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SCREENING OF AMYLOLYTIC FUNGI FROM DIFFERENT SOURCES

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Abstract

In this study, amylolytic fungi were screened from various sources such as bread, *Daucus carota* L. (carrot), *Musa* sp. (banana), *Sechium edule* Sw. (chayote), *Vitis vinifera* L. (grape) and *Cucurbita maxima* Duch. (pumpkin). and soil. Isolated fungi were cultured on the glucose yeast peptone agar medium (GYP medium) for 5 to 11 days. Amylolytic activity was screened as sharp and distinct clear zone using with Gram's iodine solution. Three different amylolytic fungi were directly taken from bread (*Amblyosporium* sp.(1) , *Aspergillus* sp. (1), *Amblyosporium* sp. (2)), four different strains from carrot (*Aspergillus* sp. (2), *Aspergillus* sp. (3), *Aspergillus* sp. (4), *Aspergillus* sp. (5)), 1 strain from banana (*Botrytis* sp.), 1 strain from chayote (*Aspergillus* sp. (6)), 2 strains from grape (*Aspergillus* sp. (7), *Aspergillus* sp. (8)), 1 strain from pumpkin (*Penicillium* sp.) and 4 strains from soil (*Aspergillus* sp. (9), *Aspergillus* sp. (10), *Aspergillus* sp. (11), *Paecilomyces* sp.), respectively. The total number of (16) amylolytic fungi were obtained.

Keywords: amylolytic fungi

Introduction

Enzymes that hydrolyze starch are known as amylases. α- Amylase catalyzes the first step in the digestion of starch, a main source of carbohydrate in the human diet. Without the enzymes in our digestive tract for example, it would take us about 50 years to digest a single meal. The first industrially amylase enzyme was produced from a fungal source in 1894, which was used for the treatment of digestive disorder (Crueger and Crueger, 1984). *Aspergillus oryzae* has been largely used in the production of food such as soy sauce, organic acid such as citric and acetic acids and commercial enzymes including α-amylase (Kammoun et. al., 2008). Fungal Amylase - α-amylases with a slightly different action pattern yield mostly maltose and some oligomers. They are an alternative to β-amylases for making maltose syrup. Fungal amylases are more heat labile than those from bacterial and plant sources (U Win, 2004).

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In this research, amylolytic fungi were isolated from different sources and screened their amylolytic activity with Gram's iodine method and they were identified and classified into genus level. The following aims and objectives of present work are:

(a) To isolate the amylolytic fungi from various sources

(b) To identify the amylolytic fungi from different sources into genus level

Meterials and Methods

Collecting samples from different sources

The soil samples were collected from University of Yangon campus. The diluted soil were cultured on starch agar medium (GYP) for isolation of amylolytic fungi. Bread, carrot, banana, chayote, grapes, pumpkin were bought from different markets. The samples are kept in room temperature for several days. After one week, fungi colonized on samples. These fungi are directly taken for isolation of amylolytic fungi on starch agar medium (GYP) and inoculated in room temperature for 5- 11days. The resulting fungi were routinely subcultures to get pure isolates and screened with Gram's iodine stain (Kasana, et al., 2008). Glass wares that used in this experiment were sterilized at 121° C in 15lbs/sq inch for 15 minutes.

Soil Dilution Method

Soil dilution method was used according to method of Johnson (1957). One gram of soil sample was placed in a 300 ml conical flask. Water was added to the soil so that a total volume of 100 ml was reached; the suspension was stirred and shaken for 30 minutes. One ml of soil sample was transferred immediately through successive 9 ml sterile water in test tubes until the desired final dilution was reached.

