ANTIMICROBIAL AND AMYLASE ACTIVITIES OF ISOLATED SOIL FUNGI FROM TAUNG THAR TOWNSHIP (MANDALAY REGION)

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Abstract

The soil microorganisms were isolated from the various soils collected from five different places: Yay Kyaw S-1, Thae Taw S-2, Kaing Yar Gyi S-3, Si Sone Kone S-4, and Kone Thar S-5, Taung Thar Township, Mandalay Region. From the analysis of soil textures, sample No.1 was found to be loamy sand, samples No. 2 and No. 3 were sand, and samples No. 4 and No. 5 were sandy loam. In this study, the soil fungi were isolated by using the feeding method and the physical treatment dilution method. Four fungi were isolated from five different soil samples by the feeding method and six fungi were isolated from five different soil samples by the physical treatment dilution method. The morphology of the isolated fungi were studied in PGA medium. These fungi were used for screening the antimicrobial activities against seven tested microorganisms. The first time, after three days of fermentation, the antimicrobial activity was tested against three microorganisms. Among them, only the isolated fungus from soil sample 1 (Yay Kyaw S-1) showed the highest activity in clear zone (27 mm) on *Agrobacterium tumefaciens,* followed by (26 mm) on *Bacillus subtilis* and (25 mm) on *Espherichia coli,* and no activity against the remaining four tested organisms: *Candida albicans, Micrococcus luteus, Pseudomonas fluorescens* and *Staphylococcus aureus.* In the second time fermentation, the isolate fungus from S-1 (32 mm) was especially active against *Agrobacterium tumefaciens.* This isolated fungus cannot hydrolyze the starch, that is, it did not show the amylase enzyme activity.

Keywords: soil fungi, soil texture, antimicrobial activity, fermentation, amylase enzyme activity

Introduction

Microorganisms live in all parts of the biosphere where there is liquid water, including soil, hot springs, on ocean floor, high in the atmosphere and deep inside rocks within the earth crust. The typical materials for microbial sources are soil, living and fallen leaves, leaf litters, dung, insect, fresh water and marine water. The soil samples are most effective and popular minerals for the isolation of fungi and actinomycetes (Kuntgmen, 1992)(Harayama and Isono, 2002). 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).

Fungi are an important component of the microbiota, typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions (Cattle *et al.*, 2002). Fungi, bacteria and actinomycetes colonize different habitats and substrates and play substantial role in plant health and productivity besides producing diseases (Aina *et al.,* 2011).

The main aim of this research is to isolate the soil fungi from various soil samples, and to investigate the antimicrobial and amylase activities of the isolated soil fungi.

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Materials and Methods

Collection of the samples

Microorganisms have different characteristics that define them and different functions in the soil they live in. So, various different soil samples were collected from Taung Thar Township, Mandalay Region, as shown in Figure 1 and listed in Table 1. The five different soil samples were collected from the different places: Yay Kyaw, Thae Taw, Kaing Yar Gyi, Si Sone Kone and Kone Thar. The different soil samples were employed for the isolation of microorganisms. The collected soil samples were washed with water followed by filtration. After cleaning, it was dried at room temperature; the dried powder sample was stored in an air-tight container to prevent moisture changes and other contamination. The dried powder sample was used to investigate the chemical and biological activities.

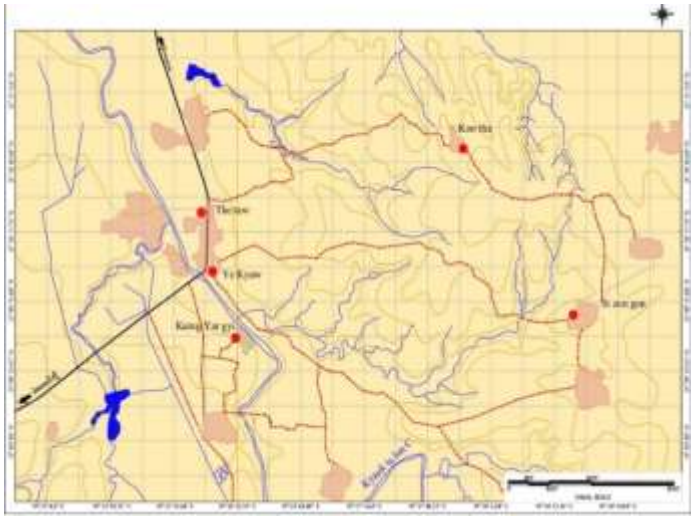


Figure 1. Map of soil samples collected area

Table 1. Five Different Soil Samples Collected from Taung Thar Township, Mandalay Region

