Taung Pone Nyar Area, Saw Township, Gantgaw District, Magway Region. Isolation of soil fungi was carried out by Chemical Treatment Dilution Method

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(Hayakawa and Kobayashi, 2005). The specimens were cultured on Low Carbon Agar and Glucose Soluble starch Yeast extract plates and incubated at room temperature.

Preliminary Study for Antimicrobial Activities of Soil Fungi

The isolated fungi were grown for 7 days on Potato Dextrose Agar medium for sporulation. The isolated fungi were inoculated on seed medium and incubated for 3 days. Twenty milliliter of seed culture was transferred into the 30 mL fermentation medium and incubated for 9 days. Thirty microliter of fermented broth was put on paper disc and placed on assay plate containing test organisms. Seed medium, fermentation medium and assay medium were employed in the studies for antimicrobial activities. Test organisms utilized for antimicrobial activity were *Staphylococcus aureus,* Methycillin Resistance *Staphylococcus aureus, Bacillus subtilis,* Chloramphenicol Resistance *E. coli* (CREC), *Micrococcus luteus, Pseudomonas fluorescens, Salmonella typhi, Candida albicans* and *Agrobacterium tumefaciens*.

Study on Microbial Growth Kinetics of the Selected Soil Fungi MMH-23

The strain MMH-23 was inoculated into the Glucose Yeast extract NZ Amine type A medium at 25 ºC and incubated for 120 h. The culture sample (10 mL) was checked in 12 h interval for the growth. The sample (10 mL) was centrifuged at 2000 rpm for 30 mins and Packed Cell Volume % (PCV %) was calculated (Omura, 1985; Crueger and Crueger, 1989).

Study on the Effects of Ages and Sizes of Inoculum on the Fermentation

According to the results of microbial growth kinetics of MMH-23, (42, 48, 54, 60, 66 and 72 h) seed culture were utilized for the fermentation. Based on the results of the ages of inoculum of MMH-23, (5 %, 10 %, 15 %, 20 %, 25 % and 30 %) of 42 h seed cultures were utilized for the fermentation. Antibacterial activity was checked by paper disc diffusion assay method with 8 mm diameter paper discs.

Study on the Effects of Initial pH on the Fermentation Medium

Fermentation media for MMH-23 were adjusted to the pH 5.0, 5.5, 6.0, 6.5 and 7.0 respectively. Antibacterial activity was checked by paper disc diffusion assay.

Results and Discussion

Isolation of Fungi from Soil Samples and their Antimicrobial Activities

A total of 15 different soil samples were collected at Shin Ma Taung (9 soil samples) and Pone Taung Pone Nyar (6 soil samples) Areas. A total of 23 fungi (MMH-01 to MMH-23) were isolated from 15 different soil samples collected at Shin Ma Taung and Pone Taung Pone Nyar Areas. The study of antimicrobial activities of isolated fungi showed that MMH-03, MMH-05, MMH-11, MMH-20, and MMH-23 strains possessed the antibacterial activity on test organisms (Figures 1-6).

Among them, MMH-03 showed the antibacterial activity against Chloramphenicol Resistance *E. coli* (23.12 mm at 7 day fermentation). MMH-05 showed the antibacterial activity against *Staphylococcus aureus* (24.53 mm at 7 day fermentation). MMH-11 showed the antibacterial activity against *Salmonella typhi* (26.29 mm at 7 day fermentation). MMH-20 showed the antibacterial activity against *Agrobacterium tumefaciens* (23.35 mm at 7 day

fermentation). MMH-23 showed the antibacterial activities against MRSA (29.13 mm at 7 day fermentation), *Staphylococcus aureus* (17.30 mm at 7 day fermentation) and *Micrococcus luteus* (16.71 mm at 7 day fermentation). MMH-23 was selected for further investigations because it showed high antibacterial activity on Methicillin Resistance *Staphylococcus aureus* (Table 1).

Soil texture and soil pH of selected soil samples are shown in Table 2. Selected fungus MMH-23 was isolated from the soil sample PT-6 of Pone Taung Pone Nyar Area. Soil texture of PT-6 was sandy loan and soil pH was 6.60.

