

Study on the Antimicrobial Activities and Utilization of Carbon and Nitrogen Sources for Fermentation of Soil Fungi

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Abstract

The soil samples were collected from five different places of Hinthada Township, Ayeyarwaddy Region. These soil samples were used for the isolation of fungi. The isolation of microorganisms is carried out by serial dilution method. Sixteen fungi were isolated from five different soil samples. According to the results of antimicrobial activities test, the fungi NS-07 showed more active on *Bacillus subtilis* IFO 90571 than other strains. Carbon and nitrogen sources were employed in this study, among them, rice powder, wheat powder, maize powder, tapioca powder, glucose, sucrose, soluble starch, glycerin, palm sugar, yeast extract, peptone, chick pea powder, malt extract, beef extract, fish extract, branny and NaNO_3 are excellent for the fermentation of NS-07. About 0.5 g per Liter are applied each source.

Keywords: isolation, antimicrobial activities, carbon and nitrogen sources

Introduction

Microorganisms are crucial to nutrient recycling in ecosystems as they act decomposers. As some microorganisms can fix nitrogen, they are a vital part of the nitrogen cycle, and recent studies that airborne microorganisms may play a role in precipitation and weather (Christner, 2008).

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis (Kingston, 2008).

Media generally contain source of carbon, nitrogen and vitamins. Glucose is the most widely utilization carbon source, and hence is the most commonly used in growth media. Fructose and mannose are the next most commonly utilized sugars by fungi and are found in media from natural sources. Sucrose may be used in some media. Nitrogen sources include yeast extract, malt extract, amino acids, ammonium and nitrate compounds. It is a good general source of nitrogen but is vitamin free contains nitrogen and a high peptone and amino acid content. Fungi have natural deficiencies for vitamins. The most common naturally occurring vitamin deficiencies are thiamin and biotin. Other organic nutrients such as glucose are often contaminated with vitamins sufficient to supply the growth requirements of fungi (Margulis, L., O. and *et.al*, 1990).

Materials and Methods

Isolation of fungi from five different soil samples

The five different soil samples were collected from the places of Shwe-taung-thaya village, Ohm-pin-su village, Thin-gan-ai village, Taman-Oo village and Kokko-su village, Hinthada Township. These soil samples were used for the isolation of microorganisms. The isolation of microorganisms was carried out by serial dilution method. Low carbon agar

medium (LCA) and potato glucose agar medium (PGA) were used for the isolation of soil fungi in this study.

Serial Dilution Method (Phay & Yamamura, 2005)

One gram of the soil and 100mL distilled water were added to the conical flask. Then the conical flask is shaken about 10 minutes. 9.0 mL of distilled water was added in each of 10 sterile test tubes. When the soil suspension in the conical flask was cooled, 1.0 mL was transferred to the first test tube. Mix the suspension gently. 1.0 mL was transferred to additional test tube containing 9.0 mL to get serially diluted suspension of 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} and 10^{-9} respectively. After taking the final test tube, nutrient agar (LCA medium) was streaked with the inoculation loop. A loop is sterilized with flame or ethanol. The loop used for picking small quantities of soil suspension from a microbial colony, and can be used to inoculate either a liquid or a solid medium. Incubate the plate for about 4-5 days at room temperature.

