Isolation of Fungi from Soil Samples and Preliminary Study of Antimicrobial Activities

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Abstract

In the course of investigation for antimicrobial metabolite, five different soil samples were collected from five different places in Pathein Industrial Zone area. After the collection of three days, five different soil samples were isolated by physical treatment serial dilution method and chemical treatment dilution method. After the isolation of 7 days their morphologies were studied. According to this study, 12 different fungi were isolated. However, fungus MS-03 showed more highly selective antibacterial activity against *E. coli*. Therefore, this strain MS-03 was selected for further investigations. This fungus MS-03 was isolated from sandy loam soil. In the fermentation studies for the antibacterial metabolite, it was found that 72 hrs ages of inoculum and 15% sizes of inoculum were suitable for the fermentation. The fermentation was performed using fermentation medium FM-3 a suitable of PH-5 at room temperature for 7 days. Maximum activity (37mm) reached at 5 days fermentation period with 72 hrs of ages and 15% of sizes of inoculum.

Keywords : isolation of fungi, antibacterial activities

Introduction

The term soil refers to the outer loose material of the earth crust. It may be regarded as a three phase's system which composes of solids, liquids and gases. On the whole the soil is composed of five major components, these include: Mineral matter, Water, Organic matter, Air and Living Organisms. Living portion of the soil body includes small animals and microorganisms but it is generally considered that its microorganisms play the most important role in the release of nutrient and carbondioxide for plant growth.Fungi are abundant in soil. These are important in the soil food sources for other, larger organisms, pathogens, beneficial symbiotic relationships with plants or other organisms and soil health. Soil is the largest source of microorganisms numerous varieties of microorganisms are living on earth and are deeply involved with human life. They are immensely diverse with respect to their habitats, material production and so on. Taxonomic identification of the soil fungi and fermentation conditions will be studied for further investigation (Manoch, 2004)

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Table 1 Soil samples collected at different places (Pathein Industrial Zone area)

Soil Sample No.	Collected place	pH	Location	Soil type
S 1	From the compound of the needle work factory.	5.82	N 16º 47.755' E 94º 45.759'	Sandy loam
S 2	Beside the sewing factory where the waste is placed	4.17	N 16º 47.313' E 94º 46.009'	Sandy clay loam
S 3	From the factory of a North Shore Group	4.62	N 16º 47.331' E 94º 46.667'	Clay loam
S 4	Before the factory of Donglong (Pathein) garment co.ltd.	6.10	N 16º 47.147' E 94º 46.188'	Sandy loam
S 5	Beside 'Myanmar Ah Hla Company' where the factory is still constructing	5.36	N 16º 47.243' E 94º 46.235'	Clay

Materials and Methods

Isolation of soil fungi by direct plate method

The collected soil samples were air-dried at room temperature for 3 days. The soil sample was ground and sieved in 2 mm screen.0.5 g of the sieved soil was cultured on the plates containing PGA medium, incubated for 1-7 days at room temperature. After incubation about 3 days, the colonies of fungi were observed.Then, re-cultured into sterilized petridish to get pure culture, finally pure strains were transferred into the test tube for further use.

Physical treatment serial dilution method

The collected soil samples were air-dried at room temperature for 3 days. The soil sample was ground and sieved in 2 mm screen.1 g of the sieved soil was suspended in 100 ml of sterile water and heated for 1 hour. The sample was vigorously agitated and then supernatant was diluted.

This dilution series were cultured on LCA medium and incubated for 5-7 days at room temperature.Single colonies from the plates were picked and purified by re-streaking.The pure strains were maintained in agar culture in test tube.

Chemical treatment serial dilution method

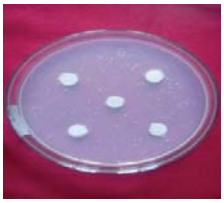
Culture in test tube.

Screening (or) Preliminary study for antimicrobial activities by paper disc diffusion assay (Tomita, 1988)

The collected soil samples were air-dried at room temperature for 3 days. The soil sample was ground and sieved in 2 mm screen.2 g of the sieved soil was then put into the test tube 4 ml of sterilized distilled water put into the tube containing soil, and settle for 6-hours to germinate early germinating soil fungi.14

ml of 70% ethanol solution was then added into the tube containing soil suspension and shaken for 1 minute and diluted with sterile water. 50 ml of soil suspension was cultured on plates containing LCA medium and then incubated for 5-7 days at room temperature. Single colonies from the plates were picked and purified by re-streaking. The pure strains were maintained in agar.