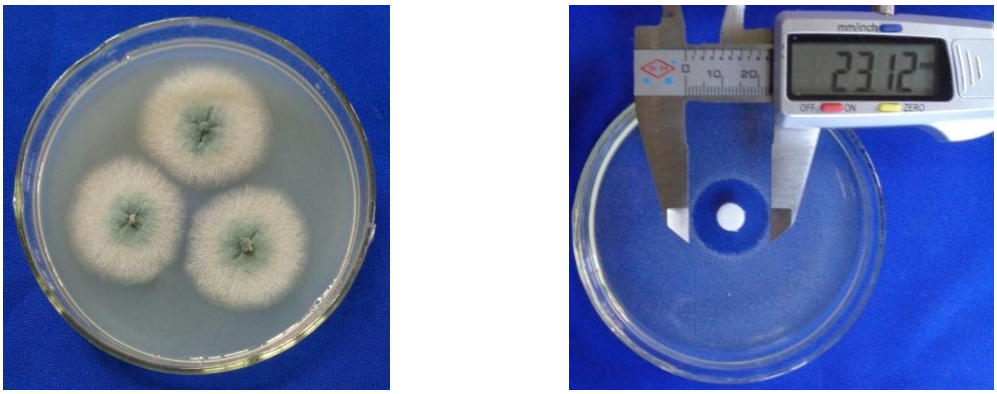


Figure 1 Morphology and activity of isolated fungus MMH-03
(7 day fermentation, Test Organism CREC)

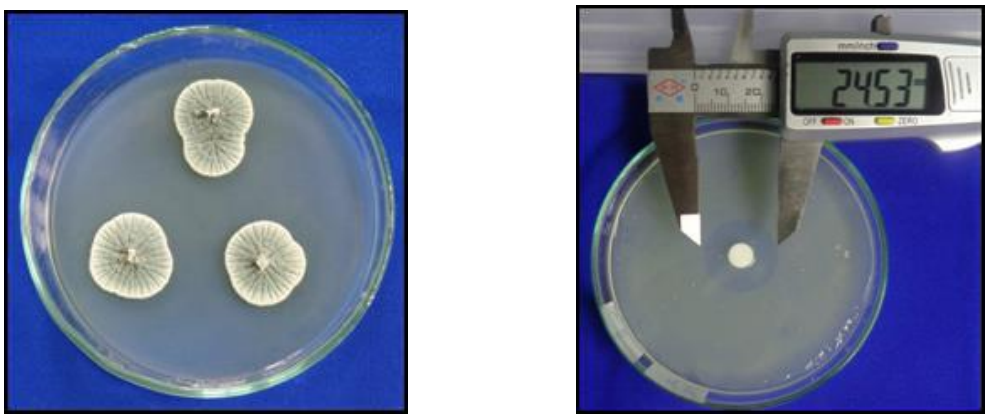


Figure 2 Morphology and activity of isolated fungus MMH-05
(7 day fermentation, Test Organism *Staphylococcus aureus*)

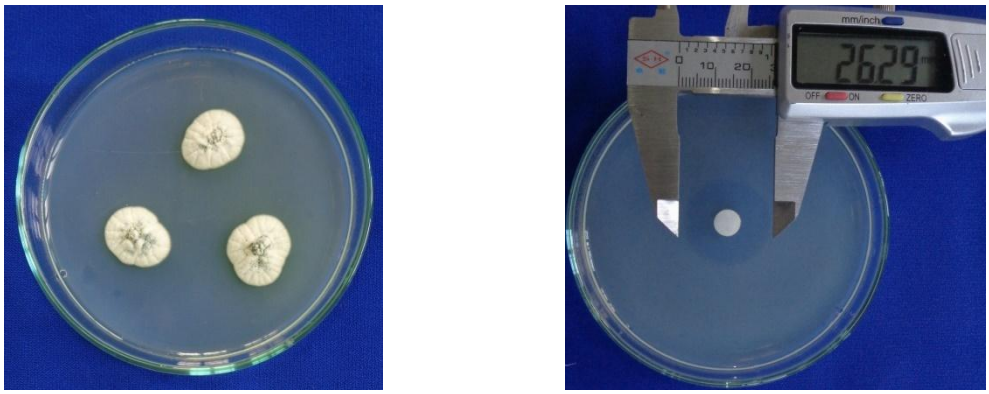


Figure 3 Morphology and activity of isolated fungus MMH-11
(7 day fermentation, Test Organism *Salmonella typhi*)

