CULTURAL CHARACTER AND ANTIMICROBIAL ACTIVITIES OF ISOLATED FUNGI FROM SOIL SAMPLES OF BELU-GYUN, CHAUNG-ZON TOWNSHIP, MON STATE

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Abstract

Soil samples from twenty different locations of Belu-Gyun, Chaung-Zon Township, Mon State were collected. These soil samples were utilized in the isolation of 37 fungal strains. They were designated as MM-1 to MM-37. Among these fungi colonies, MM-34 was found to give the highest antibacterial activity of 36.35 mm clear zone against *Micrococcus luteus.* So, MM-34 was preferred to conduct further investigation concerning the optimal fermentation conditions to produce antibacterial metabolite. In the fermentation experiments with MM-34, it was observed that the 84 hours of ages, 15% sizes of inoculums were suitable for highest antibacterial activity against *Micrococcus luteus.* The result revealed that maximum production of antimicrobial metabolite (27.27 mm clear zone) was obtained when medium was supplemented with glucose. Culture containing yeast extract showed the highest antibiotic production (27.54 mm clear zone). FM-3 gave maximum yield of antibacterial activity (30.02 mm clear zone) against *Micrococcus luteus.*

Keywords: soil fungi, antibacterial activity

Introduction

Soil is the most abundant ecosystem on Earth, but the vast majority of organisms in soil are microbes. There may be a population limit of around one billion cells per gram of soil, but estimate the number of species vary widely from 50,000 per gram to over a million per gram of soil. Soil sustains an immerse diversity of microbes, which to a large extent, remains unexplored. Bacteria including actinomycetes and fungi are most preferably used as screening sources from various habitats. Fungi are well known as prolific producers of biologically active natural products (Hara Kishore *et al.,* 2007).

The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem. Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Ainsworth, 1995).

Fungi grow best in environments that are slightly acidic. Soil fungi have been the most studied of fungi, and typical soil genera such as *Acremonium, Aspergillus, Fusarium* and *Penicillium* have shown ability to synthesize a diverse range of bioactive compounds (Asnaashari, *et al.,* 2012).

Antibiotic have an important role in human health. Most of the naturally occurring antibiotics have been isolated from soil microorganisms. Antibiotics represent the medically important group of secondary metabolites, which have been useful in our battle against infectious disease. The proper cultivation and transfer of inoculums are essential for the production of both primary and secondary metabolites. Media used in the cultivation of microorganisms must

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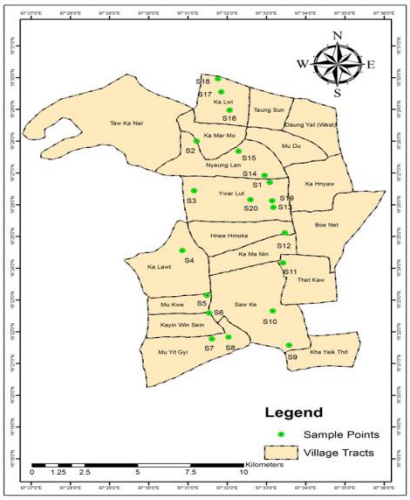
contain all elements in a form suitable for the synthesis of cell substances and for the production of metabolic products (Dale, 1984; Balows *et al.*, 1992). Therefore, the fermentation conditions such as substrates, inoculums cultivation and transfer have to be optimized for the production of primary and secondary metabolites.

The aim and objectives of this research were to study the various isolated fungi, age and size of the inoculum of fungus MM-34 and to investigate the effect of carbon and nitrogen utilization on the fermentation.

Materials and Methods



Twenty Soil samples were collected from the twenty different places of Belu-Gyun, Chaung-Zon Township, Mon State
Source: Department of Geography, Pathein University
Figure 1 Map of Collected Soil Samples from Belu-Gyun

Collection of Soil Samples

Twenty soil samples were collected from the twenty different places of Belu-Gyun, Chaung-Zon Township, Mon State. Soil samples were utilized for the isolation of fungi and the soil samples were put into plastic bag under aseptic condition. The soil type as its pH was analyzed by Department of Agriculture (Land Use) Insein.

Table 1 Some characters of soil samples

Sr No.	Soil No.	Collected Area	pH	Texture	Sr No.	Soil No.	Collected Area	pH	Texture
1	Soil-1	Ywar Lut	4.13	Silt loam	11	Soil-11	Thet Kaw	3.92	Sandy loam
2	Soil-2	Ka Mar Mo	3.60	Loamy sand	12	Soil-12	Hnee	4.74	Sandy loam
3	Soil-3	Ywar Lut	3.55	Sandy loam	13	Soil-13	Ywar Lut	4.77	Loam
4	Soil-4	Ka Lawt	3.67	Sandy loam	14	Soil-14	Nyaung	4.52	Sandy loam
5	Soil-5	Mu Kwe	4.11	Sandy loam	15	Soil-15	Nyaung	4.48	Silt loam
6	Soil-6	Kayin Win	4.11	Sandy clay	16	Soil-16	Ka Lwi	3.68	Loamy Sand
7	Soil-7	Mu Yit Gyi	4.12	Loam	17	Soil-17	Ka Lwi	3.93	Sandy loam
8	Soil-8	Mu Yit Gyi	3.42	Sandy loam	18	Soil-18	Ka Lwi	3.89	Sandy loam
9	Soil-9	Saw Ke	4.51	Sandy Clay	19	Soil-19	Ywar Lut	4.74	Sandy Clay
10	Soil-	Saw Ke	4.10	Loam	20	Soil-20	Ywar Lut	4.04	Sandy loam