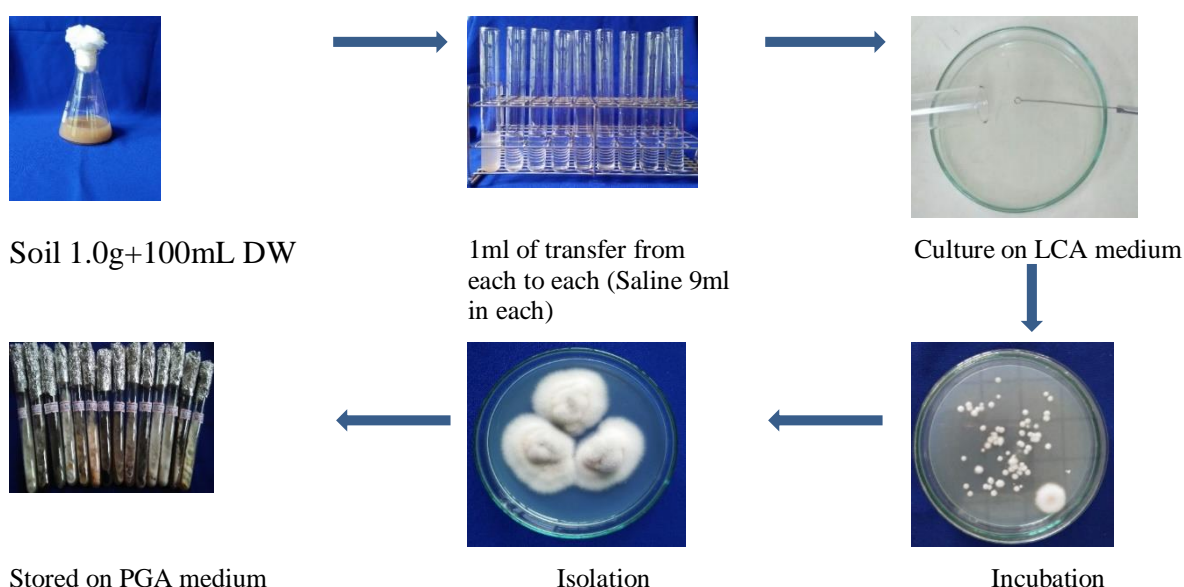


Figure (1). Serial Dilution Method (Phay & Yamamura, 2005)

Screening for antimicrobial activities of soil fungi by paper disc diffusion assay (Hokkaido Uni., 1988)

The isolated fungi were grown at room temperature for 5 days on PGA medium for sporulation. The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days. Twenty mL of seed culture was transferred into the fermentation medium and incubated at room temperature for 5 days. Twenty μ L of fermented broth was put on paper disc (8mm) and placed on assay plate containing test organisms. Paper disc having eight millimeter diameter (Advantec, Roshi Kaisha Co. Ltd., Japan) were utilized for antimicrobial assays.

The assay medium (Glucose 10 g, Polypepton 30 g, KNO_3 10 g, Agar 18 g, Distilled water 1000 mL) was used for the antimicrobial activity test. One or two drops of test organism was added to assay medium and then poured into plates. After solidification paper disc impregnated with fermented broth were applied on the agar plates and the plates were incubated for 24-36 hours at room temperature to examine the inhibitory zones.

Seed medium	
Glucose	20.0 g
Polypeptone	3.0 g
KNO ₃	1.0 g
K ₂ HPO ₄	1.0 g
DW	1000 mL
pH	6.5

Fermentation medium	
Glucose	20.0 g
Yeast extracts	8.0 g
K ₂ HPO ₄	1.0 g
MgSO ₄	0.1 g
CaCO ₃	1.0 g
DW	1000 mL
pH	6.5

BR-BDC- Screening Media (2004)

Table (1). Test organisms used in antimicrobial activities

No	Test organisms	Sources	Infections
1	<i>Agrobacterium tumefaciens</i>	NITE 09678	Plant disease
2	<i>Aspergillus paraciticus</i>	IFO 5123	Fruits disease
3	<i>Bacillus subtilis</i>	IFO 90571	Fever
4	<i>Candida albicans</i>	NITE 09542	Candidosis
5	<i>E.coli</i>	AHU 5436	Diarrhoea
6	<i>Micrococcus luteus</i>	NITE 83297	Skin disease
7	<i>Pseudomonas fluorescens</i>	IFO 94307	Rice disease
8	<i>Saccharomyces cerevisiae</i>	NITE 52847	Food spoilage
9	<i>Salmonella typhi</i>	AHU 7943A	Typhoid fever and food poisoning
10	<i>Staphylococcus aureus</i>	AHU 8465	Boils and food poisoning

