Isolation of Soil Fungi Collected from Hinthada University Campus and Their Enzyme Activity

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Abstract

Amylases are enzymes that break down starch or glycogen. Amylases have been produced by bacteria, fungi and other organisms. In this study, seven soil samples were collected from seven different places in Hinthada University Campus, Hinthada Township, in order to discover amylase enzyme produced by soil microbes. Seven soil samples were analyzed by soil texture and pH. The soil samples were isolated by soil dilution method. Thirty two soil fungi (HU-01 to HU-32) were isolated from seven soil samples. Pure colonies were inoculated into slant culture containing in Potato Glucose Agar medium. Pure fungal isolates were also tested by starch hydrolysis activity. Among them, thirty two soil fungi, except HU-30 and HU-31 did not show the hydroxyl starch or amylase enzyme activity.

Keywords: soil microbes, soil texture, starch hydrolysis activities, amylase enzyme

Introduction

Soil sample is a primary source of microorganisms. The numbers and species of microbes in soil depend on environmental conditions like nutrient availability, soil texture, presence of moisture in soil and type of vegetation cover, and their number varies according to the type of environmental condition (Atlas and Bartha,1998).

One gram of soil may harbor up to 10 billion microorganisms of possible thousands of different species. Because only a tiny fraction of soil microbes from soil are readily cultured, soil might be the greatest untapped resource for novel chemistry (Jain and Pundir, 2011). Microorganisms have significant functions in ecosystems and are found in all kinds of habitats (Cavalcanti *et al*., 2006). Such fungi are active in degrading a wide variety of biological materials present in the soil (Saranraj and Stella, 2013). A variety of microorganisms such as bacteria, fungi, yeast and actinomycetes are known to produce these enzymes (Madan *et al*., 2002). Fungi are important component of the soil micro biota. Micro fungi play a focal role in nutrient cycling by regulating soil biological activity (Abu, *et al*., 2005).

Fungi generally produce alpha-amylase (dextrinzing enzymes), beta-amylase (saccharifying enzymes), proteases and other enzymes (Sethi, *et al*., 2017 and Vihinen, *et al*., 1989). The first enzyme produced industrially was an amylase from fungal source in 1894, which was used for the treatment of digestive disorders (Pandey *et al*., 2000). Sources of amylase and protease in yeast, bacteria and molds have been reported and their properties have been described (Alkan, *et al*., 2007). Amylase enzymes are industrially important enzymes used in food, sugar, textile, pharmaceutical, paper and detergent industries (Asrat, and Girma, 2018).

The aim of the present study is to analyze the collected soil samples, isolate the soil microorganisms from different soil samples and to study the biological activities of starch hydrolysis activities.

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Materials and Methods

Collection of Soil Samples

Seven soil samples were collected from seven different places in Hinthada University Campus (Longitude: 94° 15'; latitude 96° 15'), Hinthada Township, Ayeyarwady Region, during June, 2018. The samples were taken near the tree by digging 4 inches depth under the soil. The sample were collected in sterile glass container, sealed and carefully placed in plastic bag and brought to the laboratory. The locations of map of the Hinthada University Campus and collected places were shown in figure (1) and table (1).

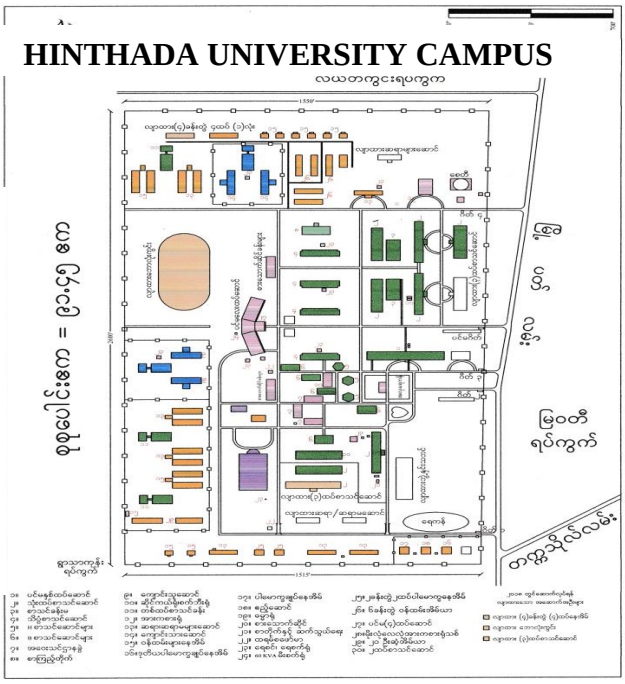


Figure (1). Map showing the soil samples collected at Hinthada University Campus (Sources: Hinthada University)