Media used for isolation of soil fungi (Ando, 2004)

Isolation medium		**Transfer Medium**	
Glucose	- 1.0 g	Glucose	- 1.2 g
Yeast extract	- 0.7 g	Yeast extract	- 0.8 g
KH_2PO_4	- 0.01 g	KH_2PO_4	- 0.01 g
KNO_3	- 0.02 g	$MgSO_4$	- 0.01 g
Agar	- 1.8 g	Agar	- 1.8 g
Distilled water	- 100 mL	Distilled water	- 100 mL

Isolation of fungi from soil samples

The isolation of soil fungi were carried out by feeding method (Hayakawa, and Kobayashi, 2005)

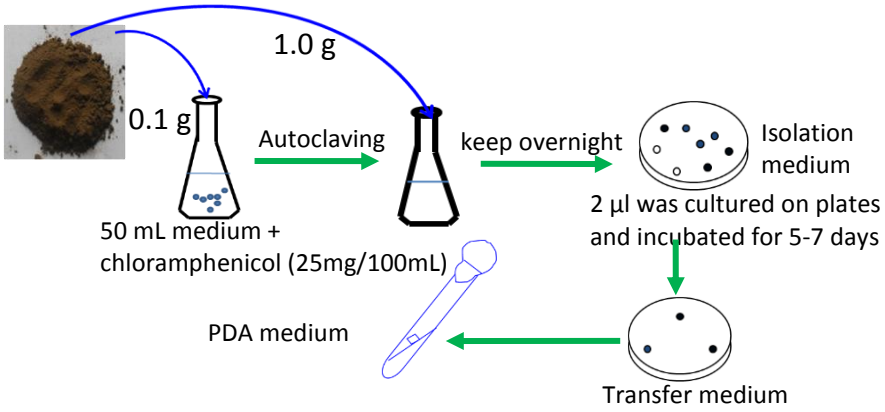


Figure 2 Feeding method (Hayakawa, and Kobayashi, 2005)

Medium used for antimicrobial activity test (Ando, 2004)

Seed Medium		**Fermentation Medium**		**Assay Medium**	
Glucose	- 1.0 g	Glucose	- 1.5 g	Glucose	- 0.8 g
Yeast extract	- 0.8 g	Yeast extract	- 0.8 g	Peptone	- 0.7 g
K_2HPO_4	- 0.01 g	Peptone	- 0.4 g	KNO_3	- 0.2 g
KCl	- 0.1 g	$MgSO_4$	- 0.001 g	Agar	- 1.8 g
DW	- 100 mL	K_2HPO_4	- 0.01 g	DW	- 100 mL
		KCl	- 0.1 g		
		DW	- 100 mL		

Screening of antimicrobial activity of isolated soil fungi by paper disc diffusion assay (Tomita, 1988)

The isolated fungi were grown at room temperature for 5 days. The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days. Seed culture (20%) was transferred into the fermentation medium and incubated at room temperature for 10 days. 20µL of fermented broth was put on paper disc. After drying, placed on assay plate containing test organism and incubated for 24-36 hours. Six pathogenic microorganisms were utilized for antimicrobial activity.

Table 2 Test Organisms utilized for antimicrobial activities

No.	Test Organisms	Disease
1	*Aspergillus flavus* IFO3290	Aspergillosis
2	*Bacillus subtilis* KY-327	Fever
3	*Micrococcus luteus* NITE83297	Skin disease
4	*Escherichia coli* AHU5436	Diarrhoea
5	*Pseudomonas fluorescens* IFO94307	Rice disease
6	*Salmonella typhi* AHU7943	Typhoid fever and food poison

Effect of age of inoculum on the fermentation of antibacterial activity by isolated soil fungus MM-34

Five days old culture of the selected fungus MM-34 was inoculated into seed medium and then transfer to fermentation medium. Age of culture with 36 hrs, 48 hrs, 60 hrs, 72 hrs, 84 hrs, 96 hrs and 108 hrs were utilized for fermentation. The antibacterial activity was carried out by paper disc diffusion assay with 8 mm paper disc.

Effect of size of inoculum on the fermentation medium of antibacterial activity of isolated soil fungus MM-34

In this study, 5%, 10%, 15%, 20%, 25%, 30% and 35% of 84 hrs of seed culture were utilized for the fermentation. Fermentation was carried out by paper disc diffusion assay with 8 mm paper disc.

Effect of carbon sources utilization on fermentation of antibacterial activity by isolated soil fungus MM-34

To study the effect of different carbon sources utilization on fermentation, glucose, sucrose, soluble starch, potato powder, tapioca powder, corn powder, rice powder were used. 2 g of each carbon sources were added to the basal medium individually.

