Screening of Antibacterial Activity Possessing Soil Fungi against *Agrobacterium tumefaciens*

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Abstract

In the isolation of fungi, ten fungi were isolated from three different kinds of soil samples at three different places. Three kinds of soil samples were collected from Pathein Chaungtha Roadside, in December, 2018. The isolation of fungi was undertaken by serial dilution method. Ten fungal strains were obtained from these soil samples. The morphological characters of isolated soil fungi were studied. In the investigation of antimicrobial activities of isolated soil fungi, *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Candida albicans* and *Agrobacterium tumefaciens* were used for the test throughout the research studies. Among them, one strain showed weak activity; three strains exhibited good activity and one strain (TPF-07) showed more highly antibacterial activity against *Agrobacterium tumefaciens.* This isolated soil fungus TPF-07 was selected for further investigation. In the fermentation parameters of selected soil fungus TPF-07, one day (24 hrs) seed culture and 20% size of inoculum were optimized and maximum activity reached at 4 days fermentation period.

Key words: isolation of fungi, antibacterial activities, fermentation period

Introduction

Microorganisms have significant function in ecosystem and are found in all kinds of habitats. There will be an increasing demand for microorganisms with unusual properties (Kurtzman, 1992; Subramanian, 1992). Microorganisms are a virtually unlimited source of novel chemical structures with many potential therapeutic applications (Behal, 2000).

Soils are the naturally occurring physical covering of the earth's surface, and represent the interface of three material states: solids (geological and dead biological materials), liquids (water), and gases (air in soil pores). Each soil is a unique product of the combination of geological parent material, glacial and geomorphologic history, the presence and activity of biota, and the history of land use and disturbance regimes. Soils are the foundation of all terrestrial ecosystems and are home to a vast diversity of bacteria, archaea, fungi, insects, annelids, and other invertebrates as well as plants and algae. These soil dwellers provide food or nutrients that support organisms that live above and below ground. Soils also play critical roles in buffering and filtering freshwater ecosystems. Consequently, soils are extremely important to human societies (Dominati *et al*. 2010)

Antibiotic is a medicinal substance used to treat infections caused by microbes that can cause disease to higher level of eukaryotic organisms. Antibiotic functions to break down the bacterial cells that cause certain diseases because antigenic substances are present on the cell wall of bacteria. Microorganisms are the most potential sources for production of natural therapeutic agents (Pannapa 2017).

For the human health and nutrition fungi are well known to produce both beneficial and deleterious natural agents and continue to be explored as useful sources of natural antimicrobial agents. In comparison to plants, microorganisms are highly diverse but narrowly explored. Till date, only about 1% of bacteria and 5% of fungi have been characterized while rest remains unexplored for their contribution to the welfare (Chioma *et al* 2016). In recent years, the isolation of fungi from soil and screening of antimicrobial activity has gained more attention. Present study worked on isolation of fungi having

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antibacterial activity of soil fungi from Pathein Chaungtha Roadside. This soil fungus has high activity against *Agrobacterium tumefaciens*. The aim and objectives are to isolate the soil fungi from different places, to investigate the antibacterial activities of soil fungi against *Agrobacterium tumefaciens* and to study the various fermentation conditions of selected soil fungus.

Materials and Methods

Isolation of fungi from soil samples

Three different soil samples were collected at Pathein Chaungtha Roadside (Figure 1) and (Table 1). The isolation of soil fungi were undertaken by serial dilution method.