7 days old culture



The isolated fungi were grown at room temperature for 7 days on PGA medium for sporulation.

20 µL of fermented broth was put on paper disc and placed on assay plate containing test organisms

100 ml conical flask containing 50 ml seed medium

The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days.

Five ml of seed culture was transferred into the fermented medium and incubated at room temperature for 7 days.

Figure 1 Procedure of antimicrobial activity test

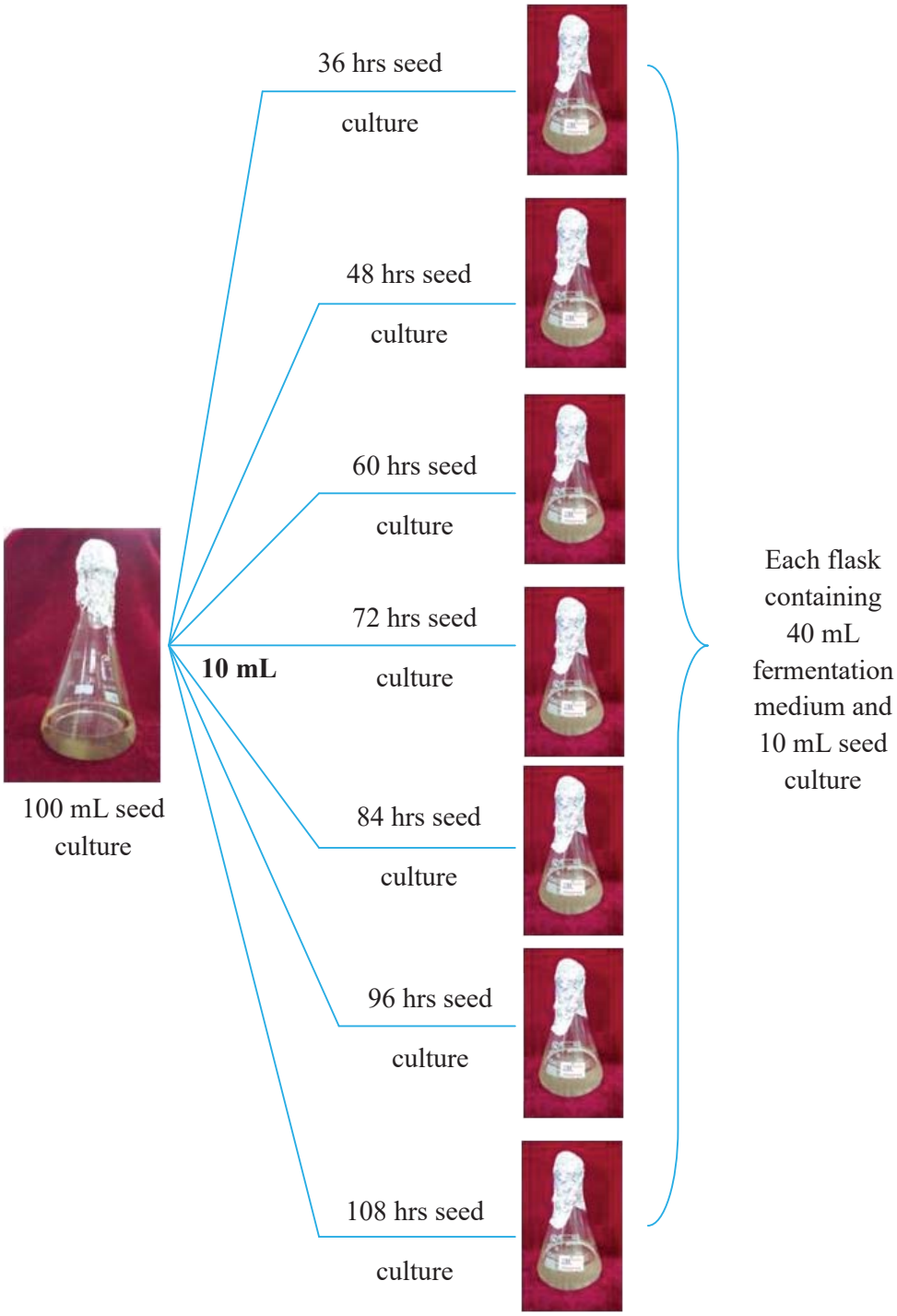


Figure 2 Procedure for the study on the effects of age of seed culture on the fermentation