Effect of nitrogen sources utilization on fermentation of antibacterial activity by isolated soil fungus MM-34

To study the effect of different nitrogen sources utilization on fermentation, yeast extract, peptone, KNO_3, NH_4Cl, NH_4SO_4, NH_4NO_3 and meat extract were used, 1 g of each nitrogen sources were added to basal medium individually.

Basal fermentation medium used in carbon sources utilization		**Basal fermentation medium used in nitrogen sources utilization**	
Yeast extract	1.0 g	Glucose	2.0 g
$MgSO_4$	0.001 g	$MgSO_4$	0.001 g
KNO_3	0.1 g	D/W	100 mL
D/W	100 mL		

Medium Selection for Fermentation

Based on the results of carbon and nitrogen sources utilization on fermentation, 6 fermentation media were composed. Fermentation was undertaken with 84 hrs age and 15% size of inoculum with six different media.

FM-1		**FM-2**		**FM-3**	
Glucose	2.0 g	Glucose	2.0 g	Potato	2.0 g
Yeast extract	1.0 g	Peptone	1.0 g	Yeast extract	1.0 g
$MgSO_4$	0.001 g	$MgSO_4$	0.001 g	$MgSO_4$	0.001 g
D/W	100 mL	D/W	100 mL	D/W	100 mL

FM-4		**FM-5**		**FM-6**	
Potato	2.0 g	Glucose	1.0 g	Glucose	1.0 g
Peptone	1.0 g	Potato	1.0 g	Potato	1.0 g
$MgSO_4$	0.001 g	Yeast extract	1.0 g	Peptone	1.0 g
D/W	100 mL	$MgSO_4$	0.001 g	$MgSO_4$	0.001 g
		D/W	100 mL	D/W	100 mL

Results

Isolation of fungi from soil samples

In the study of isolation of fungi, a total of 37 soil fungi were isolated from soil samples collected at 20 different places of Belu-Gyun, Chaung-Zon Township, Mon State.

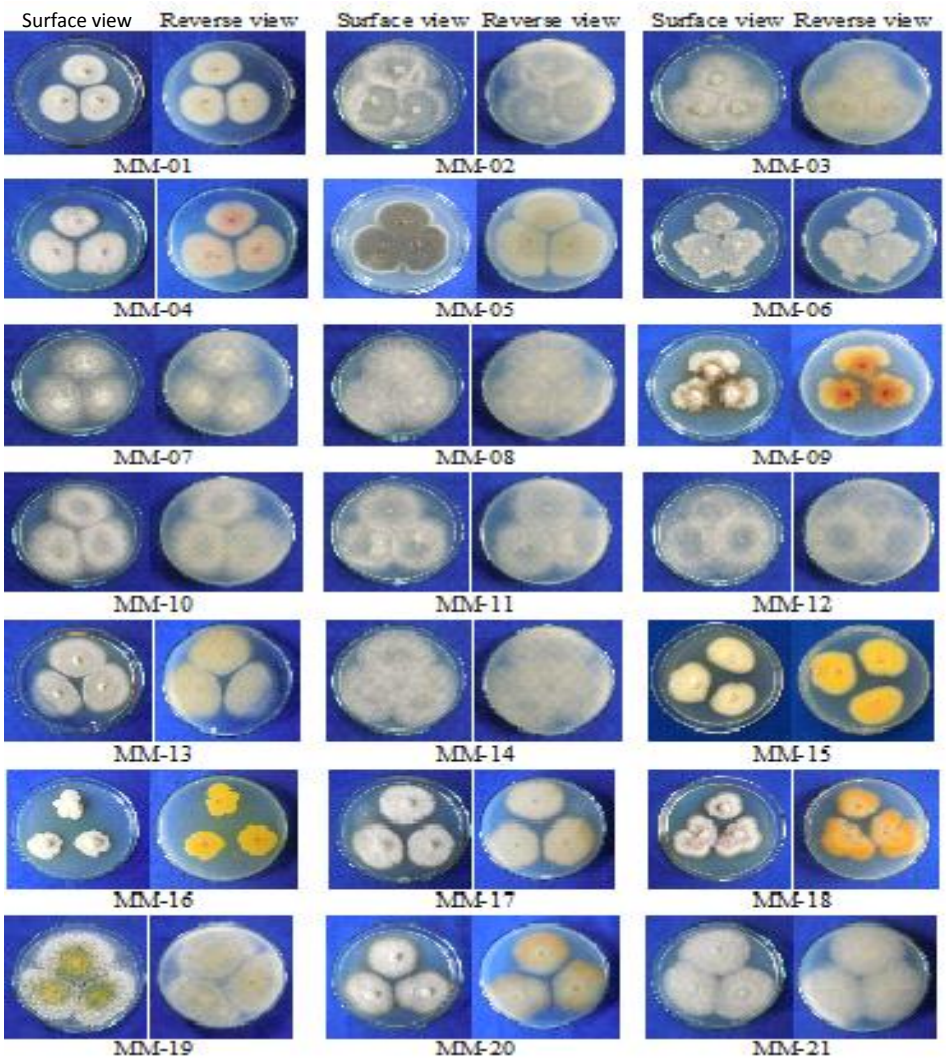


Figure 3 Morphology of isolated soil fungi MM-01 to MM-21(7 Days old culture)