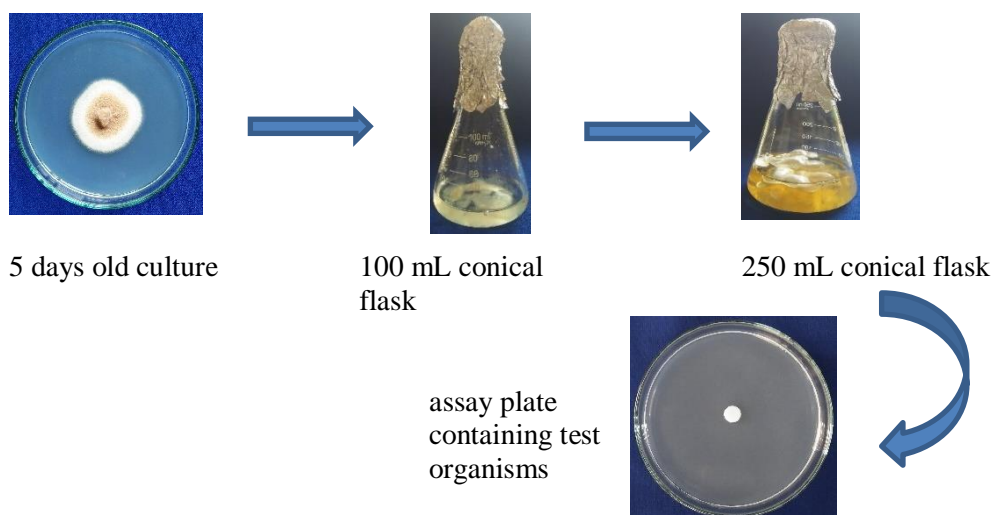


Figure (2). Procedure for the antimicrobial activity test (Hokkaido Uni., 1992)

Carbon and Nitrogen utilization

In this study, rice power wheat powder, maize powder, tapioca powder, glucose, sucrose, soluble starch, glycerin, palm sugar were employed as carbon sources for suitable media. Yeast extract, peptone, malt extract, beef extract, fish extract, chick pea powder, branny, polypepton, NaNO₃ were used as nitrogen sources. The culture of selected fungus

NS-07 were undertaken on plates containing these carbon or nitrogen sources (carbon source / nitrogen source 5g, Agar 18g, Distilled water 1000mL) for 7 days at room temperature.

Results

Isolation of fungi from five different soil samples

Five different soil samples were collected from Hinthada Township, Ayeyarwaddy Region. The soil texture of No.1 and No.5 were clay loam. The soil No.2 and 4 were sandy clay loam and No.3 soil was loam soil type. These soil samples were employed for the isolation of microorganism. In this study, sixteen fungi were collected from five soil samples by using serial dilution method. Fungi NS-01 to NS-05 were isolated from soil NO.1. Fungi NS-06 and NS-07 from soil No.2, fungi NS-08 to NS-10 from soil No.3, fungi NS-11 to NS-13 were isolated from soil No.4. Fungi NS-14 to NS-16 were isolated from soil No.5.

Table (2). Isolated soil fungi from five different soil samples

Soil No.	Soil type	Soil pH	Isolated strains
S-1	Clay loam	5.10	NS-01,02,03,04,05
S-2	Sandy clay loam	5.89	NS-06,07
S-3	Loam	5.42	NS-08,09,10
S-4	Sandy clay loam	5.71	NS-11,12,13
S-5	Clay loam	5.44	NS-14,15,16