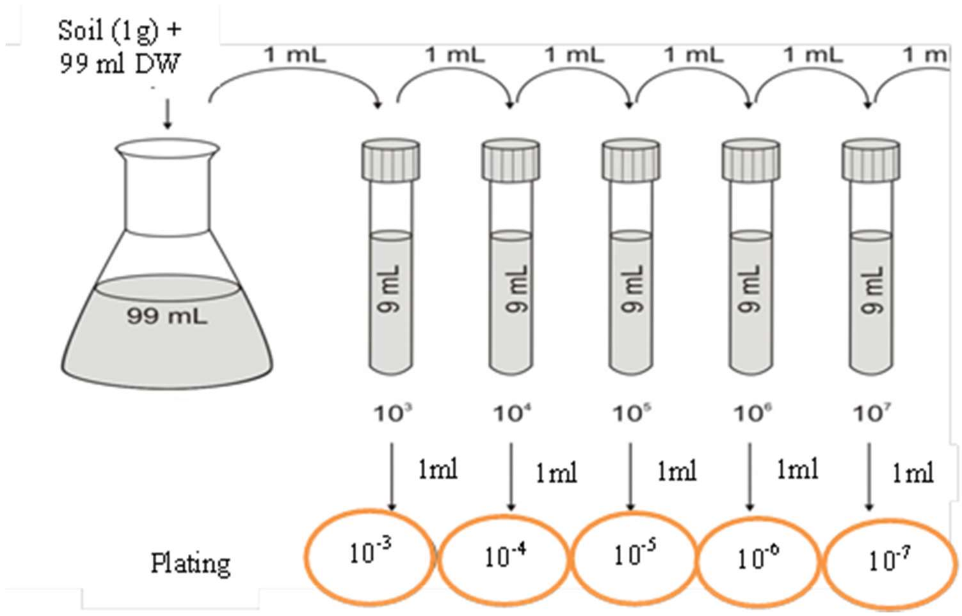


Figure.1 Soil Dilution Method (Johnson, 1957)

Preparation of GYP medium for preliminary test (GYP medium)

Glucose yeast peptone agar medium was used for amylolytic enzyme activity according to the method of Elsa and Bhimba (2012). GYP media was prepared by mixing soluble starch 2.00 g, glucose 0.1 g, yeast extract 0.01g, peptone 0.05 g, Agar 1.60 g, distilled water100.0 ml in a 250-ml conical flask. The pH of the solution was adjusted to 6.0.

Grams Iodine Stain

Grams Iodine Stain was used for screening amylolytic fungi by Kasana, et al. (2008).

Potassium iodide (KI)	- 2.0 g
Iodine	- 1.0 g
Distilled water	- 300 ml

Identification of fungi

Fungal isolates were identified on the basis of routine cultural, morphological and microscopic characters according to Watanabe (1937), Barnett (1969) and Ingold (1967).

Czapekdox agar medium

Czapekdox agar medium was used as stock culture medium or sub-culture medium for maintenance the fungus according to the method of Raper and Thom (1945). This medium were contain Sucrose 30.00 g, $NaNO_3$ 2.00 g, K_2HPO_4 1.00 g, $MgSO_4$ 0.50 g, KCL 0.50 g, $FeSO_4$ 0.10 g, Agar 20.00 g, Demineralized water 1000.0 ml. The pH of the solution is 7.0.

Results

Soil sample was collected from University of Yangon campus and starch rich sources such as bread, *Daucus carota* L. (carrot), *Musa* sp. (banana), *Sechium edule* Sw. (chayote), *Vitis vinifera* L. (grape) and *Cucurbita maxima* Duch. (pumpkin) were bought. Three different amylolytic fungi were directly taken from bread (*Amblyosporium* sp. (1), *Aspergillus* sp. (1), *Amblyosporium* sp. (2)), were shown in figure-(3), (4), (5). Four different strains were directly taken from carrot (*Aspergillus* sp. (2), *Aspergillus* sp. (3), *Aspergillus* sp. (4), *Aspergillus* sp. (5)), were shown in figure-(7), (8), (9), (10). One strain was isolated from banana (*Botrytis* sp.), was shown in figure – (12). One strain was isolated from chayote (*Aspergillus* sp. (6)), was shown in figure-(14). Two strains were isolated from grape (*Aspergillus* sp. (7), *Aspergillus* sp. (8)) were shown in figure-(16), (17). One strain was isolated from pumpkin (*Penicillium* sp.), was shown in figure – (19) and four strains were isolated from soil (*Aspergillus* sp. (9), *Aspergillus* sp. (10), *Aspergillus* sp. (11), *Paecilomyces* sp.), were shown in figure- (21), (22), (23), (24) respectively. The total number of (16) amylolytic fungi were obtained and showed in Table 1 and 2.