Soil No.	Places	Location	Collected date
S-1	Yay Kyaw	N 21˚ 10′ 05″ E 95˚ 26′ 21″	8. 6. 2022
S-2	Thae Taw	N 21˚ 10′ 39″ E 95˚ 26′ 13″	8. 6. 2022
S-3	Kaing Yar Gyi	N 21˚ 9′ 66″ E 95˚ 26′ 36″	8. 6. 2022
S-4	Si Sone Kone	N 21˚ 9′ 80″ E 95˚ 28′ 67″	1. 7. 2022
S-5	Kone Thar	N 21˚ 10′ 76″ E 95˚ 27′ 92″	1. 7. 2022

Analysis of Collected Soil Sample

Some parameters, such as soil texture, pH, nitrogen, and moisture of the collected samples, were tested at the Department of Agriculture (Land Use), Yezin, and Nay Pyi Taw. Soil textures were analyzed by the triangle method; pH of soil water suspensions was measured using 4A1-1:5; nitrogen content was determined by the Kjeldahl distillation method; and moisture content was determined by the gravimetric method.

Isolation of Fungi from Five Different Soil Samples

Isolation of microorganisms from five different soil samples was carried out by the feeding method (I) and the physical treatment dilution method (II) (Phay & Yama Mura, 2005). Low carbon agar medium (LCA) and potato glucose agar medium (PGA) were used in the procedure for the isolation of soil fungi in this study (Figures 2 & 3).

Method I

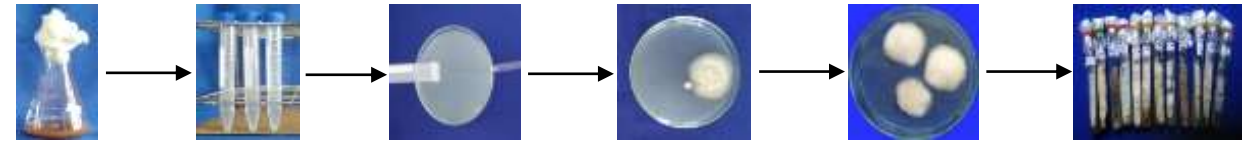


Figure 2. Isolation of soil fungi by the feeding method

Method II

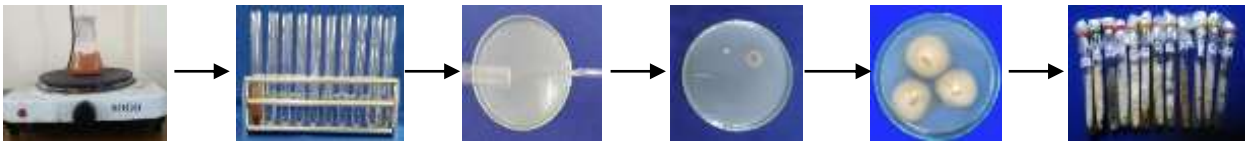


Figure 3. Isolation of soil fungi by the physical treatment dilution method

Medium Used for the Isolation of Fungi, Low Carbon Agar (LCA) Medium

(Ando, 2004)

Glucose	2.0 g
Sucrose	2.0 g
K_2HPO_4	1.0 g
KNO_3	1.0 g
KCl	0.5 g
Agar	18.0 g
D W	1000 mL
pH	6.5

Potato Glucose Agar Medium (PGA)

(Ando, 2004)

Potato	200.0 g
Glucose	20.0 g
Agar	18.0 g
DW	1000 mL
pH	6.5

(After autoclaving, chloramphenicol was added to both mediums)

Preliminary Study for Antimicrobial Activity by Paper Disc Diffusion Assay

Antimicrobial activity of isolated fungi was tested at Biological Resource and Biotechnology Development Centre, Botany Department, Pathein University. Screening of antimicrobial activity was studies by the Laboratory method of Applied Microbiology, 1992. The assay medium (glucose 10 g, polypeptone 30 g, KNO_3 10 g, agar 1.8 g, distilled water 1000 mL) was used for the antimicrobial activity test. One percent (1.5 x 10^8/mL of spore suspension) of the test organism was added to the assay medium and then poured into plates. After

solidification, paper discs (8 mm) impregnated with fermented broth were applied to the agar plates and incubated for 24-36 h at room temperature to examine the inhibitory zones.