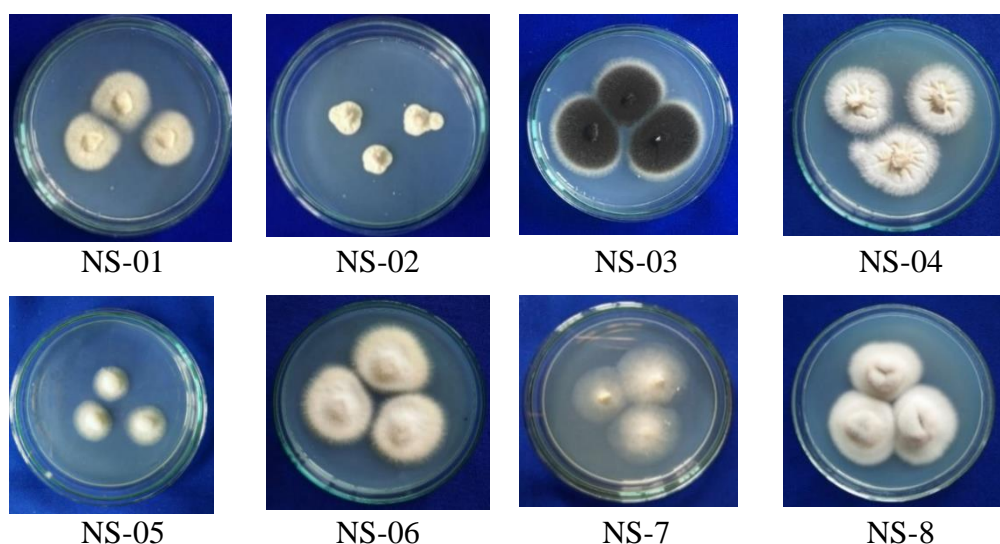


Figure (3). Morphological characters of isolated fungi NS-01 to NS-08 on PGA medium (5 days old culture)

Preliminary study for antimicrobial activities of soil fungi by paper disc diffusion assay (Hokkaido Uni., 1988)

Sixteen fungi were isolated from five different soil samples of Hinthada Township, Ayeyarwaddy Region. In this study, sixteen fungi were used in the antimicrobial activities by using the paper disc diffusion assay.

NS-01 to NS-16 showed the antimicrobial activity against *Bacillus subtilis*, *Candida albicans* and *E.coli*. According to the results of antimicrobial activities test, sixteen fungi showed the antimicrobial activities on some test organisms. Among them, the soil fungi NS-07 was selected for further investigations based on the results of the antibacterial activity especially against *Bacillus subtilis*.

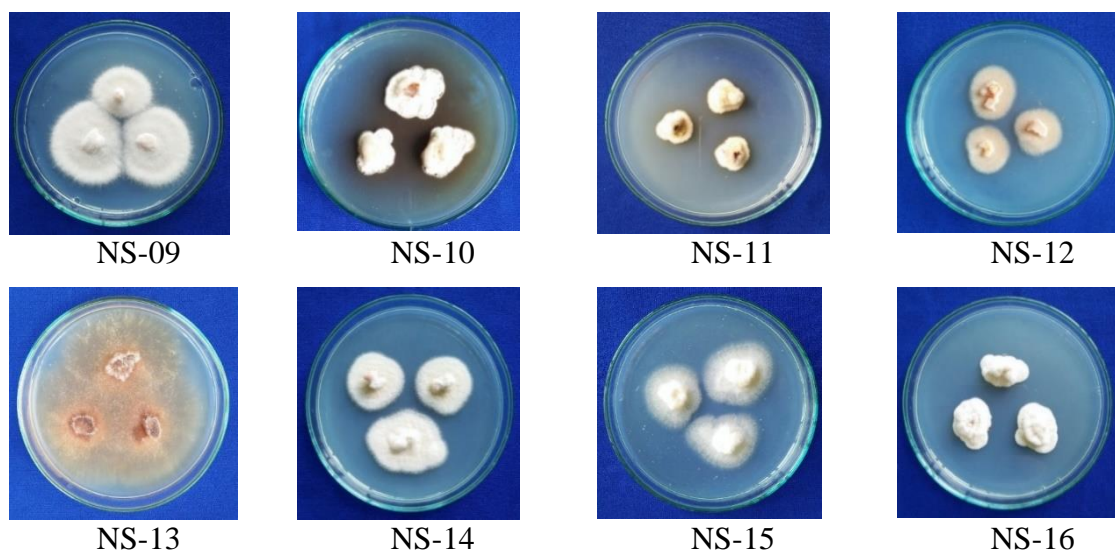


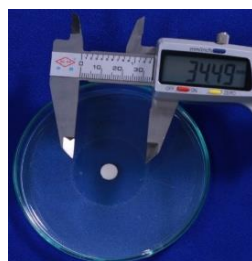
Figure (4) Morphological characters of isolated fungi NS-09 to NS-16 on PGA medium (5 days old culture)