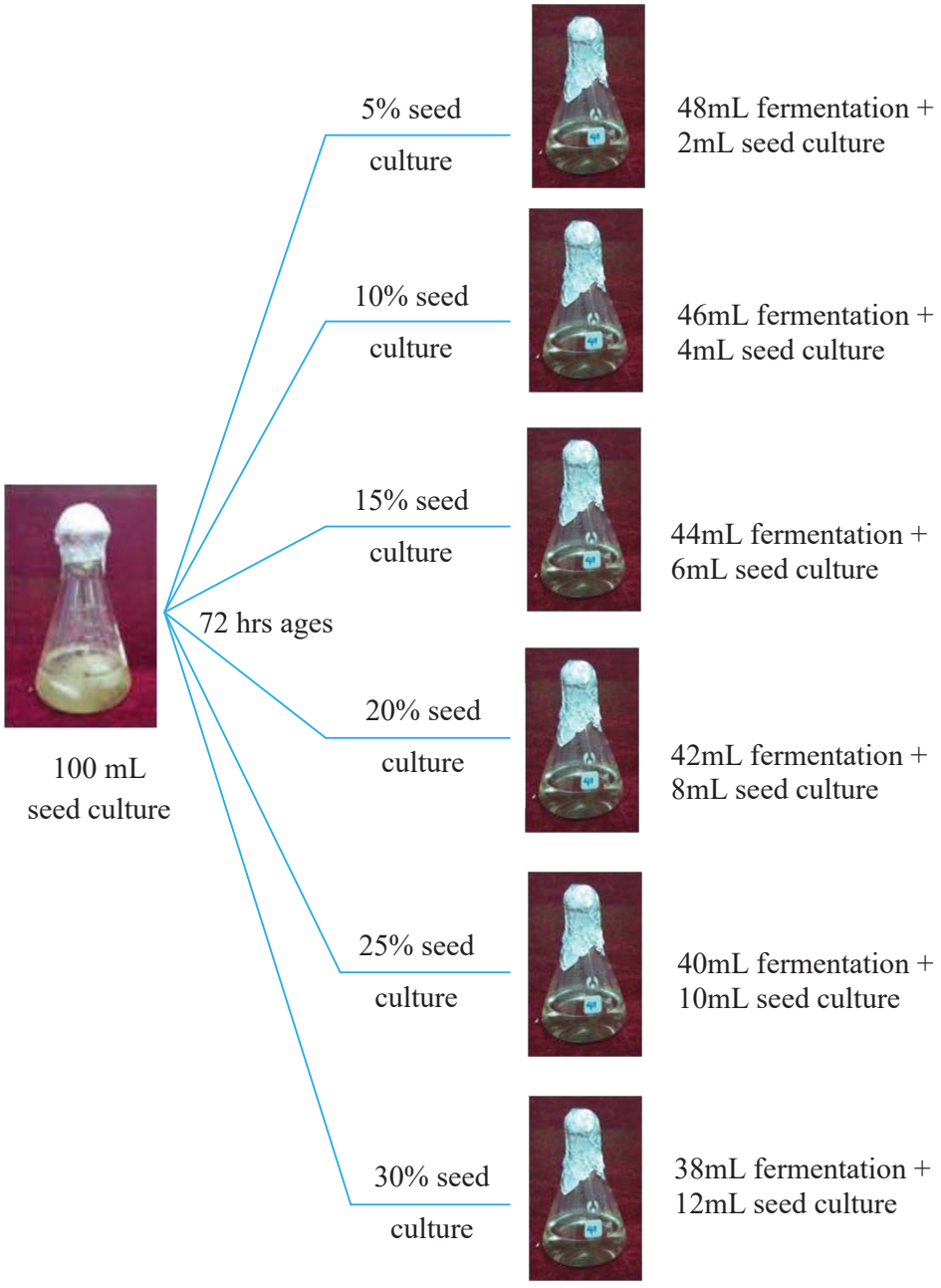


Figure 3 Procedure for the study on the effect of size of inoculum on the fermentation