Seed and fermentation media were used for screening the antimicrobial activities of isolated fungi against seven tested organisms. The tested organisms used in the paper disc diffusion assay method were *Agrobacterium tumefaciens* NTTE 09678, *Bacillus subtilis* IFO 90571, *Candida albicans* NTTE 09542, *Escherichia coli* AHU 5436, Micrococcus *luteus* NTTE 83297, *Pseudomonas fluorescens* IFO 94307, and *Staphylococcus aureus* AHU 8465 (Table 2). These tested organisms were obtained from the NTTE (National Institute of Technology and Evaluation, Kisarazu, Japan) and sc.

Table 2. Tested Organisms Used in Antimicrobial Activity Screening

No.	Tested organisms (NITE-2004)	Sources	Infections
1	*Agrobacterium tumefaciens*	NITE 09678	plant disease
2	*Bacillus subtilis*	IFO 90571	fever
3	*Candida albicans*	NITE 09542	candidosis
4	*Escherichia coli*	AHU 5436	diarrhoea
5	*Micrococcus luteus*	NITE 83297	skin disease
6	*Pseudomonas fluorescens*	IFO 94307	rice disease
7	*Staphylococcus aureus*	AHU 8465	boils and food poisoning

BR-BDC-Screening Mediums (2004)

Seed medium		**Fermentation medium**	
glucose	20.0 g	glucose	20.0 g
polypeptone	3.0 g	yeast extracts	8.0 g
KNO_3	1.0 g	K_2HPO_4	1.0 g
K_2HPO_4	1.0 g	$MgSO_4$	0.1 g
DW	1000 mL	$CaCO_3$	1.0 g
pH	6.5	DW	1000 mL
		pH	6.5

Preliminary Study on the Starch Hydrolyzing Activity of Isolated Fungi

The starch hydrolyzing activity was determined by NITE method (NITE, 2005). The preserved fungi were inoculated in 10 mL of liquid medium and incubated for five days at room temperature. Then, the iodine solution was slowly poured into the liquid culture medium. The control was also done. The change of colour was observed After adding iodine solution. If the

colour is still purple, the microorganisms cannot hydrolyze the starch. Conversely, if changes, the microorganisms can hydrolyze the starch. This test was used to determine amylase enzyme activity.

Results and Discussion

The analytical data (pH, soil texture, moisture content and nitrogen content) of the collected soil samples coded as S-1, S-2, S-3, S-4 and S-5 are shown in Table 3.

Table 3. Analytical Data of Five Different Soil Samples

Soil Samples	pH	Soil texture	Moisture (%)	Nitrogen (%)
S-1	8.12	loamy sand	1.7	0.065
S-2	8.82	sand	3.6	0.213
S-3	8.76	sand	0.6	0.013
S-4	7.63	sandy loam	1.9	0.040
S-5	8.86	sandy loam	2.3	0.065

In Table 3, the pH of all tested samples ranged between 7.63 and 8.86. It showed the basic character. The moisture contents were in the range of 0.6 % to 3.6 %. The nitrogen content of all the samples ranged from 0.013 % to 0.213 %. The soil texture of S-1 (Yay Kyaw) was loamy sand whereas S-2 (Thae Thaw) and S-3 (Kaing Yar Gyi) were sand. In addition, the soil samples of S-4 (Si Sone Kone) and S-5 (Kone Thar) were sandy loam.

Isolation of Fungi from Soil Samples

Ten fungi were isolated from five different soil samples in Taung Thar Township, Mandalay Region.