Table (1). Seven Different Soil Samples Collected at Hinthada University Campus

Soil samples	Collected places
1	In front of Chemistry Dept:
2	Behind the Main Building
3	Behind the Hostel (3)
4	Near the Main Gate
5	Near the Pathein Hostel
6	Behind the Botany Dept:
7	Near the Gate (3)

Physicochemical Analysis of Collected Soil Samples

The collected soil samples were characterized for its some physicochemical properties. The physicochemical parameters such as pH, textures were measured by standard methods.

Isolation of Soil Fungi and Bacteria from Different Soil Samples

The soil microorganisms were isolated by soil dilution method (Ando, 2014) on different media such as Low Carbon Agar (LCA) and Potato Glucose Agar (PGA).

Soil Dilution Method

The collected soil sample was air-dried at room temperature. The soil sample was grounded with mortar and pestle. Soil sample was often through a 2mm mesh to remove large debris and homogenize the sample. Two gram of soil sample was added into 150 ml of conical flask containing 18ml of sterilized (0.15% agar) water and shaken it for more than 30 min. The dilution series were cultured on LCA containing Glucose (0.20 g), Sucrose (0.20 g), K_2HPO_4 (0.10 g), $MgSO_4.7H_2O$ (0.05 g), KNO_3 (0.10 g), KCl (0.05 g), Agar (1.80 g), Distilled water (100 L) and pH (6.5) with chloramphenicol medium and incubated for 1 to 5 days at 27° C. At 6 days after the incubation, small piece colonies appeared on the medium are transferred to a fresh PGA medium containing Glucose (0.50 g), Potato (30.0 g), Yeast extract (0.10 g), Agar (1.80 g), Distilled water (100 L) and pH (6.5). Pure colonies were inoculated into slant culture containing in PGA medium.

Morphological Characters of Isolated Soil Fungi

The isolated soil fungi were inoculated on PGA medium for 7 days and photographed. The morphological characters of isolated soil fungi were described in figure (2) to (5).

Test for Starch Hydrolysis Activity (NITE, 2004)

The isolated pure fungi were inoculated in 5 mL liquid medium containing soluble starch (0.1g), K_2HPO_4 (0.1g), $MgSO_4$ (0.1g) $(NH_4)_2SO_4$ (0.2g), $CaCO_3$ (0.1g), NaCl (0.1g), and distilled water (100 mL) incubated at room temperature for 3 days. One drop of iodine solution was slowly poured onto the liquid culture medium. The blank control was also done. If the liquid medium changes the blue color, it indicates that microorganisms do not hydrolyze the starch. If the color does not change, the microorganism may produce amylase enzyme or hydrolyze the starch. This study was focused for the amylase enzyme production. The result data were shown in figure (6).

Results

Some Physicochemical Properties of Soil Samples

The physicochemical parameters of soil samples were analyzed at Department of Agriculture (Land Use) Soil Interpretation in Yangon Township. The resulting data were shown in table (2) and (3).

Table (2). Texture of Soil Samples

Soil Samples	Texture (%)				Soil Interpreter of Results
	Sand	Silt	Clay	Total	Texture
1	31.40	3.00	65.60	100.0	Clay
2	33.40	12.00	54.60	100.0	Clay
3	31.40	2.00	66.60	100.0	Clay
4	33.40	3.00	63.60	100.0	Clay
5	33.40	3.00	63.60	100.0	Clay
6	31.40	3.00	65.60	100.0	Clay
7	34.40	35.00	30.60	100.0	Clay Loam

Table (3). pH of Soil Samples

Soil Samples	pH	Soil Interpreter of Results
1	6.23	Slightly acid
2	5.67	Moderately acid
3	6.01	Slightly acid
4	6.32	Slightly acid
5	6.36	Slightly acid
6	4.99	Strongly acid
7	5.53	Moderately acid