Table 2 Test organisms used in antimicrobial activity

No.	Test organisms	Diseases
1	*Agrobacterium tumefaciens*	Grown gall disease
2	*Bacillus subtilis*	Fever, Pathogenic group, anthrax in man and animals
3	*Salmonella typhi*	Typhoid fever and food poisoning
4	*Candida albicans*	Fungal spp. Pathogenic, skin infections, vaginal candidiasis, cardiac infection, sores ringworm
5	*Aspergillus paraciticus*	Fruits disease
6	*Micrococcus luteus*	Skin disease
7	*Escherichia coli*	Diarrhoea
8	*Pseudomonas fluorescens*	Rice disease
9	*Saccharomyces cerevisiae*	Food spoilage
10	*Staphylococcus aureus*	Boils, abscesses, wound sepsis, burn food poison, pneumonia.

Results

Isolation of fungi from soil samples

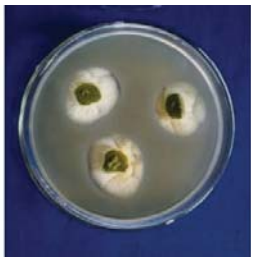
In this study, five different soil samples were processed for the isolation of soil fungi. A total of 12 soil fungi were isolated.

Table 3 Isolated fungi from different soil samples.

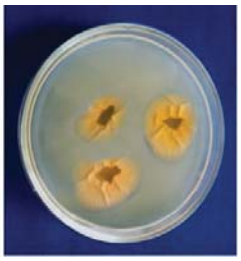
Soil Samples No.	Numbers of Isolated Fungi	
S_1	4	MS-01, MS-02, MS-03
S_2	2	MS-04, MS-05
S_3	7	MS-06, MS-07, MS-08
S_4	1	MS-09, MS-10
S_5	1	MS-11, MS-12

Table 4 Morphological Characters of isolated fungal strains

No	Fungi	Cultural Character	
		Surface color	Reverse color
1	MS-01	Dark green in centre and edge white	Yellow
2	MS-02	White	Brown in centre and edge white
3	MS-03	Pale-yellow	Yellow
4	MS-04	Green in centre and edge white	White
5	MS-05	Green in centre and edge white	Yellow
6	MS-06	Green in centre and edge white	Yellow
7	MS-07	Brown and centre and edge white	Pale-yellow
8	MS-08	Pale	Yellow
9	MS-09	Gray	Pale-yellow
10	MS-10	White	Yellow
11	MS-11	Pale brown	Yellow
12	MS-12	Green	Yellow



Front view
(5 days old culture)



Reverse view
(5 days old culture)

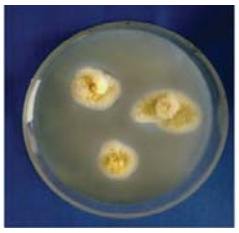
Front view
(5 days old culture)

Reverse view
(5 days old culture)

Figure 4 Morphology of isolated soil fungi MS-01 and MS-02

Front view
(5 days old culture)

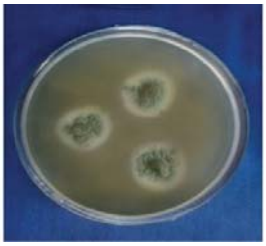
Reverse view
(5 days old culture)



Front view
(5 days old culture)

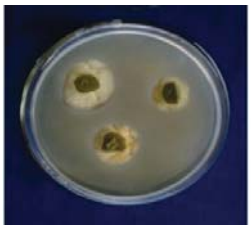
Reverse view
(5 days old culture)

Figure 5 Morphology of isolated soil fungi MS-03 and MS-04



Front view
(5 days old culture)

Reverse view
(5 days old culture)

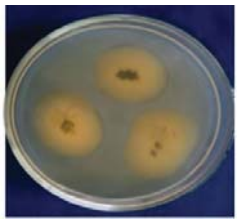


Front view
(5 days old culture)

Reverse view
(5 days old culture)

Figure 6 Morphology of isolated soil fungi MS-05and MS-6

Front view
(5 days old culture)



Reverse view
(5 days old culture)

Front view
(5 days old culture)

Reverse view
(5 days old culture)

Figure 7 Morphology of isolated soil fungi MS-7 and MS-8

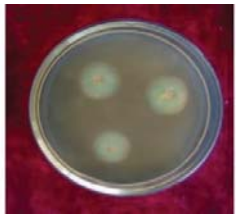
Front view
(5 days old culture)