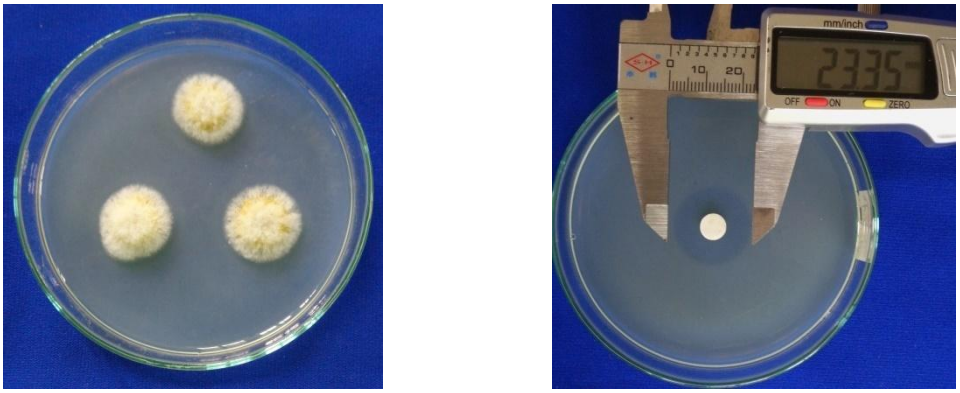


Figure 4 Morphology and activity of isolated fungus MMH-20
(7 day fermentation, Test Organism *Agrobacterium tumefaciens*)

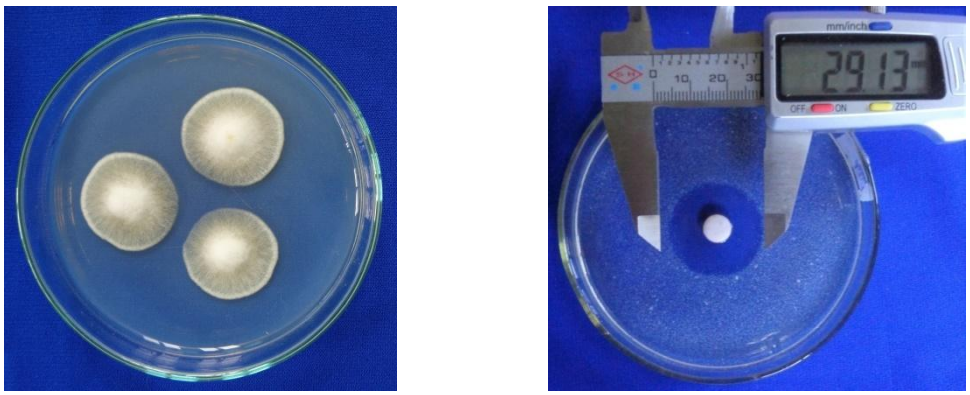
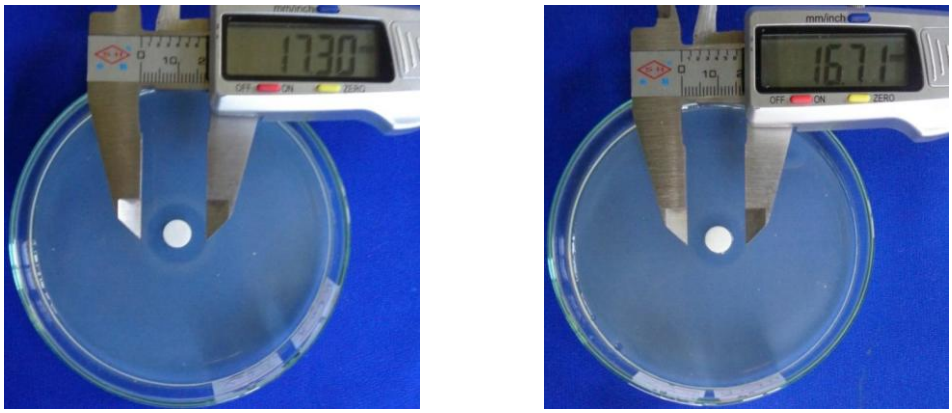


Figure 5 Morphology and activity of isolated fungus MMH-23
(7 day fermentation, Test Organism MRSA)



Staphylococcus aureus ***Micrococcus luteus***

Figure 6 Antibacterial activities of isolated fungus MMH-23
(8 day fermentation)