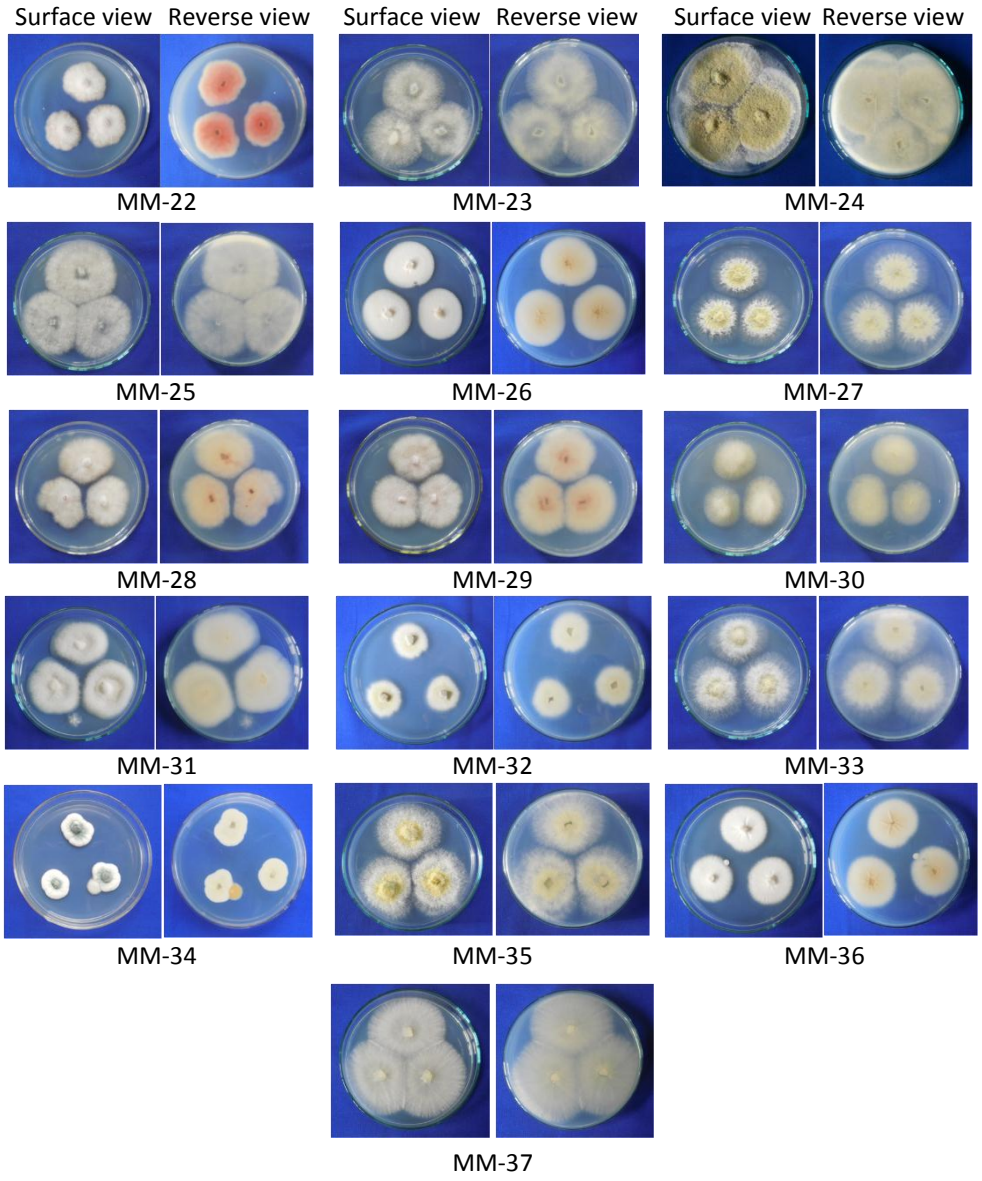


Figure 4 Morphology of isolated soil fungi MM-22 to MM-37 (7 Days old culture)

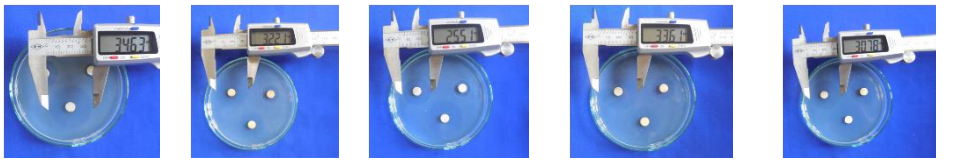
Screening of antimicrobial activity of isolated soil fungi by paper disc diffusion assay

The study of biological properties of these fungi, MM-10 and MM-21 showed antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Pseudomonas fluorescens.* Soil fungus MM-34 exhibited antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Micrococcus luteus* and *Escherichia coli.* Among them MM-34 showed the highest antibacterial activity against *Micrococcus luteus* (36.35 mm) (Table 3 and Figure 5, 6, 7).