Table 4. Ten Isolated Fungi (ND-01 to ND-10) Isolated from Five Different Soil Samples by Using Two Isolated Methods

Soil samples	Isolated fungi			
	Feeding method	Name of fungi	The physical treatment dilution method	Name of fungi
	Method (I)		(Method II)	
S-1	1	ND-01	1	ND-02
S-2	1	ND-03	1	ND-04
S-3	-		1	ND-05
S-4	1	ND-06	1	ND-07
S-5	1	ND-08	2	ND-09 ND-10
Total	4	-	6	-

In Table 4, four fungi were isolated by method I, and six fungi were isolated by method II. Four isolated fungi: ND-01, ND-03, ND-06, and ND-08 were obtained from soil samples S-1, S-2, S-4, and S-5; six isolated fungi: ND-02, ND-04, ND-05, ND-07, ND-09, and ND-10 were obtained from soil samples S-1, S-2, S-3, S-4, and S-5.

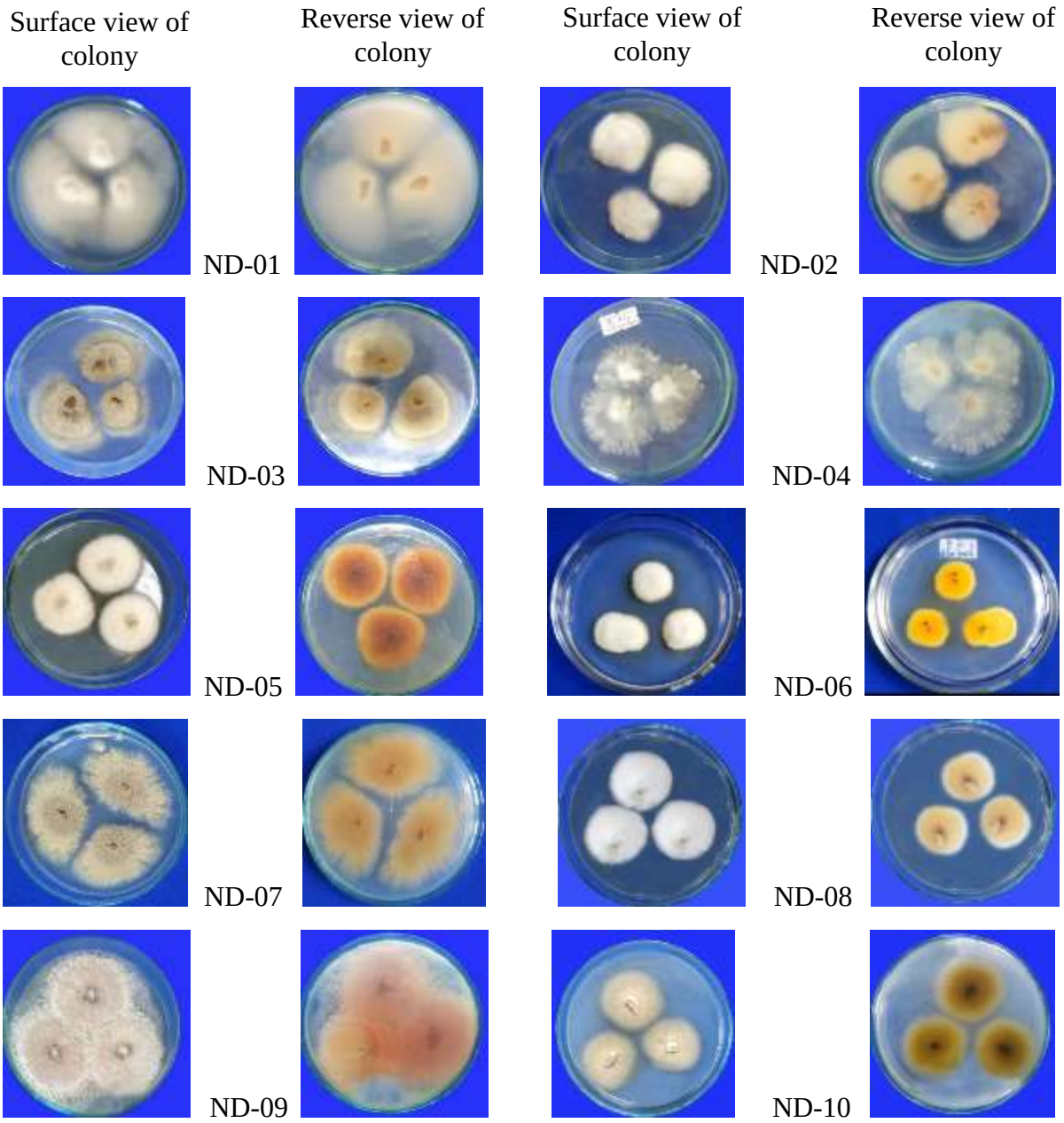


Figure 4. Morphological characters of isolated fungi ND-01 to ND-10 (5-day-old culture) on PGA Medium

In Figure 4, the morphological characters of isolated fungi ND-01 to ND-10 (5-day- old culture) were observed on PGA medium. The surface colour and back (reverse) colour of ND-01 and ND-04 were white. The surface colour and back colour of ND-03 were grey. The surface colours of ND-02, ND-05, ND-06, and ND-08 were white, and their reverse colours were light pale around white, orange, yellow, white in the middle, and light yellow around. The surface colour of ND-07 was pale yellow green and the reverse colour was orange. The surface colour of

ND-09 was centre flower mat and edge white, and the reverse colour was light pink. The surface colour of ND-10 was pale yellow, and the reverse colour was center black around the yellow.

Table 5. Screening of Antimicrobial Activity of Isolated Fungi (3-day-old culture) on Seven Tested Organisms

Isolated fungi	Inhibition zone diameters of tested organisms (mm)		