Table 1. Indentification of amylolytic fungi from different source

Sr. No.	Sampling sources	No. of amylolytic fungi	Genus
1	Bread	3	*Amblyosporium* sp. (1), *Aspergillus* sp.(1), *Amblyosporium* sp. (2)
2	Carrot	4	*Aspergillus* sp.(2), *Aspergillus* sp.(3), *Aspergillus* sp.(4), *Aspergillus* sp.(5)
3	Banana	1	*Botrytis* sp.
4	Chayote	1	*Aspergillus* sp.(6)
5	Grape	2	*Aspergillus* sp.(7), *Aspergillus* sp.(8)
6	Pumpkin	1	*Penicillium* sp.

Table 2. Identification of amylolytic fungi isolated from soil

Sr.No.	Soil concentration	Genus
1	SS-10^{-3} (1)	*Aspergillus* sp.(9)
2	SS-10^{-3} (2)	*Aspergillus* sp.(10)
3	SS-10^{-3} (3)	*Aspergillus* sp.(11)
4	SS-10^{-6} (1)	*Paecilomyces* sp.

Formation of fungal colonies on bread

Three different amylolytic fungi were directly taken from bread; they were *Amblyosporium* sp. (1), *Aspergillus* sp. (1), *Amblyosporium* sp.(2).

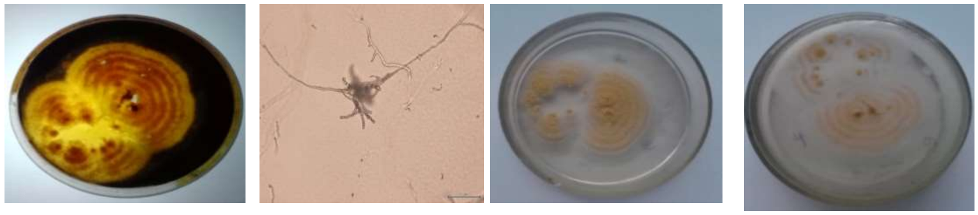
Various fungal colonies grow on bread.

Various fungal colonies grown on starch agar medium from bread

Figure 2- Various fungal colonies grow on bread and starch agar medium.

Characters of mycelium and spore formation of *Amblyosporium* sp. (1)

Mycelium pale to yellow-orange; conidiophores erect, septate, lower portion unbranched, bearing a number of irregular branches near or at the apex, from which conidial chains are formed by segmentation; conidia (arthrospores) 1-celled, hyaline or yellow-orange in mass, barrel-shaped, catenulate; saprophytic in soil or often growing on fleshy or woody basidiomycetes. The micrograph of *Amblyosporium* sp. (1) was shown in figure – (3).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Amblyosporium* sp. (1) (X 400)

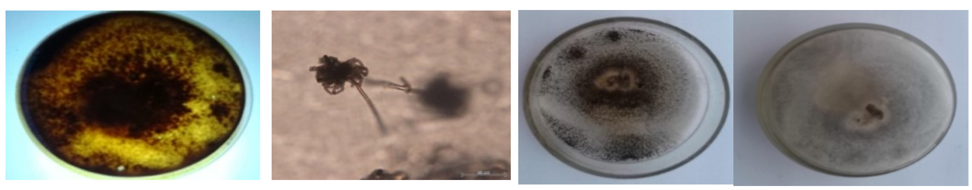
Pure fungal colony (pale yellow) 8 days old

Reverse view

Figure 3 - *Amblyosporium* sp. (1) isolated from bread

Characters of mycelium and spore formation of *Aspergillus* sp. (1)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides at the apex or radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, black colour in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (1) was shown in figure – (4).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (1) (X 400)

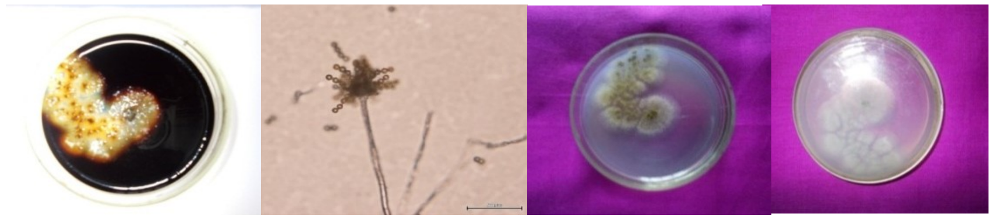
Pure fungal colony (black), 8 days old

Reverse view

Figure 4 - *Aspergillus* sp. (1) isolated from bread

Characters of mycelium and spore formation of *Amblyosporium* sp. (2)

