

ISOLATION AND FERMENTATION CONDITIONS OF SOIL FUNGUS NFO-9 FROM MANGROVE SOIL MAGYI COASTAL AREA

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Abstract

In the course of isolation of soil fungi, thirteen different kinds of fungi were isolated from five mangrove soil samples. These soil samples were collected at Magyi Channel. The isolation was undertaken by serial dilution method and using Low Carbon Agar medium (LCA medium). Potato Glucose Agar Medium (PGA medium) was utilized as transfer medium to get pure culture. Antimicrobial activities of these fungi were evaluated by agar well diffusion assay with five test organisms. Among them, eight fungi showed antimicrobial activity. Especially, NFO-9 gave the best antibacterial activity on *Pseudomonas fluorescens*. Therefore, different fermentation parameters of NFO-9 were studied by the fermentation period, age and size of inoculums, pH and fermentation medium on *Pseudomonas fluorescens*.

Keywords antibacterial activity, fermentation, soil fungi.

Introduction

Mangroves are coastal wetland forests established at the intertidal zones of estuaries, backwaters, deltas, creeks, lagoons, marshes and mudflats of tropical and subtropical latitudes. Mangrove forests are referred to as mangrove swamps, tidal forests, tidal swamp forests or mangals and are considered as transition zone between terrestrial and marine habitats. Approximately 25% of the world's coastline is dominated by mangroves distributed in 112 countries and territories encompassing an area of 181,000 sq km worldwide. Mangrove stands are located in sheltered places and the mouths of valleys in most cases. The mangrove floor varies from sandy to muddy.

Fungi from mangrove soil play an important role in the nutritive cycle and support the mangrove ecosystem. They commonly occur as saprophytes on decomposing organic matter such as wood, stem, leaf etc, and as symbiosis of plants and animals as parasites of plants in mangrove ecosystem. Fungi being universal organisms occur in all types of habitats and are the most adaptable organisms.

Marine fungi are found in all divisions of fungi and most probably evolved the association independently on many occasions. Mangrove derived microbes especially fungi have long been recognized as a potential source of novel and biologically potent metabolites (Suja *et al*, 2013; (Chioma *et al*, 2016; Sandhu *et al*, 2014). In recent years, the isolation of marine fungi and screening of antimicrobial activity has gained more attention (Styrobek, 2003).

The aims of this research were to isolate soil fungi from mangrove soil to study the isolation method and to investigate the effect of fermentation and pH of selected fungus on *Pseudomonas fluorescens*.

Materials and Methods

Material: The soil samples were collected from mangrove swamp soil of Magyi tidal creek, Shwe Thaung Yan sub-Township, Ayeyarwaddy Region. The isolation of fungi were carried out by serial dilution method (NITE, 2004).

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Method: Serial dilution method

Soil was air-dried at room temperature and then grounded and sieved. One gram of soil sample was suspended in 10 ml of distilled water and was shaken for 15 minutes. 0.1 ml of this dilution water was added to 5 ml of distilled water and shaken for 5 minutes. 0.5 ml of this dilute water (5 ml + 0.1 ml) was added to 4.5 ml of distilled water and shaken for 5 minutes. Then, 1.0 ml of dilute water (0.5 ml + 4.5 ml) was added to 4.0 ml of distilled water and shaken for 5 minutes. 0.1 ml of this solution was added to sterile agar medium for 3 days. After 3 days, the microorganisms were picked and purified by re-culture in glass plate containing PGA medium.

Preliminary study for antimicrobial activity

The isolated fungi were grown on PGA medium for 3 days. The isolated fungi were inoculated into 25 ml seed medium and incubated at room temperature for 3 days. After 3 days, 20 ml seed culture was transferred into the 80 ml of fermentation medium and incubated at room temperature. Fermentation was carried t for 3-10 days (Ando, 2004).