Isolation of Soil Fungi and Bacteria from Different Soil Samples

Three bacteria and thirty two fungi (HU-1 to HU-32) were isolated by using soil dilution method from seven different soil samples. Soil fungi HU-1 and HU-7 were isolated from soil sample 1. Soil fungi HU-8 and HU-12 were isolated from soil sample 2. Soil fungi HU-13 and HU-16 were isolated from soil sample 3. Soil fungi HU-17 and HU-21 were isolated from soil sample 4. Soil fungi HU-22 and HU-24 were isolated from soil sample 5. Soil fungi HU-25 and HU-29 were isolated from soil sample 6. Soil fungi HU-30 and HU-32 were isolated from soil sample 7. Soil fungi were cultured on LCA medium in incubated for one to seven days at room temperature. Pure colonies were inoculated into slant culture containing in PGA medium.

Morphological Characters of Isolated Soil Fungi

The morphological characters of isolated soil fungi were observed in figure (2) to (5).

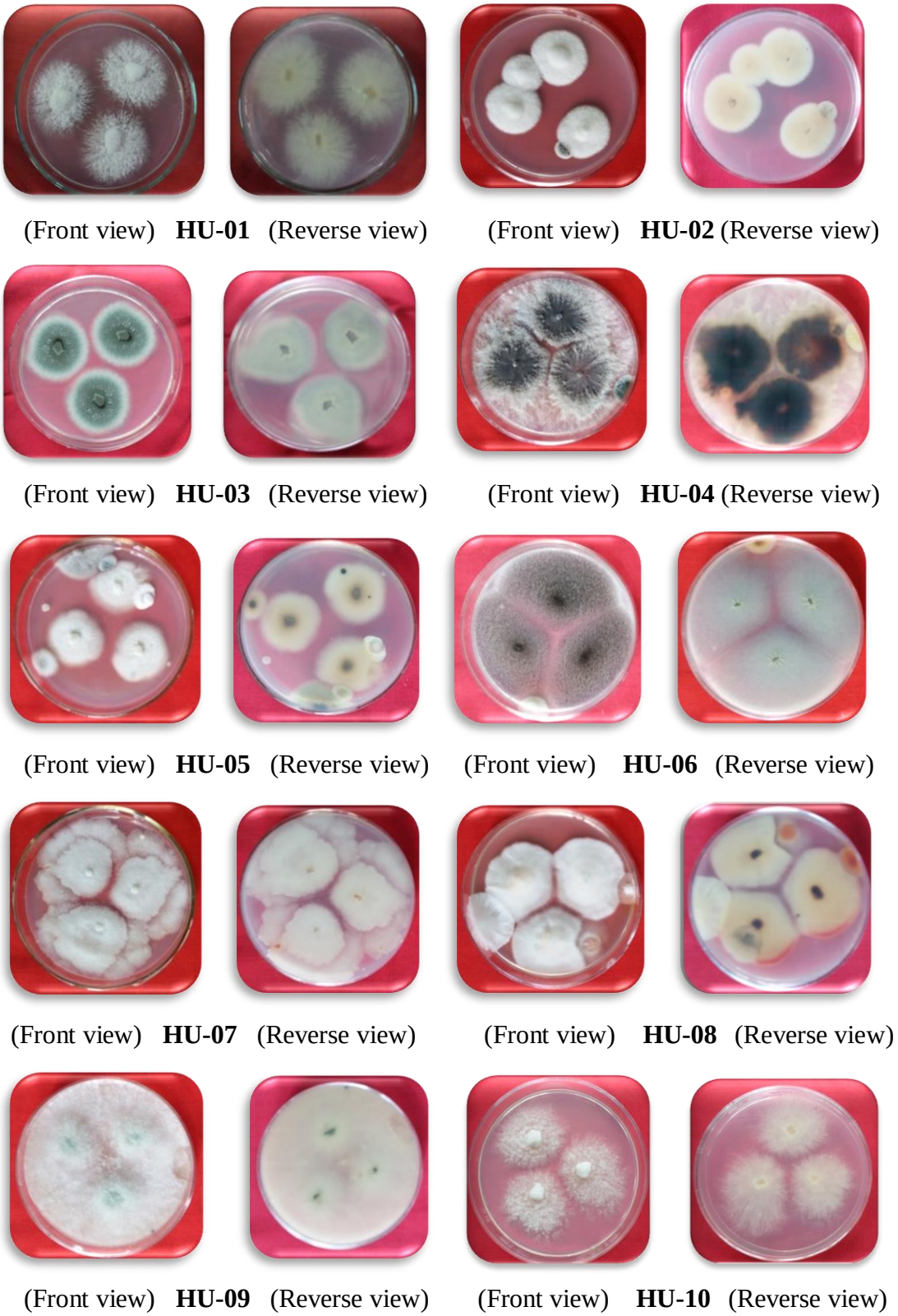


Figure (2). Morphological character of isolated soil fungi HU-01 to HU-10

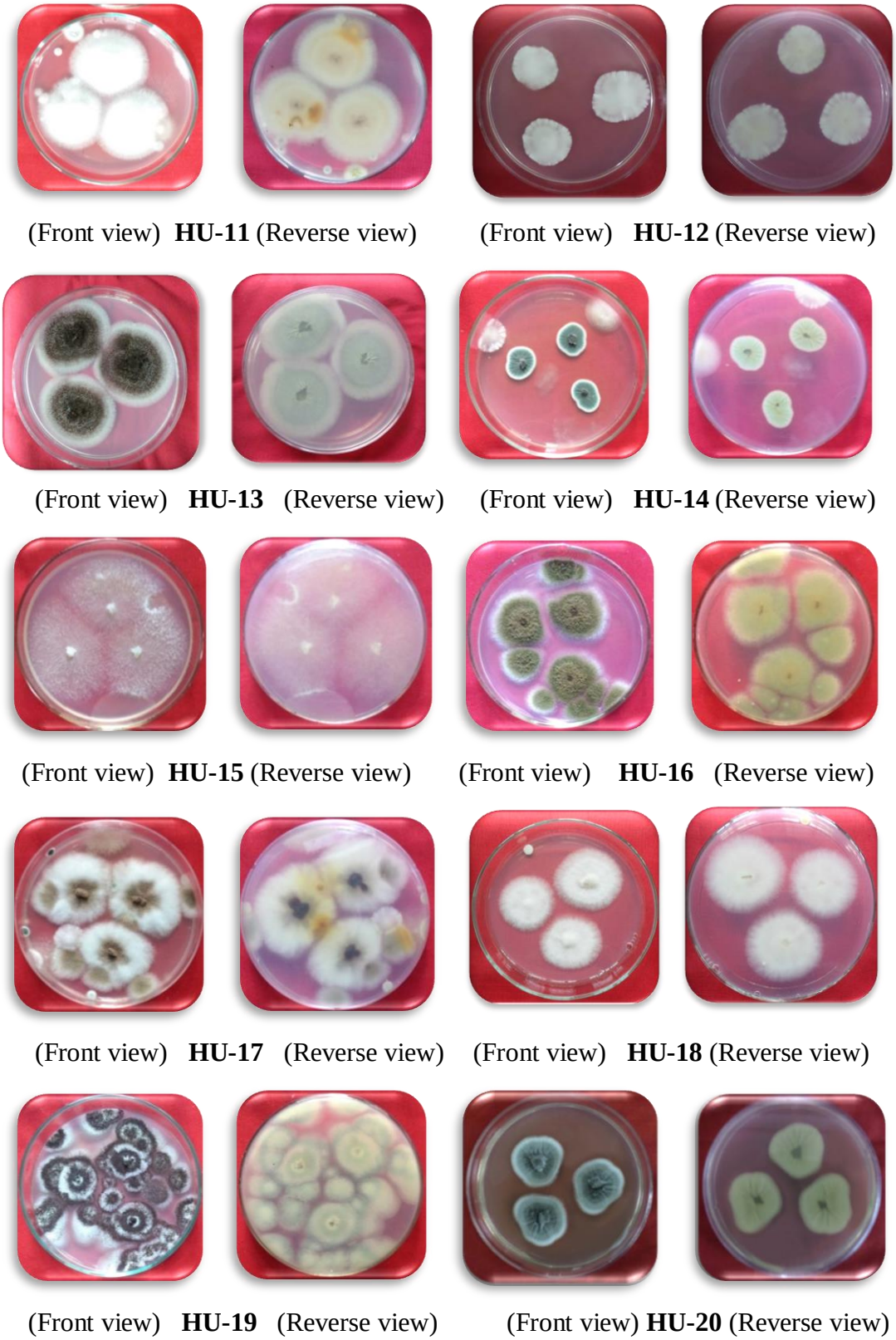


Figure (3). Morphological character of isolated soil fungi HU-11 to HU-20

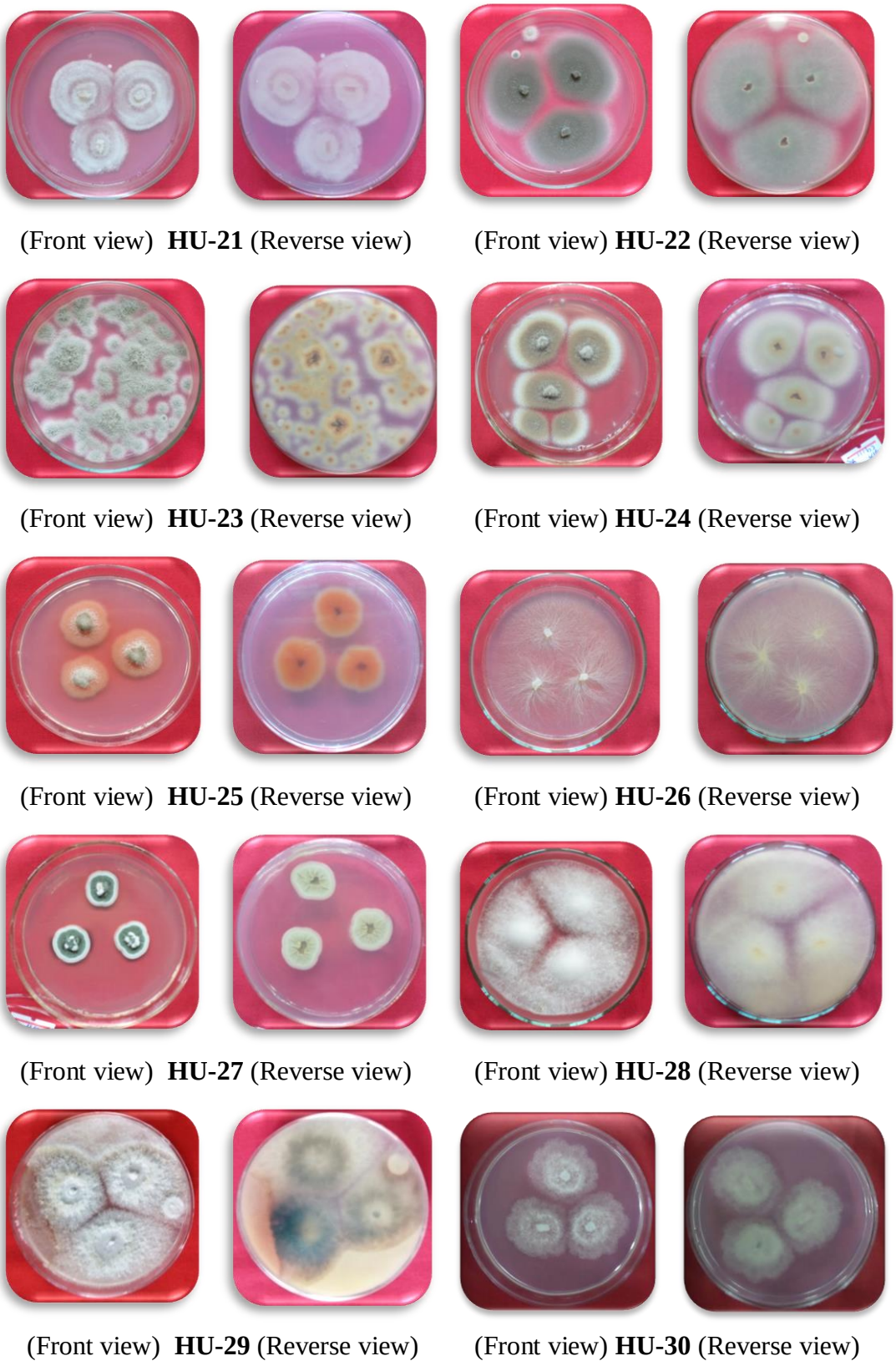
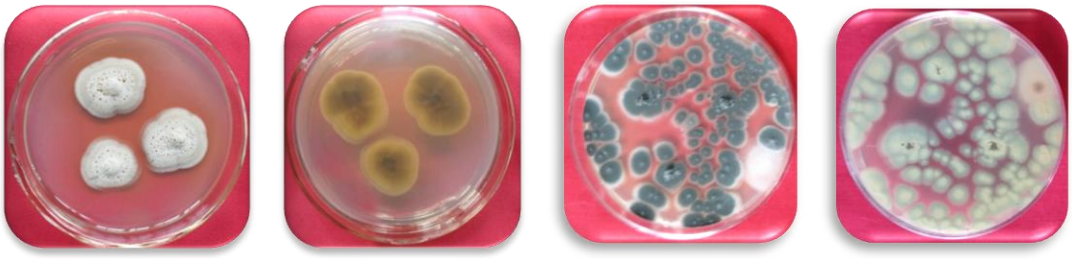