Reverse view
(5 days old culture)

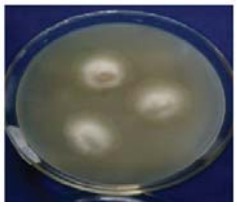
Front view
(5 days old culture)

Reverse view
(5 days old culture)

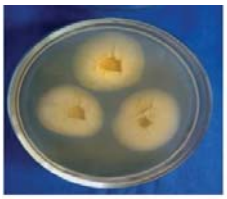
Figure 8 Morphology of isolated soil fungi MS-9 and MS-10



Front view
(5 days old culture)



Reverse view
(5 days old culture)



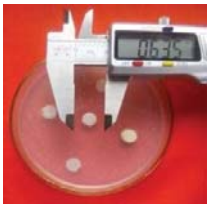
Front view
(5 days old culture)

Reverse view
(5 days old culture)

Figure 9 Morphology of isolated soil fungi MS-11 and MS-12

Table 5 Antimicrobial activities of isolated fungi

Isolated fungi	*Agrobacterium tumefaciens*	*Bacillus subtilis*	*Salmonella typhi*	*Candida albicans*	*Aspergillus paraciticus*	*Micrococcus luteus*	*Escherichia coli*	*Pseudomonas fluorescens*	*Saccharomyces cerevisiae*	*Straphylococcus aureus*
MS-01	-	-	-	-	-	-	-	-	-	-
MS-02	-	-	-	-	-	-	-	-	-	-
MS-03	16.12	23.13	15.40	19.15	13.84	-	25.80	-	-	24.06
MS-04	-	-	-	-	-	-	-	-	-	-
MS-05	-	-	-	-	-	-	-	-	-	-
MS-06	-	-	-	-	-	-	-	-	-	-
MS-07	-	-	-	-	-	-	-	-	-	-
MS-08	-	-	-	-	-	-	-	-	-	-
MS-09	13.84	-	-	13.67	-	14.50	-	16.84	-	-
MS-10	-	-	-	-	-	-	-	-	-	-
MS-11	15.78	-	15.10	15.70	-	14.48	-	11.93	-	-
MS-12	-	-	-	-	-	-	-	-	-	-



Agrobacterium tumefaciens *Bacillus subtilis* *Salmonella typhi*

Figure 10 Antimicrobial activity of isolated fungi (MS-03) against *Agrobacterium tumefaciens, Bacillus subtilis* and *Salmonella typhi*

Candida albicans

Aspergillus paraciticus

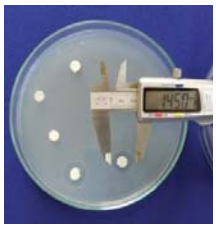
Escherichia coli

Staphylococcus aureus

Figure 11 Antimicrobial activity of isolated fungi (MS-03) against *Candida albicans, Aspergillus paraciticus, Escherichia coli,* and *Straphylococcus aureus*

Agrobacterium tumefaciens

Candida albicans



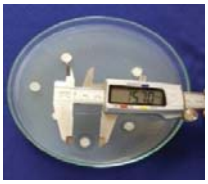
Micrococcus luteus

Pseudomonas fluorescens

Figure 12 Antimicrobial activity of isolated fungi (MS-09) against *Agrobacterium tumefaciens, Candida albicans, Micrococcus luteus* and *Pseudomonas fluorescens*

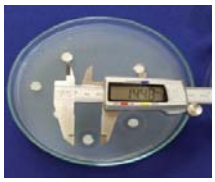
Agrobacterium tumefaciens

Salmonella typhi



Candida albicans

Figure 13 Antimicrobial activity of isolated fungi (MS-11) against *Agrobacterium tumefaciens, Salmonella typhi,* and *Candida albicans*



Micrococcus luteus

Pseudomonas fluorescens

Figure 14 Antimicrobial activity of isolated fungi (MS-11) against *Micrococcus luteus* and *Pseudomonas fluorescens*

Ages and Size of Inoculum for fermentation

In this investigation of the age of inoculum twelve different hours of 60, 72, 84, 96, and 108 hrs were for the fermentation. According to this result 72 hrs ages of seed culture was suitable for the fermentation.For the study of the size of inoculum, 5%, 10%, 15%, 20% and 25%, six different percentages were used and 15% inoculum concentration was the best for the fermentation. The size of inoculum isolated soil fungi was 15% most suitable for 72 hrs age of culture isolated soil fungi.

