Isolation, Screening, and Identification of Amylase-Producing Fungi from Selected Rice Mills for Starch Degradation

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Abstract

This study was conducted to isolate, screen, characterize, and identify amylase-producing fungi from selected rice mills in Iloilo for the degradation of starch. Amylases are classes of enzymes which have useful applications in various commercial industries with the prospering industry in Iloilo City. There were three rice mill sampling sites considered in this study. Serial dilution and spread plate method were used to isolate and characterize soil fungi from the three rice mills. A total of seven amylolytic soil fungi were isolated and identified as degraders of starch. Iodine's test after seventy-two hours of incubation was used to screen the amylolytic activity among the isolates. The range of starch degradation was described from slightly effective to very effective results. Slide culture technique was then, used for characterization and identification of active isolates, and were identified under the genera of *Aspergillus, Penecillum* and *Alternaria* species. Based on the result of the study, solate 7, *Penicillium spp.* had the highest zone of amylolytic activity while isolate 3, *Aspergillus spp.* had the lowest zone of amylolytic activity. There was also a significant difference on the zone of amylolytic activity that existed among the fungi isolates after 72 hours of incubation using Iodine test.

*Keywords***:** amylase, amylolytic activity, fungi, rice mill, starch

Nowadays, amylases (alpha-amylases, beta-amylases and glucoamylases) represent one of the most important enzyme groups within the field of biotechnology (Bansode, 2010). Starch hydrolytic enzymes comprise 30 % of the world's enzyme consumption (Sanghvi, Koyani & Rajput, 2011).

Cost of substrates on which amylases producing microbes can be cultivated has always been an important factor in production cost (Aunstrup, 1977). Urban areas have abundant supply of biological or industrial wastes that are generated from mills, bakery processing factories, flour processing units, and other cottage industries annually. At present, these wastes constitute a nuisance to the environment as they are dumped at dumpsites indiscriminately (Adeniran & Abiose, 2009). Exploitation of such wastes as substrates for amylases production can be a viable option.

Amylases are classes of enzymes which have useful applications in the food, brewing, textile, detergent and pharmaceutical industries. They are mainly employed for starch liquefaction to reduce their viscosity, production of maltose, oligosaccharide mixtures, high fructose syrup, and maltotetraose syrup (Adeniran & Abiose, 2009). In detergents production, they are applied to improve cleaning effect and are used for starch de-sizing in textile industry (Radley, 1976; Aiyer, 2005).

Recent discoveries on the use of microorganism as sources of industrially relevant enzymes have led to on increased interest in the application of microbial enzymes in various industrial processes (Varalakshmi et al., 2009). Today, a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry (Gupta, Gupta, Modi, & Yadava, 2003). This study can contribute as a source of income-generating project for the production of amylase from indigenous fungi in industrial wastes in the vicinity. Purification and screening of amylases from these fungi as efficient degraders of starch can be further patented and be commercially available not only in the growing local commercial industries in Iloilo City but also at the regional and national levels.

Agricultural countries such as the Philippines have abundant supply of agricultural wastes that are generated from rice mills and other cottage industries annually. At present, these wastes constitute a nuisance to the environment as they are left at the sites indiscriminately. Exploitation of such wastes as substrates for amylases production can be a viable option. This study makes use of the available waste materials present in the vicinity. Thus, the rice mill sites were considered in the study because one of the prime environmental hazards of the rice mill effluents is due to their foul smell which develops in the discharged effluents with the passage of time (Manogari, Daniel, & Krastanov, 2008) and degraded by various microorganisms such as fungi. The soil mixed with rice mill wastes can be a potential source of amylolytic fungi.

Amylases have many applications in various commercial industries with the prospering industry in Iloilo City. Specifically it can benefit the industrial sectors. These include bread and baking industry, starch liquefaction and saccharification for chemical industries, textile desizing for textile industries, paper industry, detergent industry, analysis in medical and clinical chemistry, and food and pharmaceutical industries (Metin et al., 2010).

Microbial enzymes, from fungal sources taken from organic wastes such as rice hull have good source of enzymes, can be associated with their broad biochemical diversity, rapid growth, limited space required for cell cultivation and the ease with which the enzymes can be genetically manipulated to generate new enzymes for various application (Nigam et al., 2012). Therefore, it is believed that search for microbial enzymes could be directed towards biological wastes that commonly serve indigenous populations for industrial purposes growing in natural environmental setting or interesting endemic locations as they are expected to harbor microbes with promising amylase-producing enzymes.