Mycelium pale to yellow-orange; conidiophores erect, septate, lower portion unbranched, bearing a number of irregular branches near or at the apex, from which conidial chains are formed by segmentation; conidia (arthrospores) 1-celled, hyaline or yellow-orange in mass, barrel-shaped, catenulate; saprophytic in soil or often growing on fleshy or woody basidiomycetes. The micrograph of *Amblyosporium* sp. (2) was shown in figure – (5).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Pure fungal colony (yellow), 8 days old

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Reverse view

Figure 5 - *Amblyosporium* sp. (2) isolated from bread

Formation of fungal colonies on carrot

Four different strains were isolated from carrot, which are *Aspergillus* sp. (2), *Aspergillus* sp. (3), *Aspergillus* sp. (4), *Aspergillus* sp. (5).

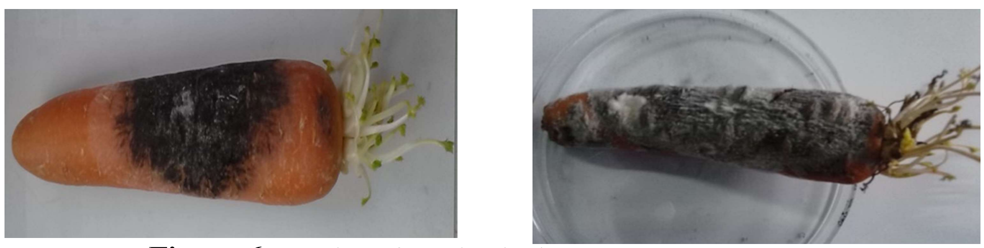
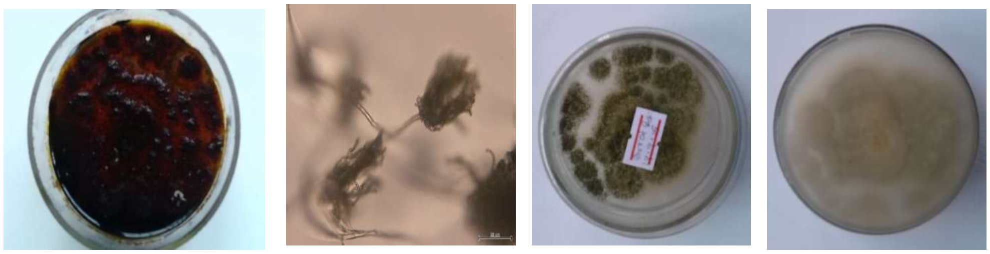


Figure 6 - Various fungal colonies grown on carrot.

Characters of mycelium and spore formation of *Aspergillus* sp. (2)

Conidiophores hyaline, simple, occasionally thick-walled, inflated globosely or ellipsoidally at the apex (called vesicles), bearing spore heads composed of catenulate conidia borne on uniseriate phialides on vesicles: conidial heads dark green, loosely columnar. Conidia phialosporous, pale brown to yellowish brown, globose, delicately rough at the surface. The micrograph of *Aspergillus* sp. (2) was shown in figure – (7).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution	Micrograph of *Aspergillus* sp. (2) (X 400)	Pure fungal colony (yellowish brown), 5days old	Reverse view

Figure 7 - *Aspergillus* sp. (2) isolated from

Characters of mycelium and spore formation of *Aspergillus* sp. (3)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides at the entire surface; conidia (phialospores) 1-celled, globose, black colour in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (3) was shown in figure – (8).

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Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution	Micrograph of *Aspergillus* sp. (3) (X 400)	Pure fungal colony (black), 5 days old	Reverse view

Figure 8 - *Aspergillus* sp. (3) isolated from carrot.

Characters of mycelium and spore formation of *Aspergillus* sp. (4)

In the conidial (*Aspergillus*) stage the conidiophore is erect and unbranched, and its apex swells into a globular head, from this there bud out a number of projections (phialides) each of which produces a growing chain of conidia with the youngest at the bottom, spores are dry, easily detached by air currents, culture of this species is bright yellow because of the pigmentation of the conidia, saprophytes in the soil and on decaying vegetable matter. The micrograph of *Aspergillus* sp. (4) was shown in figure – (9).