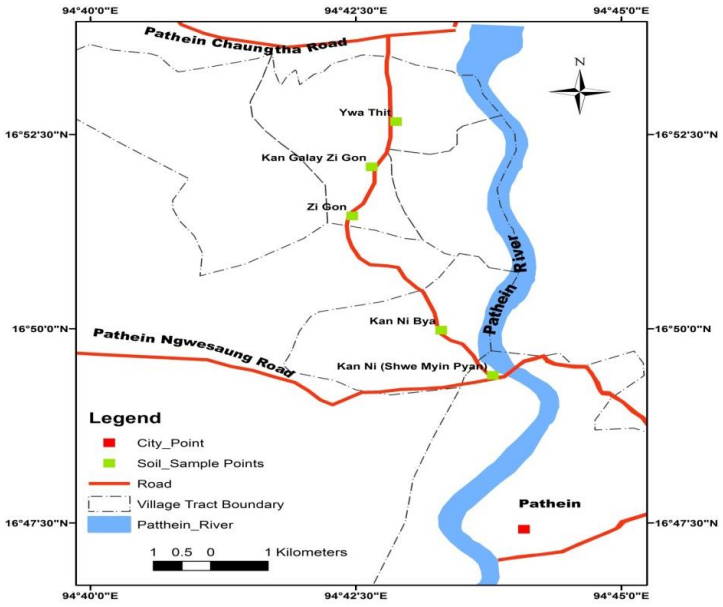


Fig (1) Location Map of Pathein-Chaungtha Roadside

Table (1) Three different soil samples collected at three different places

Soil sample No.	pH	Soil type	Collected date	Collected place
S -1	3.71	Sandy loam	8.12.2018	N 16° 49' 24" E 94º 43' 47.28" Kan Ni (Shwe Myin Pyan)
S-2	4.82	Clay Loam	8.12.2018	N 16º 49' 58.84" E 94º 43' 18.16" Kan Ni Bya
S-3	4.41	Sandy loam	15.12.2018	N 16º 51' 27.06" E 94º 42' 27.83" Zi Gon

Antimicrobial activities by paper disc diffusion assay (NITE 2005)

1. The isolated microorganisms were grown at room temperature for 5 days on culture medium.
2. The isolated microorganisms were inoculated on seed medium and incubated at 25°C for three days.
3. 5 mL of seed culture was transferred into the fermentation medium and incubated at 25°C for 1 to 10 days.
4. 20 mL of fermented broth was put on paper disc and placed on assay plate containing test organisms.

Table (2) Test organisms used in antibacterial activities

No.	Test organisms	Infections
1.	*Agrobacterium tumefaciens*	Plant disease, crown gall disease and hairy root disease
2.	*Bacillus subtilis*	Fever, tissue necrosis
3.	*Candida albicans*	Candidiasis
4.	*Escherichia coli*	Diarrhoea, urinary tract infections
5.	*Micrococcus luteus*	Skin disease, pneumonia, urinary tract infections,

Studies on microbial growth kinetics of fungus TPF-07

This study was carried out by the method of Omura, 1985 and Crueger and Crueger, 1989. The fungus TPF-07 was inoculated into the medium (Distilled water 100 mL Glucose 2.0 g, Yeast extract 0.3 g, Sucrose 0.3 g, KNO_3 0.1 g, K_2HPO_4 0.01 g, pH 6.5) and incubated for three days. The culture sample 10 mL was checked in 12 hrs intervals for the growth.

Study on the effects of size of inoculums for the fermentation

In this study, 5%, 10%, 15%, 20%, 25% and 30% of one day seed culture were utilized for the fermentation. Fermentation was carried out for 9 days and antibacterial activity was tested by paper disc diffusion assay.

Morphological, photomicrograph and distinctive characters of TPF-07

For the study of morphology and macroscopical characters, fungus TPF-07was cultured at room temperature on Potato Glucose Agar (PGA) medium and Low Carbon Agar (LCA) medium. Then, these plates were incubated at room temperature for 3 to 7 days. Colony forms, surfaces and reverse pigments of isolated selected fungi were studied for morphology at Microbiology Lab, Department of Botany, Pathein University. Photomicrograph were investigated by using high magnification of microscope at Botany Department, Pathein University.

Results

In the course of the isolation for antimicrobial metabolite producing microorganisms, 10 fungi were isolated from five different kinds of soil samples (Table-3).