Table 3 Antimicrobial activities producing fungi and their activities

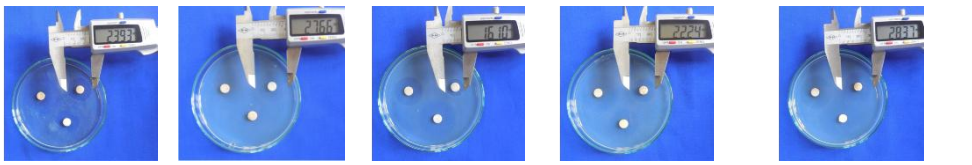
Isolated fungi	Antimicrobial activities on test organisms (inhibitory zone, mm)					
	Aspergillus flavus	*Bacillus subtilis*	*Micrococcus luteus*	*Escherichia coli*	*Pseudomonas fluorescens*	*Salmonella typhi*
MM-10	**34.63**	32.21	25.51	33.61	30.78	-
MM-21	23.93	27.66	16.10	22.24	**28.37**	-
MM-34	20.11	26.67	**36.35**	28.58	-	-

(-) = No activity



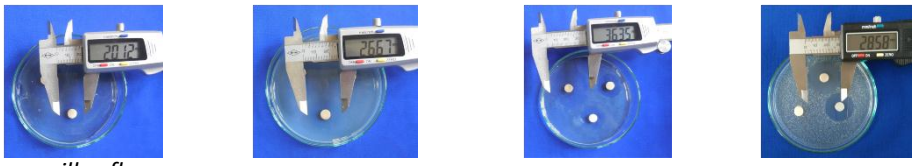
Aspergillus flavus *Bacillus subtilis* *Micrococcus luteus* *Escherichia coli* *Pseudomonas fluorescens*

Figure 5 Antimicrobial activity of isolated soil fungus MM-10



Aspergillus flavus *Bacillus subtilis* *Micrococcus luteus* *Escherichia coli* *Pseudomonas fluorescens*

Figure 6 Antimicrobial activity of isolated soil fungus MM-21



Aspergillus flavus *Bacillus subtilis* *Micrococcus luteus* *Escherichia coli*

Figure 7 Antimicrobial activity of isolated soil fungus MM-34

Study on the effects of ages of culture on the fermentation of antibacterial activity of isolated soil fungus MM-34

In this study, age of culture (36, 48, 60, 72, 84, 96, 108 hrs) were utilized for the fermentation. In the experiment it was observed that 84 hrs age of seed culture was suitable for the fermentation (Table 4, Figure 8).

Table 4 Effects of age of inoculum on the fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Seed culture (Time, hrs)	Activity (Clear zone, mm)
36	13.57
48	16.84
60	18.59
72	19.69
84	**21.98**
96	20.33
108	18.15

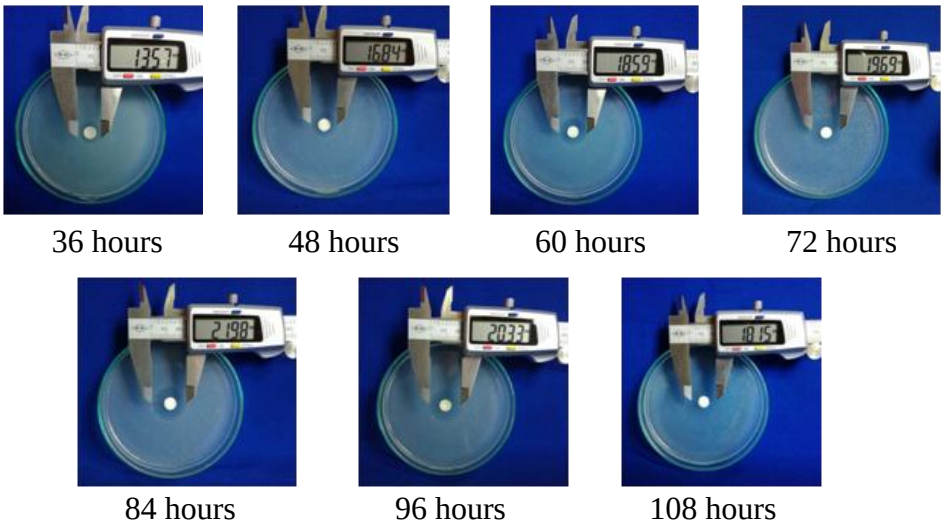


Figure 8 Effects of age of inoculum on the fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Study on the effect of size of inoculum on the fermentation of antibacterial activity of isolated soil fungus MM-34

In the study of size of inoculum, different size of inoculum (5%, 10%, 15%, 20%, 25%, 30% and 35%) were tested, 15% isolated fungi size of inoculum concentration was the best for fermentation (Table 5, Figure 9).