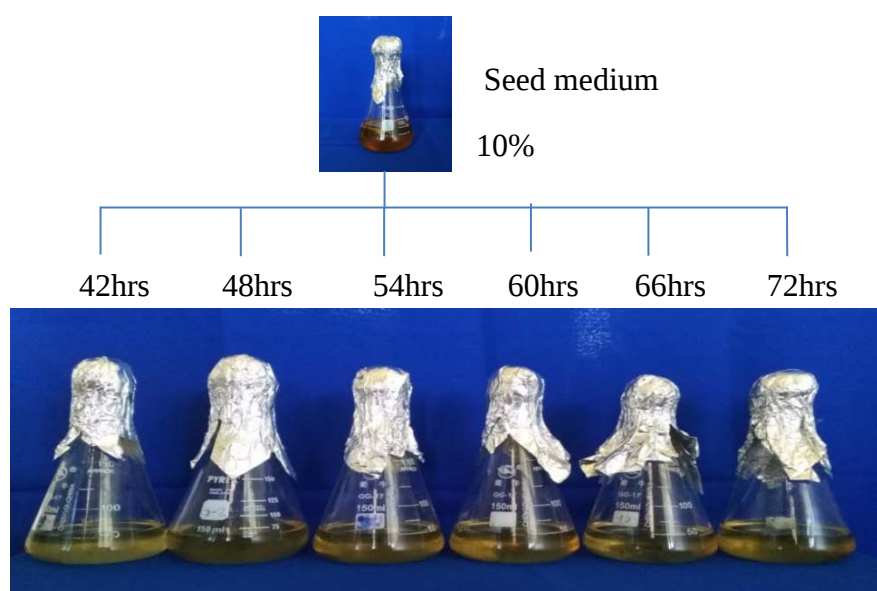


Figure 1 Procedure for the study on the effects of ages of seed culture

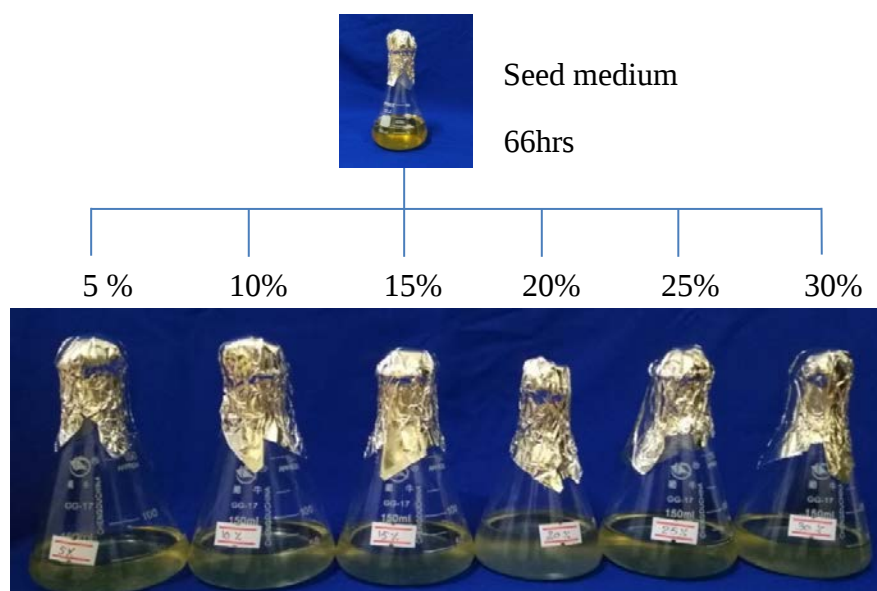


Figure 2 Procedure for the study on the effects of size of seed culture

The effect of pH on fermentation

Effects of different pH were used for antibacterial activity of pH 4, 5, 6 and 7. This different pH were adjusted by NaOH and HCL.

Study on the fermentation media of NFO-9

Fermentation was undertaken with suitable conditions of 25% sizes and 66 hrs ages of inoculum with five different media. Fermentation was carried out for 7 days and antibacterial activity test was carried out every 24 hrs.