Table (3). Antimicrobial activities of isolated fungi

Isolated No.	<i>Candida albicans</i>	<i>E.coli</i>	<i>Bacillus subtilis</i>
NS-01	26.44mm	25.23mm	26.49mm
NS-02	26.58mm	27.21mm	27.07mm
NS-03	26.33mm	24.74mm	28.20mm
NS-04	26.43mm	22.30mm	28.01mm
NS-05	24.34mm	25.37mm	25.99mm
NS-06	26.23mm	25.02mm	28.09mm
NS-07	26.89mm	24.72mm	29.38mm
NS-08	25.28mm	23.93mm	27.64mm
NS-09	23.68mm	24.32mm	25.63mm
NS-10	27.13mm	24.44mm	30.51mm
NS-11	27.01mm	25.43mm	27.76mm
NS-12	26.46mm	24.90mm	27.81mm
NS-13	25.46mm	25.76mm	26.57mm
NS-14	27.38mm	23.10mm	28.98mm
NS-15	27.45mm	25.38mm	27.54mm
NS-16	26.37mm	23.80mm	26.99mm



NS-07

Clear zone-34.49 mm NS-07 on
*Bacillus subtilis*Figure (5). Antibacterial activity of selected fungi NS-07 on *Bacillus subtilis* IFO 90571

Carbon and Nitrogen Sources utilization for Fermentation condition of isolated fungi NS-07

The cultures for NS-07 were undertaken on plates containing carbon and nitrogen sources for 7 days at room temperature. To determine the suitable carbon sources for NS-07 fungus. Among these potato is the best nitrogen source.

Nitrogen sources of yeast extract, peptone, beef extract, fish extract, polypepton and NaNO_3 are excellent for suitable media of NS-07 fungus and chick pea powder served as moderate medium. In this investigation, carbon sources of rice powder, wheat powder, maize powder, soluble starch and palm sugar were more suitable for NS-07 on fermentation medium.

Table (4). Morphological characters of NS-07 on various nitrogen sources

Nitrogen sources	Color	Growth
Yeast extract	White	Excellent
Peptone	White	Excellent
Malt extract	White	Poor
Beef extract	White	Excellent
Fish extract	White	Excellent
Chick pea powder	White	Moderate
Branny	White	Poor
Polypepton	White	Excellent
NaNO_3	White	Excellent

Table (5). Morphological characters of NS-07 on various carbon sources

Carbon sources	Color	Growth
Wheat powder	White	Moderate
Rice powder	White	Moderate
Maize powder	White	Moderate
Tapioca powder	White	Poor
Glucose	White	Poor
Sucrose	White	Poor
Soluble starch	White	Moderate
Glycerin	White	Poor
Palm sugar	White	Moderate