Thus, this study was conducted to (1) isolate amylase-producing fungi from selected rice mills; (2) screen the isolated fungi for degradation of starch; (3) characterize the amylase-producing fungi and identify the species of characterized amylase-producing fungi that can degrade starch; and (4) describe and assess the amylolytic activity among the starch-degrading fungal isolates.

Materials and Methods

Description of the Sampling Site

Tigbauan is situated in Iloilo, Region 6, Philippines, seated about 18 km west of province capital Iloilo City, and its geographical coordinates are 10° 56' 0" North, 122° 27' 0" E. The land area has a total 8,889 has. 93.78% is devoted to agriculture and allied activities (Torres et al., 2004). Around 60 hectares are allocated to urban uses are residential, commercial, and institutional. The municipality has a flat terrain. The plain is approximately 57% of the total area, covering about 6,667 has along the Northwest site of the Sibalom River. Along the boundaries of Guimbal, Leon, and Tubungan are rolling hills covering an area, of about 1,518 has. The highest point is 200 meters above sea level and lowest is one (1) meter above sea level. The town has two main rivers, Sibalom and Tacuyong River. It has two seasons, the wet and the dry: the first, starts early June and ends early November while the second, starts early of November and ends in the month of May. Influenced by these two seasons, the municipality has two basic industries- agriculture and fishing.

Soil Sample Collection

Soil samples were collected in containers under sterile conditions from soil with organic or cellulose-based or starch-based wastes from the three separate rice mills in Tigbauan, Iloilo. The three rice mills were provided with codes from A to C. Rice hulls that cover the top layer of soil were carefully removed with the aid of sterile spatula. Soil samples were dug from 3 to 4 cm depth. Samples were transferred to sterile plastic bags and maintained in aseptic conditions. Soil sample were characterized based on some selected physicochemical parameters as shown in Table 2.

Preparation of Starch-potato Dextrose Agar Plates

In one liter solution, 23 g/L of potato dextrose agar and 2.76 g/L of starch (Vaseekaran, Balakumar, & Arasaratnam, 2010) were thoroughly mixed. A 150 mg per liter of solution of ciprofloxacin was added to inhibit the growth of bacteria. It was mixed, constantly stirred and heated until completely dissolved. It was then autoclaved at 15 psi, 15 minutes at 121^0 C. Afterwards, it was then transferred aseptically to sterile Petri dishes.

Isolation and Initial Screening and Purification of Amylase-Producing Fungi

A procedure by Alariya, Sethi, Gupta and Lal Gupta (2013) was followed with modification. One gram of soil sample was serially diluted in sterilized normal saline solution. Serial dilutions from 10^{-1} to 10^{-10} dilutions were made and all even dilutions were spread-plated on starch agar plates by spreading 0.1ml of the diluted sample. Then, the plates were kept for incubation at 37°C for 72 hours. After incubation, the individual plate was flooded with Gram's iodine for 30 seconds on the starch agar plate. According to Kaur, Kaur, Samyal, and Ahmed (2012), the presence of blue color around the growth indicates negative result, and a clear zone of hydrolysis around the growth indicates positive result. The isolates which produced clear zones of hydrolysis were considered as amylase producers. Single colonies with zones of hydrolysis of different morphological characteristics such as size, shape, color, elevation and margin were then isolated from the different plates. The fungal isolates were further sub-cultured to obtain pure culture. Pure fungal isolates were maintained in starch agar slants at 4ºC.

Starch Iodine Confirmatory Test of Amylase-Producing Fungi

Pure isolates of amylase-producing fungi were cultured in nutrient broth solution and incubated for 24 hours. The 24-hour old broth culture of each isolate was matched with 0.5 McFarland Standard. After obtaining the desired turbidity, 0.1 ml of the culture was spread-plated to a plain PDA plate and incubated for 72 hours. After incubation, a block of the isolate measuring $10mm^2$ was placed at the center of the surface of the starch agar plate. The starch agar plate was then incubated for 5 days. After 5 days of incubation, the individual plate was flooded with Gram's iodine with dropper for 30 seconds. A clear zone of hydrolysis around the growth indicates positive result. The zone of amylolysis was measured with a millimeter ruler. This was done in three trials with three replicates each. Pure fungal isolates for rice mill soils were the experimental groups of the study. The positive control groups consist of the *Trichoderma harzianum, Aspergillus oryzae*, and *Aspergillus niger* while the negative control group was plain agar disc only.