A Medium Used for the isolation of soil fungi NITE (2004)

LCA Medium		PGA Medium	
Glucose	1.5 g	Potato	20g
Glycerol	1.0 mL	Glucose	1.5g
Yeast extract	0.8 g	Agar	1.8g
Polypeptone	0.4 g	DW	100 ml
K_2HPO_4	0.001g	pH	6.5
$MgSO_4 \cdot 7H_2O$	0.001g		
Agar	1.8g		
DW	100 mL		
pH	6.5		
Seed Medium		Fermentation Medium	
Glucose	1.5 g	Potato Dextrose Broth	3.9g
Glycerol	1.0 mL	Glycerol	1.2 ml
Yeast extract	0.8 g	Peptone	0.6 g
Polypeptone	0.4 g	$NaNO_3$	0.8g
K_2HPO_4	0.001g	$MgSO_4 \cdot 7H_2O$	0.001g
$MgSO_4 \cdot 7H_2O$	0.001g	K_2HPO_4	0.001g
DW	100 mL	DW	100 ml
pH	6.5	pH	6.5

(After autoclaving chloramphenicol was added to the medium.)

Result

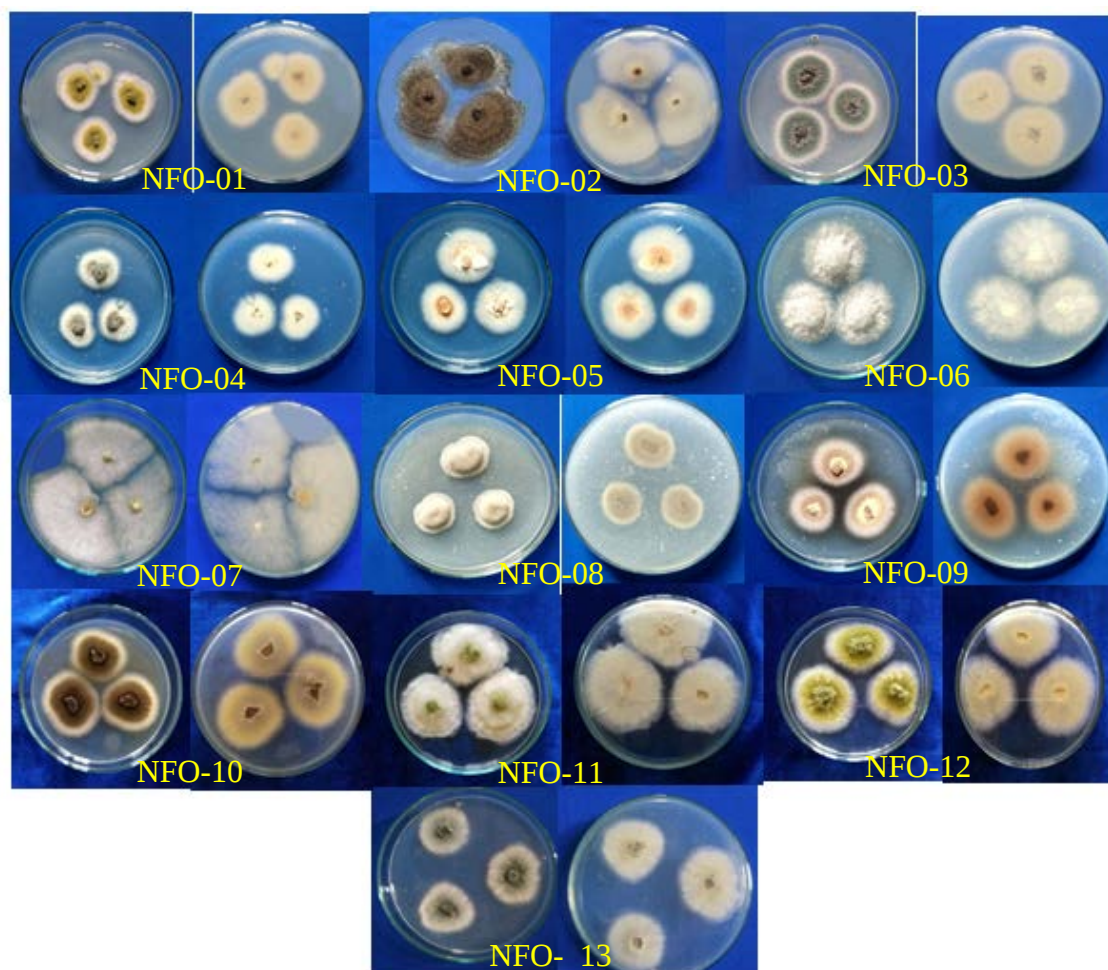


Figure 3 Morphology of fungus NFO-01 to NFO-13 (7 days old culture on PGA medium)

Table 1 Morphology of Isolated fungi NFO-1 to NFO-13

Sr No.	Isolated Fungi	Front view colour	Reverse view colour	Spore colour
1.	NFO-1	Greenish yellow	Pale yellow	Greenish yellow