Table 5 Effects of size of inoculum for fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Size of inoculum	Activity (clear zone mm)
5 %	21.65
10 %	21.00
15 %	**25.62**
20 %	23.70
25 %	23.04
30 %	23.21
35 %	25.57

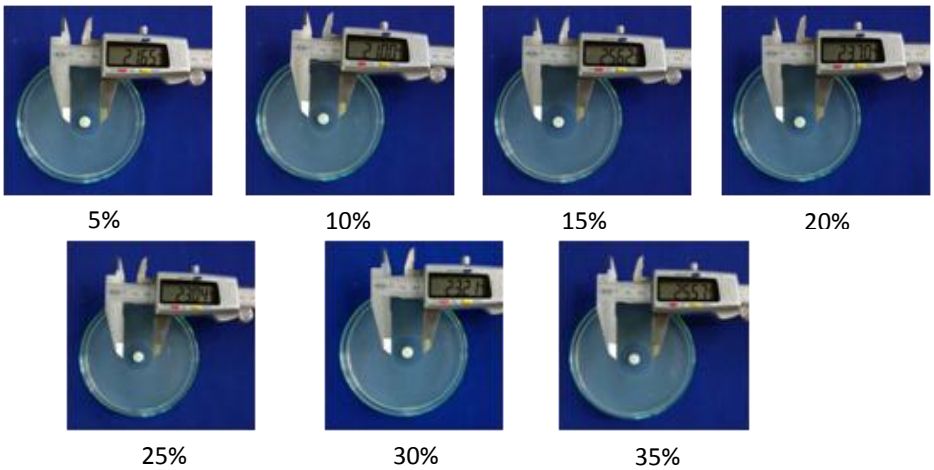


Figure 9 Effect of size of inoculum on fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Study on the effect of carbon source utilization on fermentation of antibacterial activity of isolated soil fungus MM-34

In the study of carbon source utilization on fermentation, glucose, sucrose, soluble starch, potato powder, tapioca powder, corn powder, rice powder were used. Among them, glucose was found to be the best for the fermentation. (Table 6, Figure 10).

Table 6 Effects of carbon sources utilization on fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Carbon sources	Inhibitory zone, mm
Glucose	**27.27**
Sucrose	23.90
Soluble starch	25.40
Potato powder	25.71
Tapioca powder	25.22
Corn powder	25.22
Rice powder	24.57

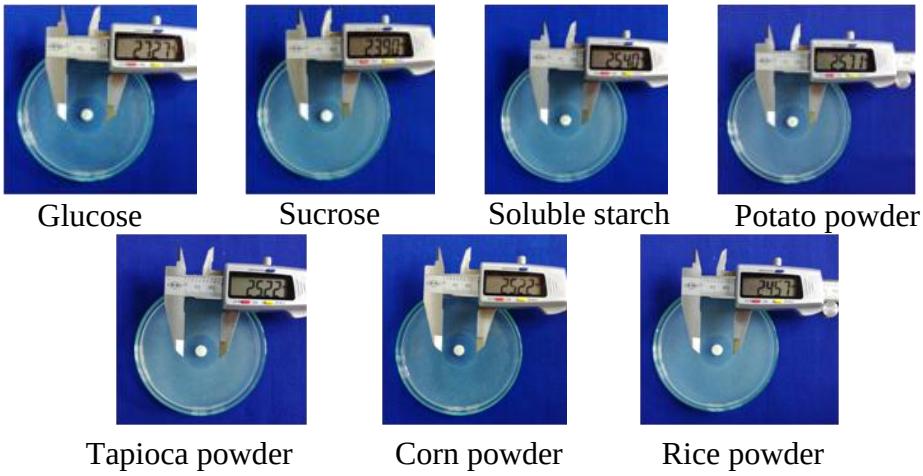


Figure 10 Effect of carbon sources utilization on fermentation of antibacterial activity of isolated soil fungus MM-34

Study on the effect of nitrogen source utilization on fermentation of antibacterial activity of isolated soil fungus MM-34

In this study, different nitrogen sources (yeast extract, peptone, KNO_3, NH_4Cl, NH_4SO_4, NH_4NO_3 and meat extract) were utilized for the fermentation. In the experiment it was observed that yeast extract was most suitable for the fermentation. (Table 7, Figure 11)

Table 7 Effects of nitrogen utilization on fermentation of antibacterial activity of isolated soil fungus MM-34 against *Micrococcus luteus*

Nitrogen sources	Inhibitory zone, mm
Yeast extract	**27.54**
Peptone	19.32
KNO_3	13.97
NH_4Cl	13.90
NH_4SO_4	14.70
NH_4NO_3	13.73
Meat extract	15.08