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (4) (X400)

Pure fungal colony (yellow), 5days old

Reverse view

Figure 9 - *Aspergillus* sp. (4) isolated from carrot.

Characters of mycelium and spore formation of *Aspergillus* sp. (5)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides at the apex or radiating from the apex; conidia (phialospores) 1-celled, globose, often variously colored in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (5) was shown in figure – (10).

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (5) (X400)

Pure fungal colony (bright green), 5 days old

Figure 10- *Aspergillus* sp. (5) isolated from carrot.

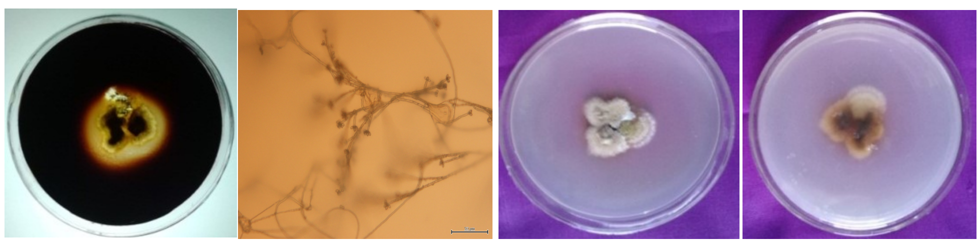
Formation of fungal colonies on banana

One amylolytic fungi strain was isolated from banana, it was *Botrytis* sp.

Figure 11 - Fungi grown on banana

Characters of mycelium and spore formation of *Botrytis* sp.

Conidiophores tall, slender, determinate, pigmented, branched irregularly in upper portion, apical cells enlarge or rounded, bearing clusters of conidia; conidia (botryoblastospores) hyaline or gray in mass, ovoid; black irregular sclerotia often present; causing "gray mold" on many plants or saprophytic. The micrograph of *Botrytis* sp. was shown in figure – (12).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution	Micrograph of *botrytis* sp. (X400)	Pure fungal colony (white), 6 days old	Reverse view

Figure 12 - *Botrytis* sp. isolated from banana.

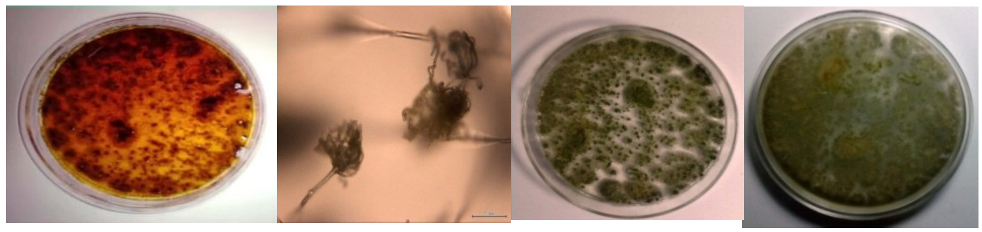
Formation of fungal colonies on chayote

One amylolytic fungi strain was isolated from chayote; it is *Aspergillus* sp. (6).

Figure 13 - Fungi grown on chayote

Characters of mycelium and spore formation of *Aspergillus* sp. (6)

Conidiophores erect, simple, and rough in the surface, with foot cells basally, inflated at the apex forming globose vesicles, bearing radiate conidial heads composed of catenulate conidia borne on uniseriate or rarely biseriate phialides: conidial heads yellowish green, radiate, columnar. Conidia phialosporous, pale green, globose, echinulate. The micrograph of *Aspergillus* sp. (6) was shown in figure – (14).



Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution | Micrograph of *Aspergillus* sp. (6) (X400) | Pure fungal colony (green) 7 days old | Reverse view

Figure 14 - *Aspergillus* sp. (6) isolated from chayote.

Formation of fungal colonies on grape

Two amylolytic fungi strains were isolated from grapes which are *Aspergillus* sp. (7) and *Aspergillus* sp. (8).