2.	NFO-2	Black	Gray	Black
3.	NFO-3	Green	Pale yellow	Green
4.	NFO-4	White	White	White
5.	NFO-5	Brown	Brown	Brown
6.	NFO-6	White	White	White
7.	NFO-7	White	White	White
8.	NFO-8	Gray	Gray	Gray
9.	NFO-9	Pale brown	Pale brown	Pale brown
10.	NFO-10	Black	Gray	Black
11.	NFO-11	White	White	White
12.	NFO-12	Greenish yellow	Pale yellow	Greenish yellow
13.	NFO-13	Black	Gray	Black

Isolated Fungi and their Antibacterial Activity

In this study, eight fungi strains were tested with *Pseudomona fluorescens* by agar well diffusion method. NFO-9 gave the best activity on *Pseudomonas fluorescens*.

Table 2 Antibacterial activity of ten days fermentation of NFO-9 on *Pseudomonas fluorescens*

Fermentation period	Clear zone (mm)
1-3 days	No activity
4 day	18.21
5 day	20.94
6 day	24.31
7day	29.57
8 day	25.17
9-10 days	No activity

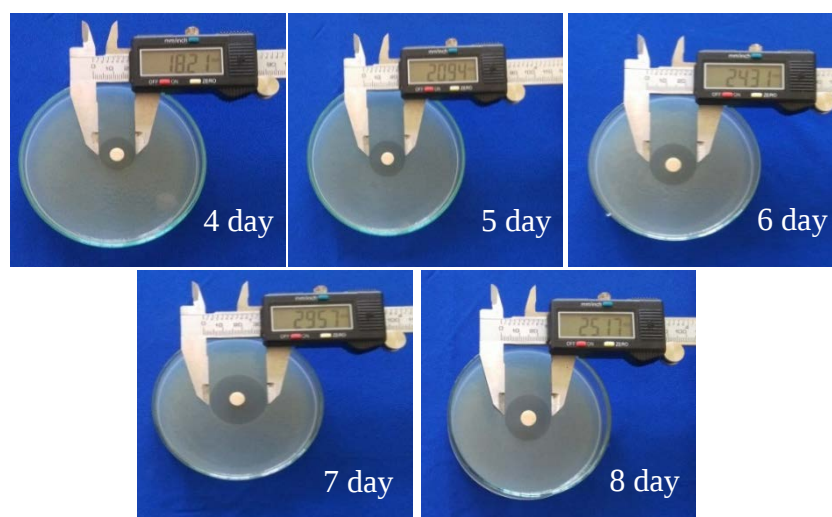


Figure 4 Antibacterial activity of ten days fermentation of NFO-9 on *Pseudomonas fluorescens*.