Table (3) Isolated fungi from three different soil samples by serial dilution method

No. of Soil samples	Isolation method	Isolated fungi
S-1	Serial dilution method	TPF-01, TPF-02, TPF-03
S-2	Serial dilution method	TPF-04, TPF-05, TPF-06
S-3	Serial dilution method	TPF-07, TPF-08, TPF-09, TPF-10
Total		**10**

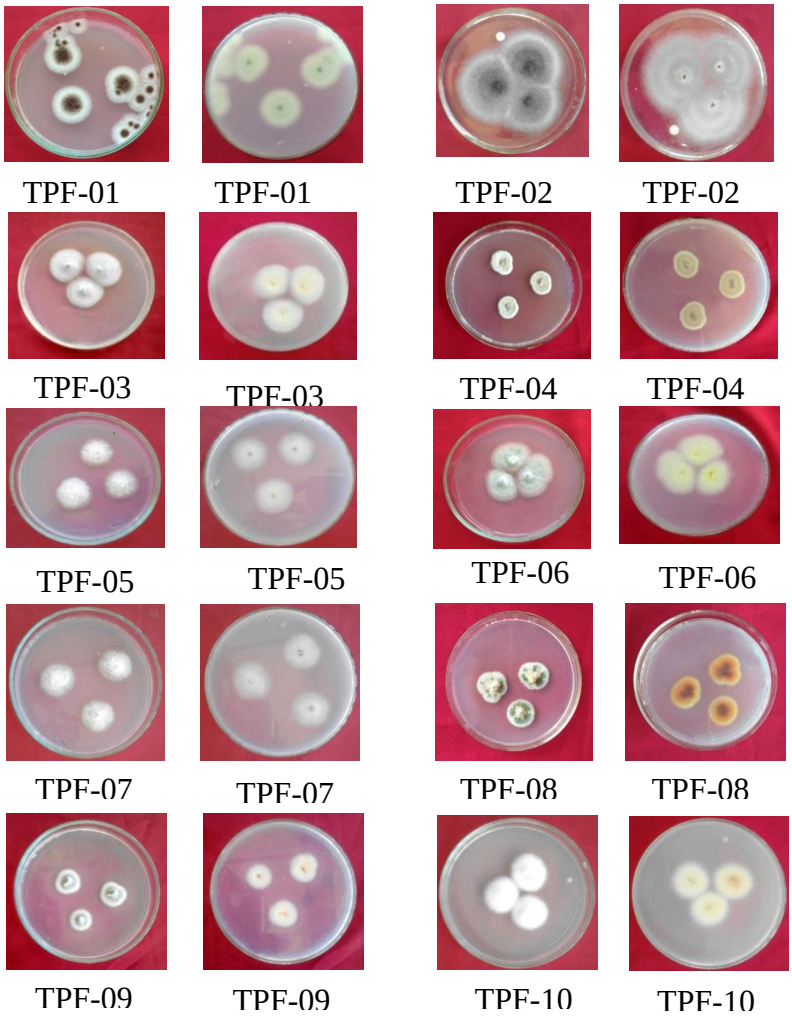


Fig (2) Morphologies of isolated fungi (TPF-01 to TPF-10) on PGA media

Study of antimicrobial activities by paper disc diffusion assay

In the course of the investigation of fungi, 10-fungi were isolated from 3 different soil samples collected at Pathein-Chaungtha Roadside. During the study of antimicrobial activities of these fungi, one isolated fungus (TPF-01) showed weak activity, four isolated fungi (TPF-07, 08, 09 and 10) against good activities, among them two strains TPF-07 and TPF-10 exhibited antibacterial activities against *Agrobacterium tumefaciens*.

Among them, fungus TPF-07 showed more highly selective antimicrobial activity against *Agrobacterium tumefaciens* than other isolated fungi. Therefore, this strain TPF-07 was selected for further investigations such as fermentation for the growth kinetics of antibacterial metabolites.