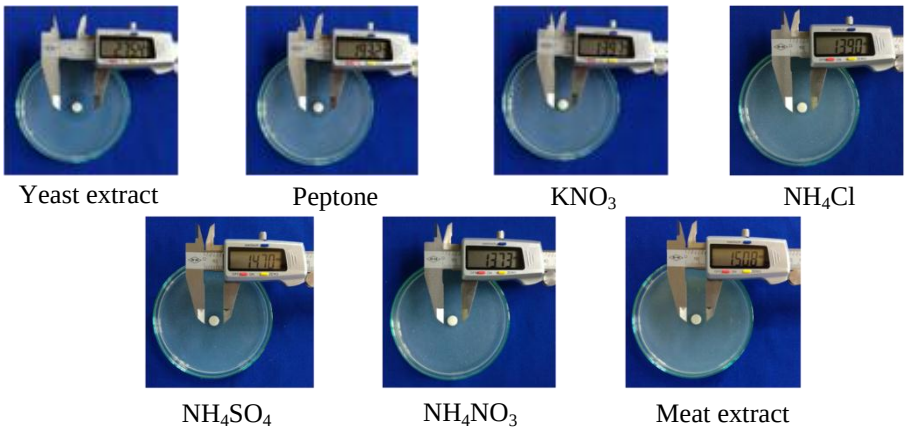


Figure 11 Effect of nitrogen utilization on fermentation of antibacterial activity of isolated soil fungus MM-34 against *Micrococcus luteus*

Medium selection for fermentation

In order to select the best fermentation medium for maximum antibiotic production, six different fermentation media FM-1 to FM-6 were tested. Fermentation medium FM-3 gave maximum yield of antibacterial metabolites against *Micrococcus luteus* (Table 8, Figure 12).

Table 8 Effects of media on fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Medium	Activity (Clear zone, mm)
FM-1	16.56
FM-2	19.06
FM-3	**30.02**
FM-4	22.39
FM-5	27.01
FM-6	22.51

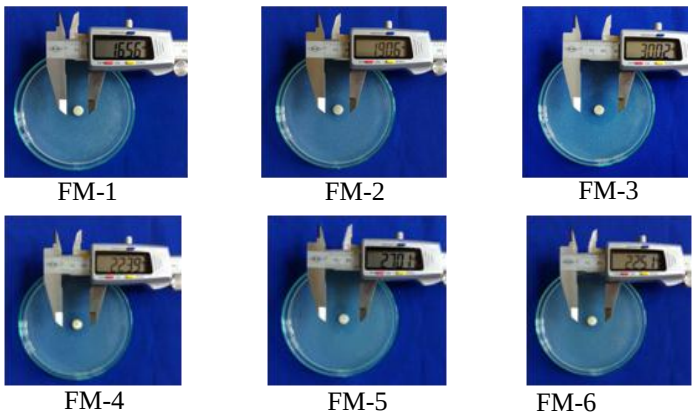


Figure 12 Effects of media on fermentation of antibacterial activity of isolated soil fungus (MM-34) against *Micrococcus luteus*

Discussion and Conclusion

In the course of investigation, 37 fungi were isolated from twenty different soil samples which were collected from Belu-Gyun, Chaung-Zon Township, Mon State. Feeding method was used in the isolation of soil fungi. Isolated 37 fungi their morphological character and reverse color were found to be different. Among 37 soil fungi, MM-10, MM-21 and MM-34 showed antimicrobial activity. During the study of biological properties of these fungi, MM-10 and MM-21 exhibited antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Micrococcus luteus, Escherichia coli* and *Pseudomonas fluorescens.* Soil fungus MM-34 showed antimicrobial activity against *Aspergillus flavus, Bacillus subtilis, Micrococcus luteus* and *Escherichia coli*. Soil fungus MM-10 was isolated from soil sample No.6. MM-10 showed the highest antifungal activity against *Aspergillus flavus* (34.63 mm). Soil fungus MM-21 was isolated from soil sample No.13. MM-21 showed the highest antibacterial activity against *Pseudomonas fluorescens* (28.37 mm). Soil fungus MM-34 was isolated from soil sample No.19. MM-34 exhibited the highest antibacterial activity against *Micrococcus luteus* (36.35 mm). Therefore, this strain MM-34 was selected for further investigation such as age of inoculum, size of inoculum, the effect of different carbon and nitrogen sources and medium selection of fermentation. This isolated soil fungus MM-34 was collected from Ywar Lut, Belu-Gyun, Chaung-Zon Township, Mon State.

From the present result, it was observed that the most suitable parameter such as 84 hrs age of inoculum and 15% size of inoculum were optimized to produce the antibacterial activity during fermentation. The different carbon and nitrogen source supplement to the culture broth strongly influenced the growth and biosynthesis of active metabolite by MM-34. On studying the effect of different carbon sources, the results indicated that glucose affected the antibacterial production (27.27 mm clear zone). This finding was coincide with those of Buchanan, *et al.,* (1984) where simple sugar such as glucose, fructose, sucrose, glycerol enhance growth as well as secondary metabolite production by microorganisms rather than complex carbon sources like starch, galactose, xylose, mannitol, etc.

Other than the carbon, the source of nitrogen is important for the production of antibiotic substances. MM-34 produced the antibacterial metabolites with most of nitrogen sources, but yeast extract was most suitable for antibacterial metabolite production (27.54 mm clear zone). Similar results were shown by Mathan *et al.,* (2013) where yeast extract promoted the secondary metabolite biosynthesis. The results were in good agreement with early studies by Kalyani *et al.,* (2016) have shown that yeast extract as nitrogen source, promoted the biosynthesis of secondary metabolites. Likewise, Zain *et al.,* (2009) reported that yeast extract showed the best secondary metabolite production. Similarly, Alatwani R.H (2016) reported that yeast extract was found to be a best and most suitable nitrogen source for the optimum production of bioactive metabolites. The results of antibacterial tests indicated that maximum antibacterial production was obtained in culture supplemented with glucose as carbon source and yeast extract as nitrogen source.