Figure 15 - Fungi grow on grape

Characters of mycelium and spore formation of *Aspergillus* sp. (7)

Conidiophores hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically, composed of catenulate conidia (over 15 conidia/chain) borne on uniseriate or biseriate phialides on pale brown, globose vesicles and phialides acutely tapered at apex. Conidia phialosporous, brown, black in mass, globose, minutely echinulate. The micrograph of *Aspergillus* sp. (7) was shown in figure- (16).

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution	Micrograph of *Aspergillus* sp. (7) (X400)	Pure fungal colony (black color), 11 days old	Reverse view

Figure 16 - *Aspergillus* sp. (7) isolated from grape.

Characters of mycelium and spore formation of *Aspergillus* sp. (8)

Conidiophores hyaline or pale brown, erect, simple, thick-walled, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically, composed of catenulate conidia (over 15 conidia/chain) borne on uniseriate or biseriate phialides on pale brown, globose vesicles and phialides acutely tapered at apex. Conidia phialosporous, brown, black in mass, globose, minutely echinulate. The micrograph of *Aspergillus* sp. (8) was shown in figure- (17).

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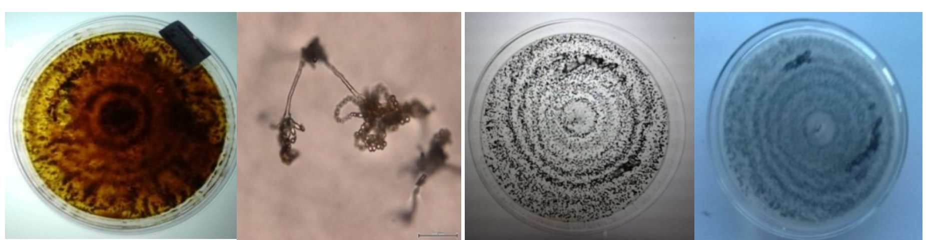

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution	Micrograph of *Aspergillus* sp. (8) X400	Pure fungal colony (black color), 11 days old	Reverse view

Figure 17 - *Aspergillus* sp. (8) isolated from grape

Formation of fungal colonies on pumpkin

One amylolytic fungi strain was isolated from pumpkin, it was *Penicillium sp.*.

Figure 18 - Fungal colony grown on pumpkin

Characters of mycelium and spore formation of *Penicillium* sp.

Conidiophores hyaline, branched penicillately at the apexes with verticillate metula, terminal phialides and catenulate conidia on each phialide, forming rather divergent conidial heads: Conidia phialosporous, subglobose, 1-celled, and smooth. The micrograph of *Penicillium* sp. was shown in figure-(19).

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution	Micrograph of *Penicillium* sp. (X400)	Pure fungal colony (green inside and white periphery), 7 days old	Reverse view

Figure 19 - *Penicillium* sp. isolated from pumpkin

Isolation of fungi from soil

There are six different kinds of fungi were isolated from soil serial dilution (10^{-2} to 10^{-7}). Some amylolytic fungi can be seen in every dilution plates and pure four different fungi were isolated from soil dilution 10^{-3} and 10^{-6}.

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Figure 20 - Various fungal colonies grown on starch agar medium from soil dilution 10^{-3}

Character of mycelium and spore formation of *Aspergillus* sp. (9)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, often variously colored in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (9) was shown in figure- (21).

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution

Micrograph of *Aspergillus* sp. (9) (X400)

Pure fungal colony from soil dilution 10^{-3} (dark green) 5 days old

Reverse view

Figure 21 - *Aspergillus* sp. (9) isolated from soil dilution 10^{-3}

Character of mycelium and spore formation of *Aspergillus* sp. (10)

Conidiophores hyaline, simple, occasionally thick-walled, inflated globosely or ellipsoidally at the apex (called vesicles), bearing spore heads composed of catenulate conidia borne on uniseriate phialides on vesicles: conidial heads dark green, loosely columnar. Conidia phialosporous, pale brown to yellowish brown, globose, delicately rough at the surface. The micrograph of *Aspergillus* sp. (10) was shown in figure- (22).

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Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution | Micrograph of *Aspergillus* sp. (10) (X400) | Pure fungal colony from soil dilution 10^{-3} (Yellowish brown), 5 days old | Reverse view

Figure 22 - *Aspergillus* sp. (10) isolated from soil dilution 10^{-3}

Character of mycelium and spore formation of *Aspergillus* sp. (11)

Conidiophores upright, simple, terminating in a globose swelling, bearing phialides radiating from the apex or the entire surface; conidia (phialospores) 1-celled, globose, often variously colored in mass, in dry basipetal chains. The micrograph of *Aspergillus* sp. (8) was shown in figure-(23).