Table 1 Comparison of Antibacterial Activity Possessing Isolated Fungi

Soil No.	Isolated Fungus	Fermentation Period (Day)	Test Organism	Antibacterial Activity (mm)
SMT-2	MMH-03,GSY	7	Chloramphenicol Resistance *E. coli*	23.12
SMT-3	MMH-05,LCA	7	*Staphylococcus aureus*	24.53
SMT-7	MMH-11,GSY	7	*Salmonella typhi*	26.29
PT-4	MMH-20,GSY	7	*Agrobacterium tumefaciens*	23.35
		8	*Pseudomonas fluorescens*	15.56
		8	*Staphylococcus aureus*	17.30
PT-6	MMH-23,GSY	8	Methicillin Resistance *Staphylococcus aureus*	29.13
		8	*Micrococcus luteus*	16.71

Table 2 Soil Texture and Soil pH of Five Selected Soil Samples

Soil No.	Soil Texture	Soil pH
SMT-2	Sandy Loam	6.62 (Near neutral)
SMT-3	Sandy Clay Loam	9.24 (Extremely alkaline)
SMT-7	Sandy Loam	8.26 (Moderately alkaline)
PT-4	Sandy Loam	8.27 (Moderately alkaline)
PT-6	Sandy Loam	6.60 (Near neutral)

Microbial Growth Kinetics of MMH-23

In the growth kinetics study of fungus MMH-23, it was found that growth phase was between 36 h and 72 h .Thus, the ages of inoculum (42, 48, 54, 60, 66 and 72 h) were suitable for the optimization of fermentation (Figure 7).

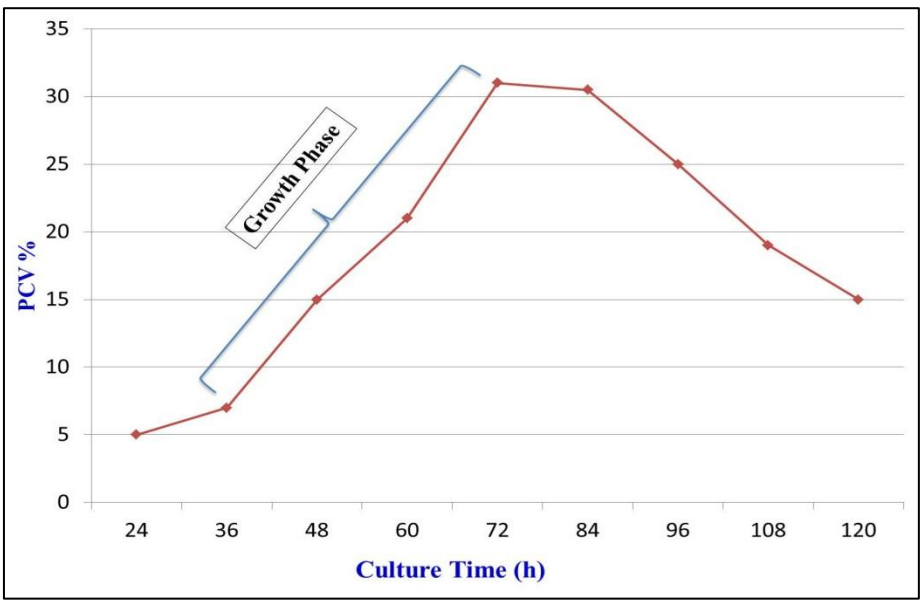


Figure 7 Microbial growth kinetics of the fungus MMH-23

Fermentation Optimization

In the study of ages of inoculum on the fermentation of MMH-23, 42 h seed culture showed the best activity on MRSA (Figure 8). Therefore, 42 h seed culture was selected for the fermentation. In the study of sizes of inoculum on the fermentation, 20 % size of culture showed the best activity on MRSA (Figure 9). Therefore, 20 % size of seed culture was selected for the fermentation. Fermentation medium adjusted to the pH 6.0 showed the highest antibacterial activity (Figure 10).