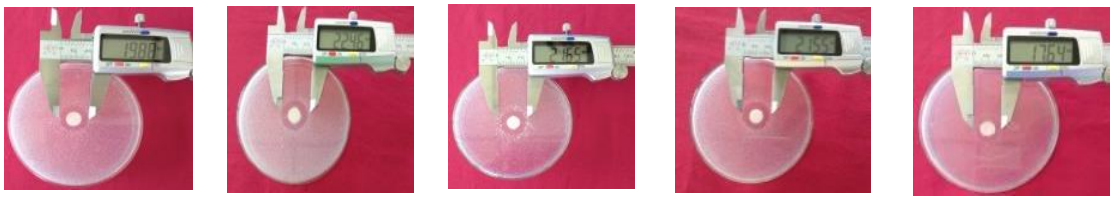
Study on the effects of age of seed culture on the fermentation

It was found that growth phase was between 12 hrs and 60 hrs. According to Crueger and Crueger (1989), age of inoculums (12, 24, 36, 48 and 60 hrs) with 20 % sizes of inoculum were utilized for the fermentation. Based on the results (Table 4 and Figure 3) 24 hrs age of seed culture was selected for fermentation, highly activity reached at 4 days period against *Agrobacterium tumefaciens*.

Table (4) Effects of age of seed culture on the fermentation by soil fungus TPF-07

Culture time (hr)	Activity (clear zone, mm)
12	19.88
24	**22.46**
36	21.65
48	21.55
60	17.64

Test organism was *Agrobacterium tumefaciens*



12 hrs | 24 hrs | 36 hrs | 48 hrs | 60 hrs

Fig (3) Study on the effects of age of inoculum for fermentation

Study on the effects of size of inoculum on the fermentation

Based on the results of (Table 5) and (Figure 4), 20% size of inoculum was selected for fermentation, highly activity reached at 4 days period against *Agrobacterium tumefaciens.*

Table (5) Effects of size of inoculum on the fermentation by soil fungus TPF-07

Size (%)	Activity (clear zone, mm)
5	19.25
10	23.42
15	24.63
20	**30.80**
25	24.07
30	22.97

Test organism was *Agrobacterium tumefaciens*

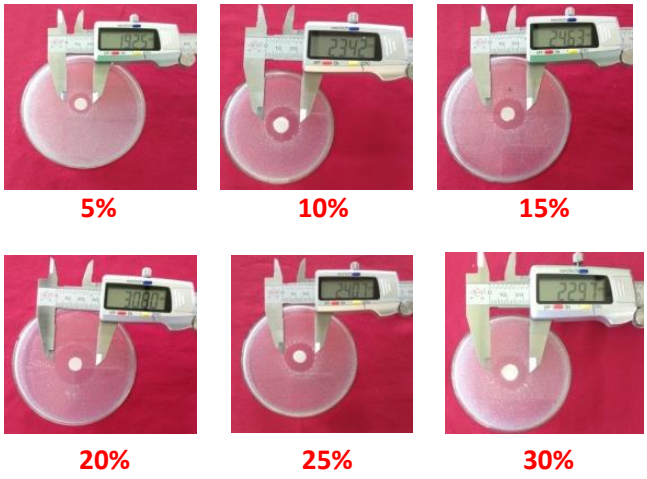
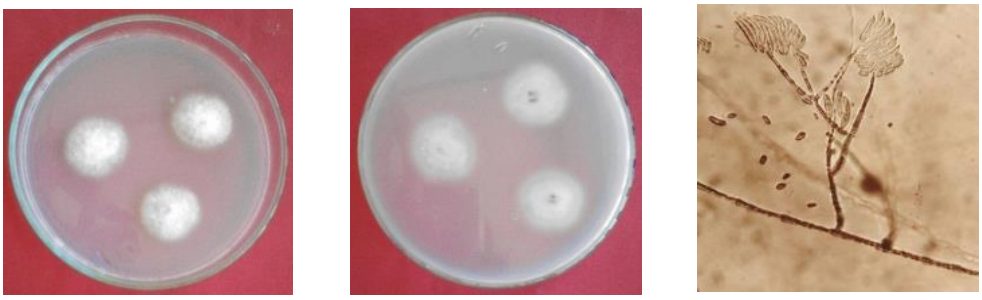


Fig (4) Study on the effects of size of inoculum on the fermentation

Morphological, photomicrograph and distinctive characters of TPF-07

The surface colour and reverse colour of TPF-07 was the same colour white. After 5 days of cultivation, it was observed that white colonies reach 1.5 cm diameter at room temperature on PGA medium. Distinctive character of soil fungus TPF-07 was found that conidium lacking septa(Ameroconidium), one celled, hyaline, fusiform, smooth, thallic type, conidiophores with septa, elongated and branched, pale brown in color (Figure 5).