The effect of ages of inoculum on the fermentation

In the effect of age of inoculum, NFO-9 was investigated by using 42, 48, 54, 60, 66 and 72 hrs old age of inoculums. The result showed that 66hrs age of inoculum gave the highest activity (29.35mm).

Table 3 The Effects of Ages of inoculums on the Fermentation for NFO-9

Hour (hrs)	Size (mm)
2	12.55
48	6.33
54	17.90
60	26.07
66	29.35
72	26.47

The effects of sizes of inoculums on the fermentation for NFO-9

In this research work, the effect of size of inoculum was studied by using 5%, 10%, 15%, 20%, 25% and 30% inoculum. Using 25% inoculums showed significantly the highest activity (35.20 mm).

Table 4 The Effects of Size of inoculums on the Fermentation for NFO-9

Sizes %	Size (mm)
5%	24.93
10%	20.40
15%	30.84
20%	34.45
25%	35.20
30%	33.81

The effect of pH on the fermentation conditions of NFO-9

In this study, the highest antibacterial activity was obtained at pH-6 (37.62mm) against *Pseudomonas fluorescens*

Table 5 The Effects of pH of inoculums on the Fermentation for NFO-9

Ser.No.	pH	Antibacterial Activity (mm)
1.	4.0	24.83
2.	5.0	28.36
3.	6.0	37.62
4.	7.0	27.92

Antibacterial activity of NFO-9 on Fermentation media

In the fermentation medium (FM), the best antifungal activity was obtained by using sucrose and peptone in FM-2 (36.47 mm) followed by 27.51 mm, FM-1 (Glycerol and peptone).

Table 6 Antibacterial activity of NFO-9 on various fermentation medium

Fermentation media	Inhibitory zone (mm)
FM-1	27.51
FM-2	36.47
FM-3	23.65
FM-4	20.94

Discussion and Conclusion

Mangrove stands are located in sheltered places and in the mouths of valleys in most cases. The mangrove floor varies from sandy to muddy. Mangrove soils are of marine transported as sediment and deposited by rivers and the sea. In this isolation of marine fungi from mangrove soil samples were collected at Magyi, Shwe Thaung Yan Sub-Township, Ayeyarwaddy Region. Marine fungi were isolated by soil dilution method. In this study, 13 marine fungi were isolated. Three fungi were isolated from soil sample No.1, three from soil sample No. 2, two from soil sample No.3, two from soil sample No.4 and three from soil samples No.5. These soil fungi NFO- 06, 07, 08 and 11 have white color. NFO- 02 and 10 black colour.

Other fungi have different colour. Among them, the selected fungus NFO-9 showed potent antibacterial activity against *Pseudomonas fluorescens*.

Therefore, NFO-9 was selected for the study of the optimum fermentation condition. To study the optimization of inoculum age, the highest antibacterial activity was found at 66 hrs (29.35 mm). In the proper size of inoculum, 25% was the most suitable and the maximum activities of NFO-9 reached up (35.20 mm) followed by 20% and 30% respectively. The effect of pH was studied by varying from pH 4, 5, 6 and 7. The best antibacterial activity was found at pH-6 (37.62 mm). The change in pH is also important for the enzyme activity of microorganisms, for the intermediate products, their dissociation and solubility (Rizk *et al.*, 2007). Fermentation media (FM) were studied and FM-2 gave the highest activity (36.47 mm). The choice of a good fermentation medium is virtually as important to the success of an industrial fermentation as is the selection of an organism to carry out the fermentation (E1-Tayeb *et al.*, 2004). Thus, the results of the optimum fermentation tests indicated that antimicrobial metabolites obtained from NFO-9 may be produced optimally in the presence of 7th days fermentation period, 66 hrs age of inoculums and 25% inoculums size, pH- 6 and FM-2. It was concluded that the present study revealed to observe the fermentation period of isolated fungi and to investigate the optimization parameters of fermentation condition on NFO-9 against *Pseudomonas fluorescens*.

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