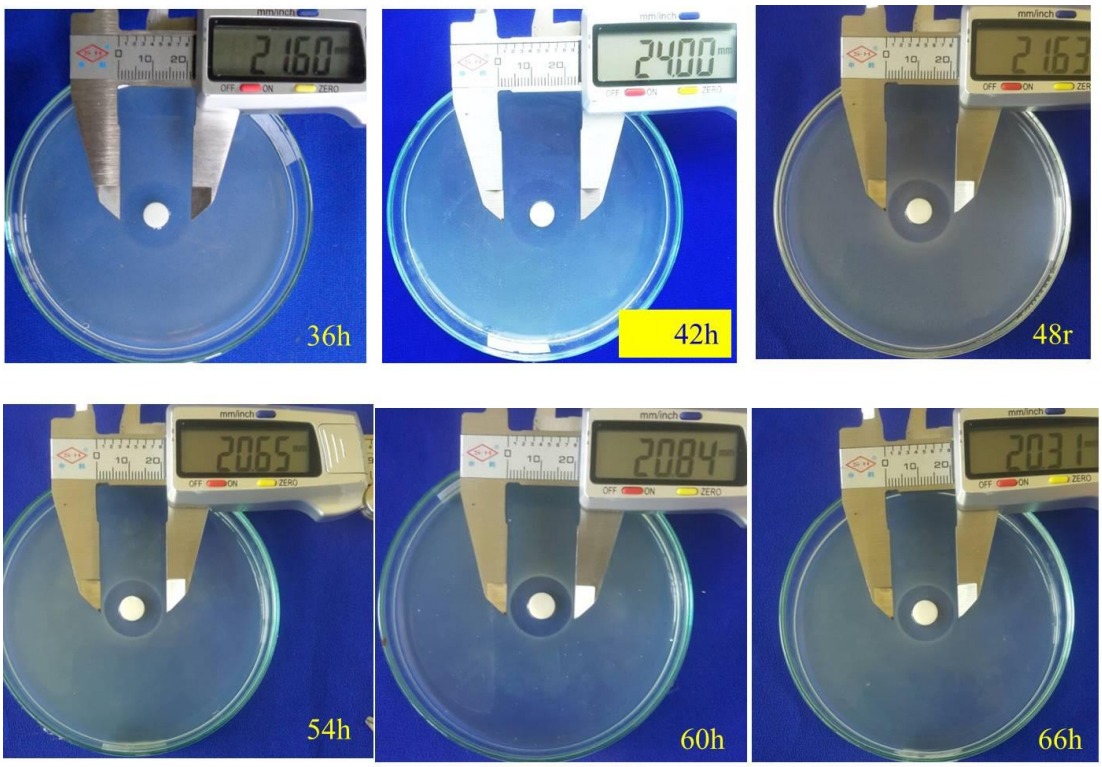


Figure 8 The effects of ages of culture on fermentation of MMH-23

Table 3 The Effects of Ages of Culture on the Fermentation

Ages of Culture (h)	Activity (mm, clear zone)
36	21.60
42	24.00
48	21.63
54	20.65
60	20.84
66	20.31