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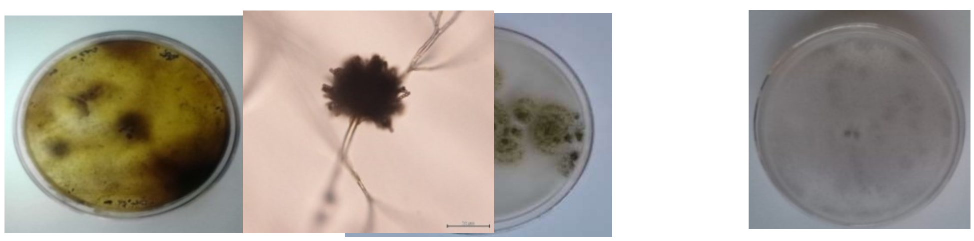

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution | Micrograph of *Aspergillus* sp (11) (X400) | Pure fungal colony (black), 5 days old | Reverse view

Figure 23 - *Aspergillus* sp. (11) isolated from soil dilution 10^{-3}.

Characters of mycelium and spore formation of *Paecilomyces* sp.

Conidiophores (phialides) simple or rarely branched, erect, hyaline, 1-septate basally, tapering from base toward apex, bearing over 10 catenulate conidia apically. Conidia phialosporous, hyaline, ovate, 1-celled, slightly apiculate at one end. The micrograph of *Paecilomyces* sp. was shown in figure- (24).

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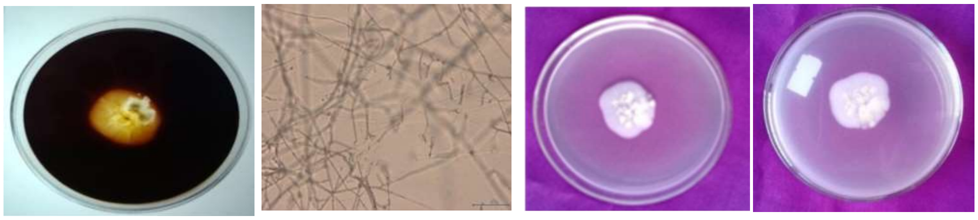

Preliminary test of enzyme secretion on starch agar medium stained with Gram's iodine solution | Micrograph of *Paecilomyces* sp. (X400) | Pure fungal colony (white), 7 days old | Reverse view

Figure 24 - *Paecilomyces* sp. isolated from soil dilution 10^{-6}.

Discussion and Conclusion

In this study, amylolytic fungi were screened from different sources. Fungi which possess amylolytic activity were selected based upon the clear zone around the fungal colony. Twelve different amylolytic fungi were directly collected from bread, *Daucus carota* L. (carrot), *Musa* sp. (banana), *Sechium edule* Sw. (chayote), *Vitis vinifera* L. (grape) and *Cucurbita maxima* Duch. (pumpkin) The appearance of clear zone can be seen after the flooding with Gram's iodine.This is the evidence that the fungi showed amylolytic activity due to hydrolysis of starch by α-amylase enzyme.

Four amylolytic strains were obtained from soil sample which was collected from Botanical garden at University of Yangon campus by soil dilution method. According to the results, Gram iodine is the best plate assay method for determining amylolytic activity and gives the best result with prominent and distinct zone within 3-5 minutes. Amylolytic fungi in the plate break down the polysaccharide which surrounded by fungal colony were exhausted with polysaccharides so monosaccharide and disaccharides were remained. Florencio *et al*., (2012) reported that mono and disaccharide cannot bind with dyes efficiently, so clear zone around the colony can be seen. In the present investigation, the total number of amylolytic fungi were (16) strains which were (11) strains of *Aspergillus,* (2) strains of *Amblyosporium*, (1) strain of *Penicillium,* (1) strain of *Botrytis*, and (1) strain of *Paecilomyces* were isolated from different sources. Among them, the best amylolytic activity of fungi will be used for the future investigation.

Acknowledgements

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