Table 6 Effects of Age of culture for fermentation (Size of Agar well 8 mm)

Seed culture (Time, hrs)	Activity (Clear zone, mm)
36	26.99
48	28.73
60	29.35
72	30.54
84	30.08
96	28.79
108	26.75

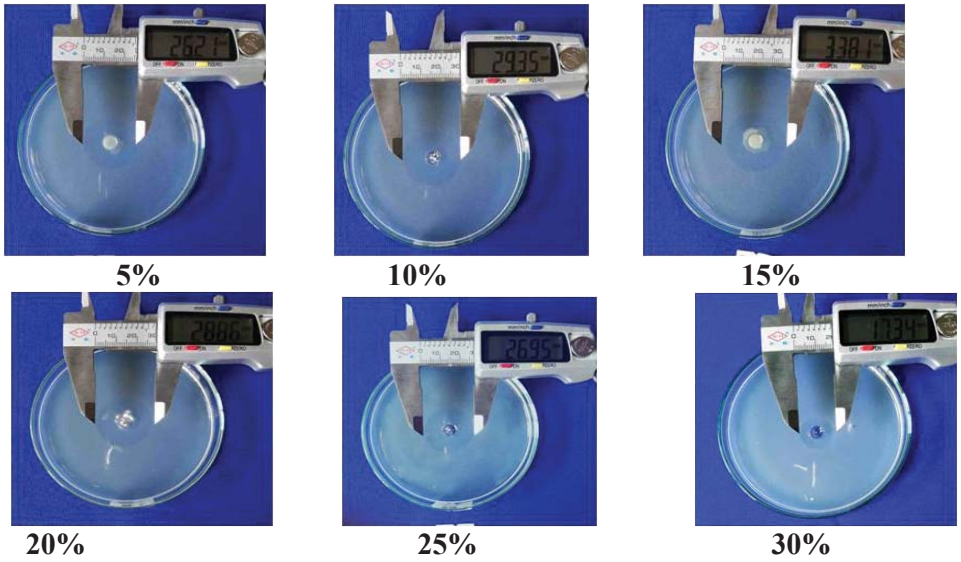
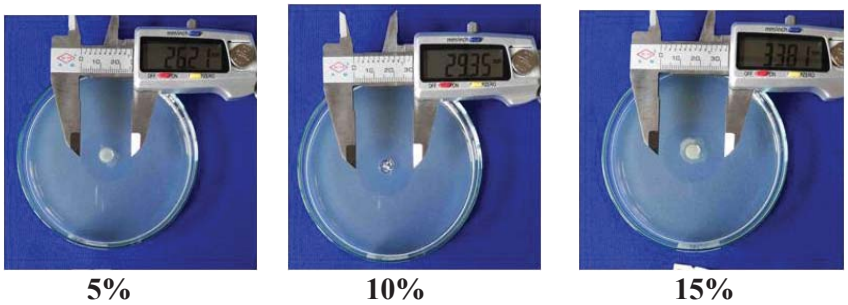
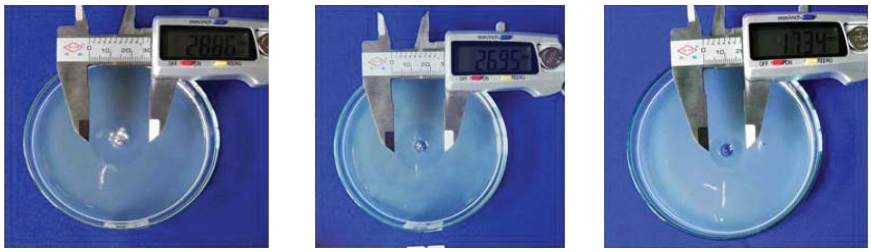


Figure 15 The effects of age of inoculums on the fermentation (MS-03) against *Escherichia coli*

Table 7 Effects of Size of inoculum for fermentation (Size of Agar well 8 mm)

Seed of inoculum	Activity (Clear zone, mm)
5%	26.21
10%	29.35
15%	33.81
20%	28.86
25%	26.95
30%	17.34