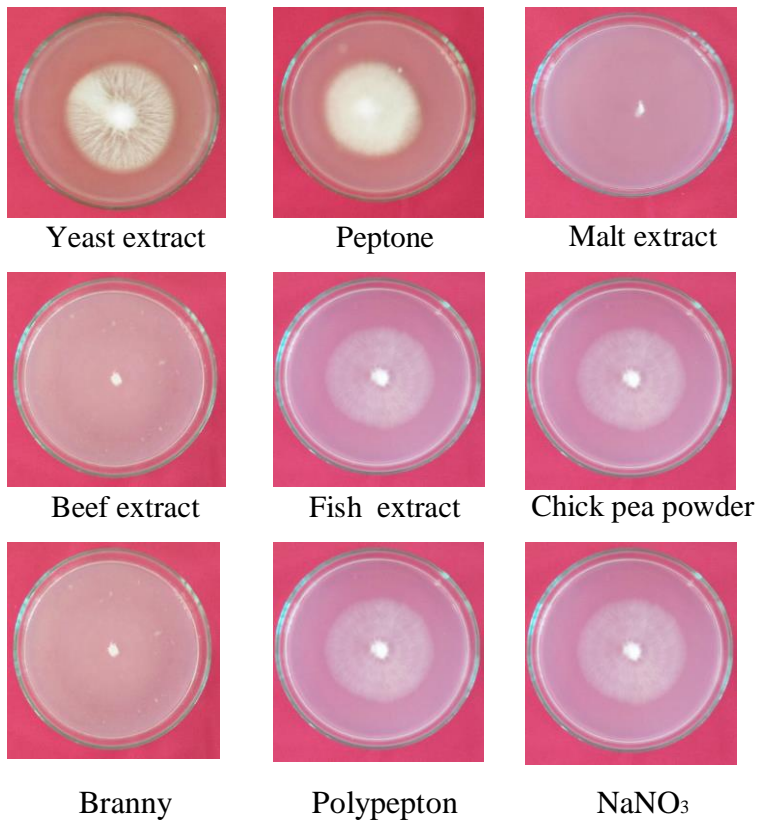


Figure (6). Morphological characters of NS-07 on various nitrogen sources

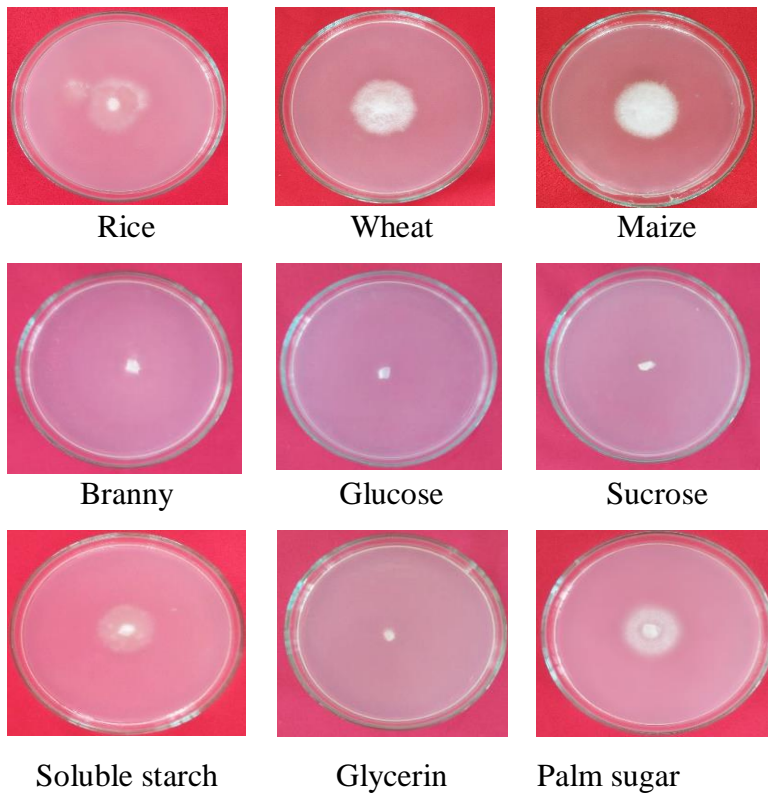


Figure (7). Morphological characters of NS-07 on various carbon sources

Discussion and Conclusion

Five different soil samples were collected from Hinthada Township, Ayeyarwaddy Region. These soil samples were employed for the isolation of microorganisms. According to the data of Department of Agriculture (Land use), soil sample No.1 and 5 are clay loam, soil sample No.2 and 4 are sandy clay loam and soil sample No.3 is loam respectively.

During the procedure work, sixteen fungi were isolated from five different soil samples by using serial dilution method. Among these samples NS-07 from soil sample No.2 was selected for the production of antimicrobial metabolite.

According to the results of antimicrobial activities test, fungus NS-07 showed more active on *Bacillus subtilis* (IFO 90571) than other strains. Therefore, this isolated strain NS-07 fungus selected for further investigations on the fermentation and purification of metabolite especially against *Bacillus subtilis* (IFO 90571).

Carbon sources of rice powder, wheat powder, maize powder, soluble starch, palm sugar and nitrogen sources of yeast extract, peptone, beef extract, fish extract, polypepton, and NaNO₃ were more suitable for NS-07 fungus for fermentation medium.

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