In order to select the best fermentation medium for maximum antibiotic production, six different fermentation media FM-1 to FM-6 were tried. FM-3 medium was the best fermentation condition of isolated soil fungus MM-34 for the production of antibacterial activity (30.02 mm clear zone).

The choice of a good fermentation medium is virtually as important to the success of an industrial fermentation as is the selection of an organisms to carry out the fermentation (EL-

Tayeb *et al.*, 2004). The constituents of a medium must satisfy the elemental requirements for cell biomass and metabolite production (Stanbury *et al.*, 1997). The result of the present work agree with EL-Tayeb *et al.*, 2004 and Stanbury *et al.*, 1997.

In conclusion, the isolated soil fungus MM-34 was selected for further investigation and to find out the nature of metabolites those can kill the test organism.

Acknowledgements

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APPENDIX

DEPARTMENT OF AGRICULTURE (LAND USE)
SOIL ANALYTICAL DATA SHEET

Division - မွန်ပြည်နယ်
Township - သီလဝကျွန်း

ခေါ်မြေဝင်းမင်းမျိုး (၁.၁၂.၂၀၁၇)

Sheet No. 1
Sr No. S 1-10 / 17-18

Sr No.	Sample plot	Moisture %	pH Soil : Water 1 : 2.5	Texture				SOIL INTERPRETATION OF RESULTS	
				Sand %	Silt %	Clay %	Total %	pH Soil : Water 1 : 2.5	Texture
	မြေနမူနာ								
1	Soil-1	1.32	4.13	26.30	63.90	8.30	98.50	Extremely acid	Silt Loam
2	Soil-2	2.99	3.60	74.50	20.10	4.30	98.90	Extremely acid	Loamy Sand
3	Soil-3	1.84	3.55	59.75	21.15	17.45	98.35	Extremely acid	Sandy Loam
4	Soil-4	2.48	3.67	52.55	39.50	6.90	98.95	Extremely acid	Sandy Loam
5	Soil-5	1.26	4.11	61.10	18.30	19.35	98.75	Extremely acid	Sandy Loam
6	Soil-6	1.13	4.11	57.80	17.40	23.75	98.95	Extremely acid	Sandy Clay Loam
7	Soil-7	2.91	4.12	44.90	30.70	23.25	98.85	Extremely acid	Loam
8	Soil-8	0.91	3.42	73.80	18.35	6.70	98.85	Extremely acid	Sandy Loam
9	Soil-9	0.72	4.51	75.50	1.10	22.20	98.80	Strongly acid	Sandy Clay Loam
10	Soil-10	3.45	4.10	51.50	38.30	9.10	98.90	Extremely acid	Loam

စိုင်ငံမော
ဒု-ညွှန်ကြားရေးမှူး
မြေဆီလွှာဗေဒလုပ်ငန်း

DEPARTMENT OF AGRICULTURE (LAND USE)
SOIL ANALYTICAL DATA SHEET

Division - မွန်ပြည်နယ်
Township - သီလဝကျွန်း

ခေါ်မြေဝင်းမင်းမျိုး (၁.၁၂.၂၀၁၇)

Sheet No. 2
Sr No. S 11-20 / 17-18

Sr No.	Sample plot	Moisture %	pH Soil : Water 1 : 2.5	Texture				SOIL INTERPRETATION OF RESULTS	
				Sand %	Silt %	Clay %	Total %	pH Soil : Water 1 : 2.5	Texture
	မြေနမူနာ								
11	Soil - 11	1.36	3.92	71.90	22.50	4.50	98.90	Extremely acid	Sandy Loam
12	Soil - 12	3.85	4.74	56.15	33.65	8.55	98.35	Strongly acid	Sandy Loam
13	Soil - 13	1.64	4.77	37.05	38.30	22.70	98.05	Strongly acid	Loam
14	Soil - 14	1.02	4.52	54.15	36.45	7.90	98.50	Strongly acid	Sandy Loam
15	Soil - 15	2.22	4.48	13.00	71.75	14.15	98.90	Extremely acid	Silt Loam
16	Soil - 16	1.99	3.68	78.95	18.35	1.00	98.30	Extremely acid	Loamy Sand
17	Soil - 17	0.96	3.93	75.50	12.90	9.95	98.35	Extremely acid	Sandy Loam
18	Soil - 18	1.75	3.89	72.30	24.10	2.30	98.70	Extremely acid	Sandy Loam
19	Soil - 19	1.51	4.74	58.05	17.50	22.85	98.40	Strongly acid	Sandy Clay Loam
20	Soil - 20	1.53	4.04	44.55	48.40	5.35	98.30	Extremely acid	Sandy Loam

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