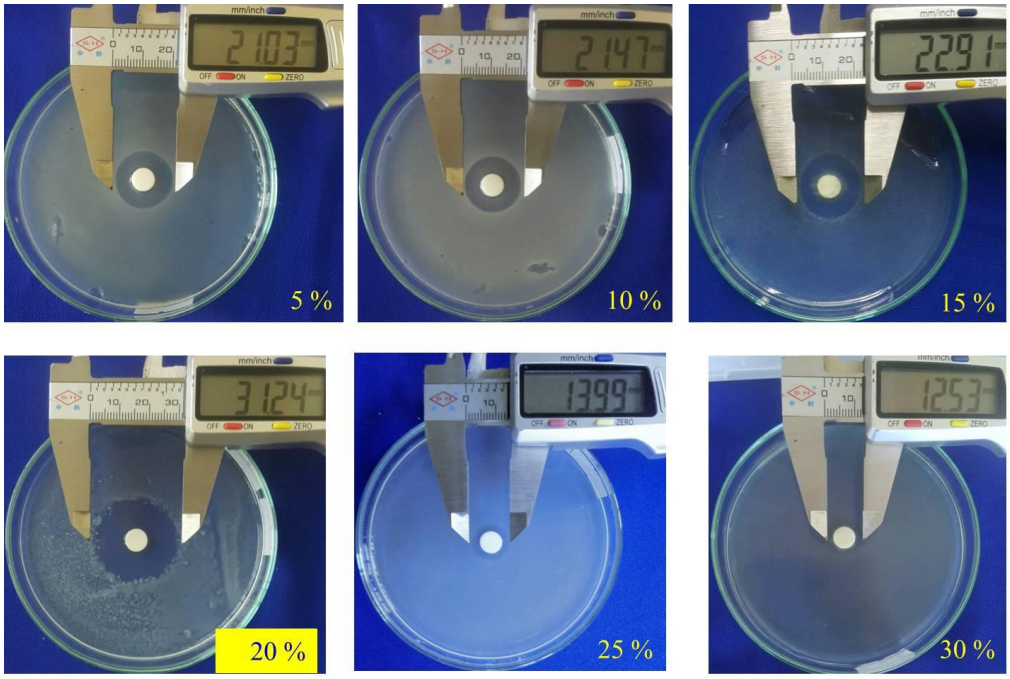


Figure 9 The effects of sizes of culture on fermentation of MMH-23

Table 4 The Effects of Sizes of Culture on the Fermentation

Sizes of Culture (%)	Activity (mm, clear zone)
5	21.03
10	21.47
15	22.91
20	31.24
25	13.99
30	12.53

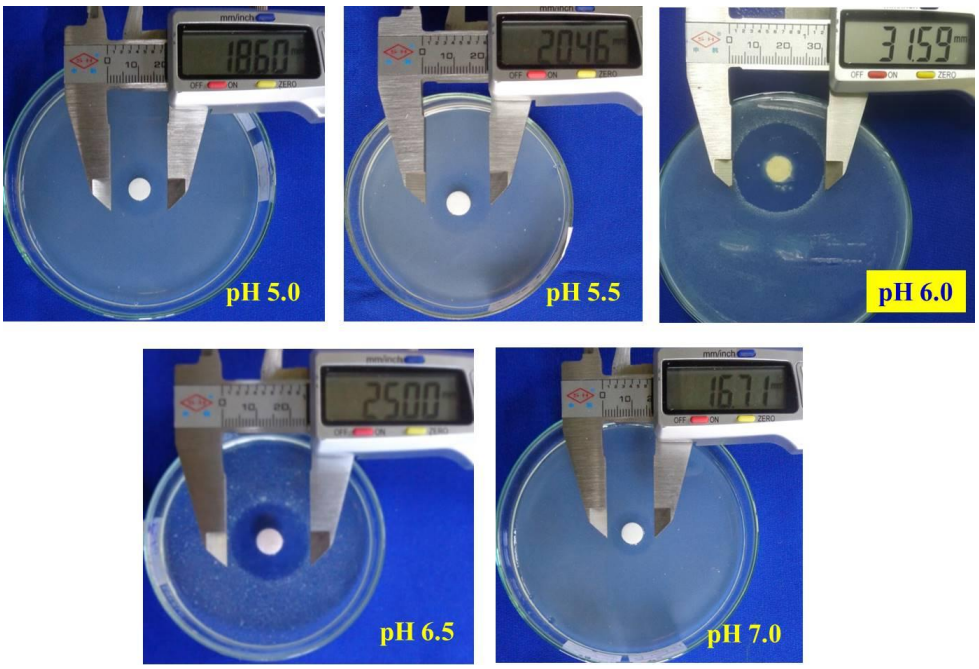


Figure 10 The effects of initial pH on fermentation of MMH-23

Table 5 The Effects of Initial pH on Fermentation of MMH-23

Initial pH	Activity (mm, clear zone)
5.0	18.60
5.5	20.46
6.0	**31.59**
6.5	25.00
7.0	16.71

Conclusion

In this research, sample collection was carried out from Shin Ma Taung and Pone Taung Pone Nyar Area, Magway Region. Soil samples are mostly sandy gravel and rocky in these areas. Soil fungi were isolated by Chemical Treatment Dilution Method.

MMH-03, MMH-05, MMH-11, MMH-20, and MMH-23 showed the antibacterial activity on test organisms. MMH-23 was selected for further investigations because it showed high antibacterial activity on MRSA. It was found that growth phase of MMH-23 from soil No. PT-6, Pone Taung Pone Nyar Area, was between 36 h and 72 h. In the study of the fermentation optimization of MMH-23, 42 h and 20 % size of seed culture showed the best activity on MRSA. It was found that fermentation medium adjusted to the pH 6.0 showed the highest antibacterial activity.

Drug-resistant pathogens are increasing. Therefore, the need for antimicrobial discovery and better treatments of these infections is becoming a rapidly growing concern. Further study is needed to isolate and to characterize the bioactive compounds responsible for antimicrobial activity.

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