Figure (4). Morphological character of isolated soil fungi HU-21 to HU-30

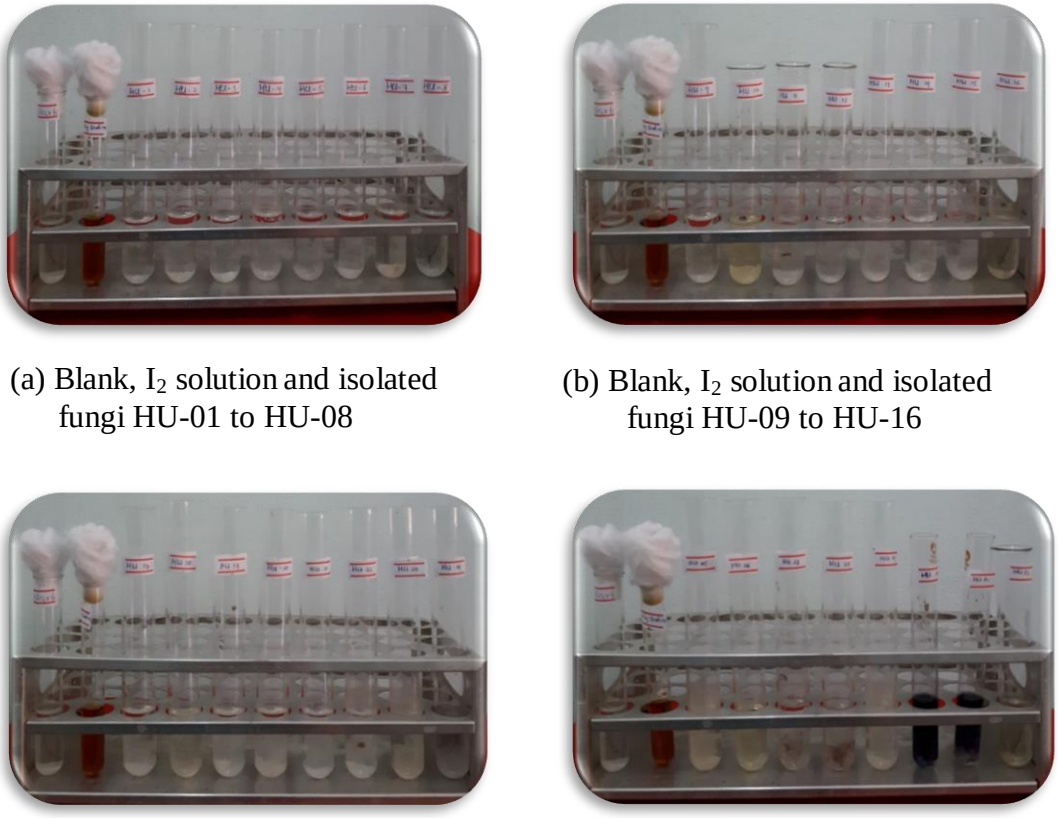


(Front view) **HU-31** (Reverse view) (Front view) **HU-32** (Reverse view)

Figure (5). Morphological character of isolated soil fungi HU-31 to HU-32

Starch Hydrolysis Activities of Isolated Soil Fungi HU-01 to HU-32 (NITE, 2004)

Isolated soil fungi HU-01 to HU-32 were tested for starch hydrolysis activity. Amylases are enzymes that break down starch or glycogen. In this study, except HU-30 and HU-31, thirty soil fungi did not change the color and these soil fungi may produce amylase enzyme or hydrolyze the starch. The experimental data were shown in figure (6) and table (4).