	Agrobacterium tumefaciens	***Bacillus subtilis***	***Escherichia coli***
ND-01	18	22	24
ND-02	**27**	**26**	**25**
ND-03	19	25	23
ND-04	25	22	23
ND-05	26	19	22
ND-06	24	24	23
ND-07	25	22	23
ND-08	24	18	22
ND-09	22	25	18
ND-10	24	22	20

In Table 5, the isolated fungi ND-01 to ND-10 showed the antibacterial activity against *Agrobacterium tumefaciens* inhibition zone diameters ranging between 18 and 27 mm, *Bacillus subtilis* ranging between 18 and 26 mm and *Escherichia coli* ranging between18 and 25 mm. No activity was found against the remaining four tested organisms: *Candida albicans, Micrococcus luteus, Pseudomonas fluorescens* and *Staphylococcus aureus*. In Figure 5, the fungus ND-02 showed the highest antibacterial activity against *Agrobacterium tumefaciens* (27 mm), *Bacillus subtilis* (26 mm) and *Escherichia coli* (25 mm) respectively. According to the results, ND-02 fungus showed the highest antibacterial activity out of ten strains.

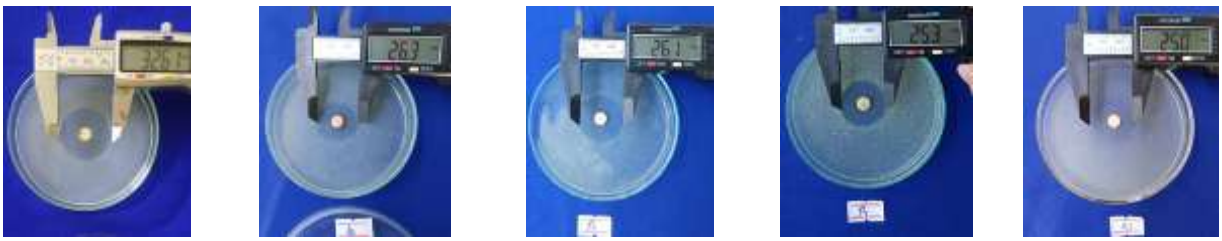
(a) (b) (c)

Figure 5. Antibacterial activity of the isolated soil fungi ND-02 (3-day-old culture) against (a) *Agrobacterium tumefaciens*, (b) *Bacillus subtilis* and (c) *Echerichia coli*

Table 6. Inhibition Zone Diameters of Selected ND-02 Fungus (within 3 to 7 days culture) on *Agrobacterium tumefaciens*

Fungus	Inhibition zone diameters of ND-02 fungus (mm) within 3 -7 days				
	3	**4**	**5**	**6**	**7**
ND – 02	**32.61**	26.3	26.1	25.3	25.0

The antibacterial activity of ND-02 fungus was determined by paper disc diffusion method. In this study, ND-02 exhibited distinct clear zone against *Agrobacterium tumefaciens* within 3 to 7 days culture as shown in Figure 6 and Table 6.