Front View of TPF-07 Reverse View of TPF-07 Photomicrograph (X400)

Morphology on PGA medium (5 days old culture)

Fig (5) Morphology and Photomicrograph of TPF-07

Discussion and Conclusion

In the course of the investigation of fungi, 10 fungi were isolated from three different soil samples collected at Pathein - Chaungtha Roadside. According to Table 3, fungi TPF-01, 02 and TPF-03 were isolated from soil sample 1, fungi TPF-04, 05 and TPF-06 from soil sample 2, fungi TPF-07, 08, 09 and TPF-10 from soil sample 3.

During the study of antimicrobial activities of these fungi, four fungi (fungus TPF-07, TPF-08, TPF-09 and fungus TPF-10) exhibited antibacterial activities against *Agrobacterium tumefaciens.* Among them, fungus TPF-07 exhibited the more highly activity against *Agrobacterium tumefaciens* (16.09 mm clear zone) than other fungus.

In the investigation of fermentation for the antibacterial metabolite; it was found that growth phase was between one to three days. According to Crueger and Crueger, (1989), this growth phase time hours (12, 24, 36, 48 and 60 hrs) was employed for fermentation. In this experiment it was observed that one day (24hrs) age of seed culture was suitable for fermentation. In the study of size of inoculum (transfer of seed culture 5%, 10%, 15%, 20%, 25% and 30%), 20% concentration was the best for fermentation. The highest activity reached at 4 days fermentation with one day (24hrs) age and 20% size of inoculum (30.80 mm, clear zone).

In conclusion, it was found that there is high potential to discover useful antibiotics producing soil fungi from the study site with some possibly novel strains. Therefore, more research works can be conducted on this remote area in order to explore novel antibiotics and their producing microbes which were residing in it.

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References

Ando, K. (2004). Isolation and identification of fungi, Workshop , BDBRC-Pathein University,

Behal, V. 2000. Bioactive products from *Streptomyces*. Malaysian Journal of Microbiology 1, 1-6.

Chioma N, Njoku EN, Pharamat T. 2016. Antimicrobial activity of secondary metabolites of fungi isolated from leaves of bush mango. J Next Gener Seq Appl. 3(3): 1-6.

Crueger, W., and A. Crueger, (1989). Methods of fermentation, in Biotechnology, A Textbook of Industrial Microbiology, 64-74.

Dominati, E. M. Patterson, A. Macky, (2010). A framework for classifying and quantifying the natural capital and ecosystem services of soils Ecological Economic, 69, pp. 1858-1868.

Gallo, M.L., AM. Seldesand and G.M. Cabrera, (2004). Antibiotic long-chain α-unsaturated aldehydes from the sulture of the marine fungus *Caldosporium* sp. *Biochem Systemat. Ecol* 32: 554-559

Kurtzman, C. P. 1992. Impact of mycology on the needs of the 21th century, In Processing of the Asian Mycological Symposium, Oct 1-4, Seoul, Korea, 1-6.

Omura, S. (1985). Microbial growth kinetics and secondary metabolites, J . Fermentation Technology, 46: 134-140

Pannapa P, and Pattra S. (2017). Antimicrobial and enzyme activity produced by *Bacillus* sp. Isolated from soil. Int J Pharm Sci. 9 (3): 205-10.

Subramanian, C.V. 1992. Tropical mycology and biotechnology. Current Science 63,167-172.

NITE (2005). Medium for fermentation to produce the metabolites.