(a) Blank, I_2 solution and isolated fungi HU-01 to HU-08

(b) Blank, I_2 solution and isolated fungi HU-09 to HU-16

(c) Blank, I_2 solution and isolated fungi HU-17 to HU-24

(d) Blank, I_2 solution and isolated fungi HU-25 to HU-32

Figure (6). Starch hydrolysis activities of isolated soil fungi HU-01 to HU-32

Table (4). Starch Hydrolysis Activities of Isolated Soil Fungi HU-01 to HU-32

Fungi No.	Amylase enzyme Activity (min)	Fungi No.	Amylase enzyme Activity (min)
HU-01	3	HU-17	+
HU-02	10	HU-18	4
HU-03	9	HU-19	2
HU-04	+	HU-20	3
HU-05	28	HU-21	2
HU-06	6	HU-22	3
HU-07	+	HU-23	+
HU-08	+	HU-24	35
HU-09	5	HU-25	7
HU-10	+	HU-26	+
HU-11	+	HU-27	+
HU-12	9	HU-28	40
HU-13	11	HU-29	+
HU-14	+	HU-30	ND
HU-15	+	HU-31	ND
HU-16	10	HU-32	+

(+) No change in color (Highly amylase enzyme activity)

- Change in colour (within 1 hour)

- ND (not detected)

Discussion and Conclusion

Many different species of fungi inhabit the soil, especially near the soil surface where aerobic conditions prevail (Saranraj and Stella, 2013). In this study, the samples were taken near the tree by digging 4 inches depth under the soil. Soil texture represents one of the most important factors influencing the structure of microbial communities as well as, pH, cation exchange capacity, and organic matter content, can affect microbial community structure directly by providing a suitable habitat for specific microorganisms which in turn making a maximum degradation process (Najmadeen, *et al*., 2010). Soil properties like pH, organic matter and moisture content etc., affects the density and diversity of microbes in the soil (Pietikainen *et al.,)*. Therefore, it is important to study the relation between soil physicochemical properties and abundance of indigenous microorganisms.

In this study, the physicochemical properties of soil used for isolation of microbial species were analyzed. From the texture analysis, clay and clay loam were generally observed. The values of soil pH were found to be in the range from 4.99 to 6.36. This indicated that the seven soil samples were slightly acidic (pH 6.01-6.36), moderately acidic (pH 5.53-5.67) and strongly acidic (pH 4.99). Most of the fungi grow within the pH range 4-

8; whereas many fungi grow over a wider range, and a few have been reported to have a narrower range.

In this investigation period, seven soil samples were isolated by soil dilution method. Three bacteria and thirty two fungi (HU-01 to HU-32) were isolated by using soil dilution method from seven different soil samples. Soil fungi were cultured on LCA medium in incubated for one to seven days at room temperature. Pure colonies were inoculated into slant culture containing in PGA medium.

Thirty two isolated soil fungi were tested for starch hydrolysis activity. In this study, pure fungi HU-4, HU-7, HU-8, HU-10, HU-11, HU-14, HU-15, HU-17, HU-23, HU-26, HU-27, HU- 29 and HU-32 showed the highest effective because these fungi did not change the blue colour. Pure fungi HU-1to HU-3, HU-5, HU-6, HU-12, HU-13, HU-16, HU-23 to HU-25, HU-28, HU-30 and HU-31 showed the moderately effective because these fungi changed the blue colour. However, the blue colour was discharged within one hour. Pure fungi HU-30 and HU-31 showed the ineffective because these fungi change the blue colour within hours. The result data were shown in Figure 6 and Table 4.

In conclusion, amylase enzyme has received a great deal of attention because of their economic and technological significance. Because of the importance of amylases, isolation of new microorganisms suitable for amylase production could provide potential new sources of the enzyme. In this study, thirty two soil fungi were isolated; thirty fungi showed the amylase enzyme activities. Therefore, potential fungus was selected for the further optimization and characterization of amylase enzyme should be studied.

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