3 days 4 days 5 days 6 days 7 days

Figure 6. Antibacterial activity of ND-02 fungus (within 3 to 7 days culture) against *Agrobacterium tumefaciens*

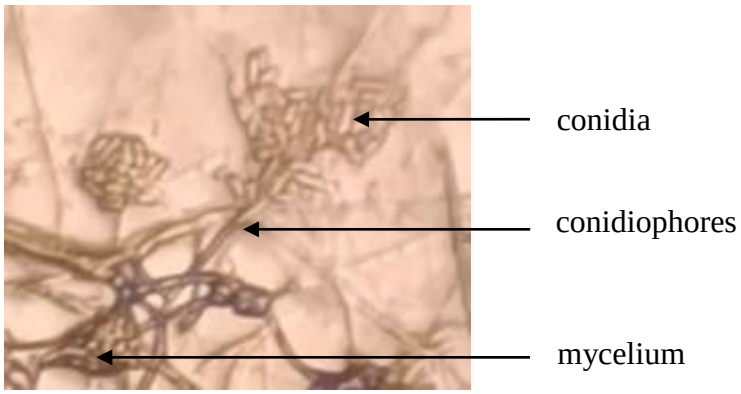


Figure 7. Photomicrograph of isolated fungus ND-02 from the soil (S-1) Yay Kyaw Village, Taung Thar Township (400X)

In Figure 7, photomicrograph of isolated fungus ND-02 consists of conidia, conidiophores and mycelium. The typical fungus character showed that ND-02 was fusarium species, and the surface colour of ND-02 was white and the reverse colour was light pale around white.

The Amylase Enzyme Activity of Isolated Fungi by Iodine Test

The hydrolyzing starch activity of isolated fungi was tested by iodine test (NITE, 2004-2005). Change of iodine colour indicates the action of amylase activity in hydrolysis of starch. After adding iodine solution to the isolated fungi ND-01 to ND-10, all samples except ND-02 showed active with iodine. In this study, ND-01, 03, 04, 05, 06, 07, 08, 09 and 10 can hydrolyze the starch (Figure 8). Therefore, these isolated nine fungi showed that may produce amylase enzyme. But only one fungus ND-02 cannot hydrolyze the starch and not possess amylase enzyme activity.

Figure 8. The amylase enzyme activity of isolated fungi

Conclusion

The soil samples (S-1 to S-5) were collected from five different places in Taung Thar Township, Mandalay Region. The soil types of the collected soil samples were loamy sand (S-1), sand (S-2 and S-3), and sandy loam (S-4 and S-5). The pH of all tested soil samples varied from 7.63 to 8.86. Moisture content ranged from 0.6% to 3.6%. Nitrogen content in all samples was between 0.013% and 0.213%. Two fungi, ND-01 and ND-02, were isolated from soil sample S-1; ND-03 and ND-04 from soil sample S-2; ND-05 from soil sample S-3; ND-06 and ND-07 from soil sample S-4; and ND-08, ND-09, and ND-10 from soil sample S-5. Therefore, a total of ten fungus isolates were obtained. A total of ten fungi colonies cultured on LCA medium were transferred to PGA medium by using the feeding method and the physical dilution method. Morphological characters of isolated fungal ND-01 to ND-10 were found to have different shapes, surface colours, and reverse colours significantly. The isolated fungus ND-02 consists of conidia, conidiophores, and mycelium. In this research, after three days of fermentation, ten fungal strains were tested for their antimicrobial activity against seven tested

organisms. ND-01 to ND-10 showed activity only against *Agrobacterium tumefaciens* (27mm), *Bacillus subtilis* (26mm), and *Escherichia coli* (25 mm). The antibacterial activity of ND-02 fungus was found to be higher than the other. All isolated fungi except ND-02 showed amylase enzyme activity by starch hydrolysis test.

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