20% 25% 30%

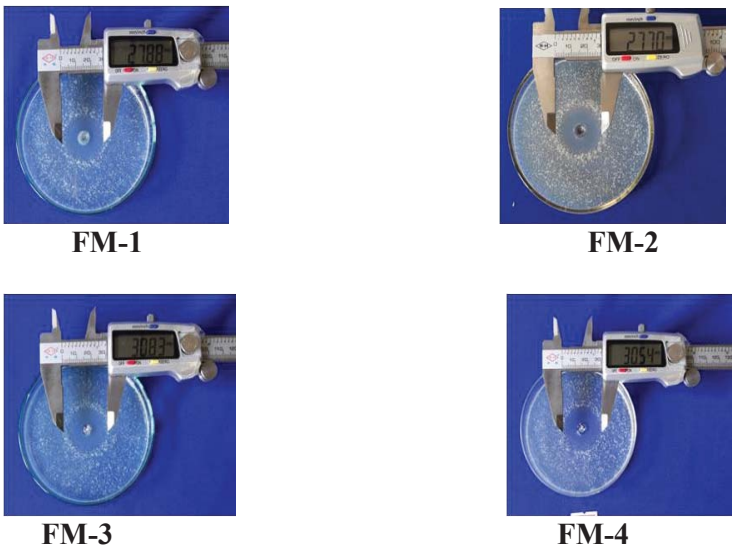
Figure 16 The effects of size of inoculums on the fermentation(MS-03) against *Escherichia coli*

Fermentation Condition for the production of Antibacterial compound by the Fungus MS-03 against *E. coli*.

In the study, four kinds of fermentation media are used. According to the results of antibacterial activity, fermentation medium FM-1 showed the inhibitory zone of 22.12 mm, FM-2 showed the inhibitory zone of 40.61 mm, FM-3 showed the inhibitory zone of 21.28 mm, and FM-4 showed the inhibitory zone of 28.47 mm. Therefore, FM-2 medium was selected for fermentation to produce antibacterial compound.

Table 8 The effect on media on the fermentation (size of agar well 8 mm)

Medium	Activity (Clear zone, mm)
FM-1	27.88
FM-2	27.70
FM-3	30.83
FM-4	30.54



FM-1 FM-2

FM-3 FM-4

Figure 17 The effects of fermentation condition for the production of metabolite

Discussion and Conclusions

In the investigation for isolation of soil fungi, 25 kinds of soil fungi were isolated from five different places of soil samples at Pathein Industrial Zone Area. In the preliminary study of antimicrobial activity, it was observed that fungi MS-03, showed the antimicrobial activity against on *Agrobacterium tumefaciens, Bacillus subtilis, Salmonella typi, Candida albicans, Aspergillus paraciticus* and *Escherichia coli, Straphylococcus aureus.* Fungi MS-09 showed the antimicrobial activity against on *Agrobacterium tumefaciens, Candida albicans, Micrococcus luteus* and *Pseudomonas fluorescens;* fungi MS-11 showed the antimicrobial activity against on *Agrobacterium tumefaciens, Candida albicans, Salmonella typhi, Micrococcus luteus* and *Pseudomonas.* Fungi isolated having potential of producing antimicrobial metabolites will be repeatedly tested against the test organism in order to confirm the antimicrobial activity. Age of culture and size of inoculum were studied for fermentation to produce the antibacterial compound against *Escherichia coli*, it was observed that 72 hours age of culture and 15% of size of seed culture were suitable for the fermentation.Optimal fermentation condition such as proper age and size of inoculum are very important for the production of metabolites (Omura 1985). Therefore, fermentation condition studies were carried out. Fermentation undertaken with suitable condition of 72 hrs age and 15% of inoculums with four different media. Fermentation medium FM-3 is the best medium. In the study of effect of pH on the fermentation, pH-5.0 is the best for the production of metabolite. The soil fungi will be the investigation for potential bioactive compounds, in future studies.

Acknowledgements

I would like to express my sincere thanks to Dr Aung Kyaw, Rector of Maubin University, for his permission to perform this paper. I would like to express my deepest appreciation and special thanks to Pro-rector. Dr Win Myint Thein and Dr Lwin Lwin Myint, Maubin University, for their kind permission to submit this paper. I am greatly thankful to express my sincere gratitude to professor Dr Mar Mar Aye, Head of the Department of Botany, Maubin University for her suggestion. I wish to express my sincere gratitude to Professor Dr Hla Hla Win, Department of Botany Maubin University for her encouragement and advice.

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