Data Collection Procedure

Measurement of zone of amylolytic activity. After 72 hours of incubation, the zone of amylolytic activity was described and interpreted based on the descriptive scale using the formula:

$$\text{Zone of Amylolytic Activity} = \frac{X-Y}{Z}$$

where: X= Diameter of Petri dish (85mm)
Y= Diameter of agar disc with fungi (10mm)
Z= number of descriptive scales (4).

Table 1 shows the level of effectiveness of the zone of amyloytic activity of the isolated fungi isolates based on the formula as indicated above. The level of effectiveness was arbitrarily described from inactive to very active along with the numerical scales presented.

Table 1

Level of Effectiveness of Zone of Amylolytic Activity

Descriptive Scale	Interpretation
56.33-85.00mm	Very active
37.57-56.32mm	Moderately active
18.76-37.56mm	Partially active
0-18.75mm	Inactive

Cultural characterization of amylase-producing fungi. The purified colonies were described and noted based on colonial characteristics on agar media as seen with the naked eye as described by Crous, Verkley, Groenewald, and Samson (2009). The colonies were described according to form, elevation, margin, colony pigmentation or color, agar pigmentation, texture, colony shape, texture, spore accumulation (present or absent), appearance in culture anamorph in mold form or teleomorph with the presence of spores), presence of liquid at the surface of the medium, and spore accumulation.

Preparation of slides for microscopic examination of amylase-producing fungi isolates. Slide preparation procedure by Harley (2011) was followed with slight modification. The surface of the slides and cover slips were flame-sterilized. Wooden sticks were used to transfer the sufficient melted paraffin to each slide to support the cover slip about 1mm over the surface of the slide. Then the paraffin was cooled and hardened. Cover slip was heated sufficiently to form a seal when set over the hardened paraffin. Potato dextrose agar tube was melted and cooled. Sterile pipette was used to transfer 0.5 ml of the culture suspension with the agar tube. The inoculated agar was flooded under the cover slip of the prepared slide to half fill the chamber before the agar hardens. Two circles of paper towel were moistened to firm the bottom of the petri dish and the one slide culture was placed. Slide was supported above the moist paper by 2 wooden sticks. The Petri dishes were incubated for 2-4 days. After incubation, the slides were stained with lactophenol cotton blue. The slides were observed under low power objective and high power objective. The organization of the hyphae, structure, and organization of the spores were noted for proper characterization of these fungi.

Characterization and identification of amylase-producing fungi. Identification and characterization of the different fungal isolates were observed under the microscope utilizing a set of dichotomous keys and a set of picture keys by Ainsworth, Sparrow, and Sussman (1973) and Arx (1981).

Data Collection Analysis

Descriptive and Inferential Statistics were both employed in the study.

For the descriptive data analysis, (a) mean and standard deviation were used to determine the zone of amylolytic activity of amylase-producing fungi; (b) A scale was used to evaluate the mean zone of amylolytic activity in the three rice mill soil bacterial isolates as indicated in Table 1.

For inferential data analysis, One-Way Analysis of Variance was used to determine any significant difference on the zone of amylolytic activity among the fungi in isolates in three Rice Mill soils Tigbauan, Iloilo. The level of significance was set at 0.05 alpha level.

Results and Discussion

Table 2 shows the summary of the three rice mill sites and the corresponding physicochemical characteristics of the soil present. There were 12 fungal isolates with amylolytic activity taken from the rice mill sites. Rice mill A (f=7) has the most number of fungal isolates while rice mill B (f=2) has the least number of isolates.

Table 2

Characteristics of Rice Mill Sites and Frequency of Fungal Isolates

Rice Mill	Site and Geographical Coordinates	Area (sq. m.)	Date and Time of Collection	Soil Characteristics			Total Isolates
				Texture	Color	pH	
A	Bagumbayan, Tigbauan Iloilo N 10^0 41‘ 25.743“ E 122^0 23’ 14.127”	1,500 m^2	Feb. 2, 2016; 9:03AM	Medium sand; Granular, day, loose soft	Brownish black	slightly acidic (5)	7
B	Bitas, Tigbauan, Iloilo N 10^0 41‘ 58.767“ E 122^0 23’ 34.52”	300 m^2	Feb. 2, 2016; 9:24AM	Very coarse sand; Structureless, single grain, very friable	Grayish brown	slightly acidic (5)	2
C	Cordova Sur, Tigbauan, Iloilo N 10^0 41‘ 48.385“ E 122^0 23’ 44.733”	2000 m^2	Feb. 2, 2016; 9:58AM	Medium sand; Granular, moist, friable	Brownish black	slightly acidic (5)	3

Table 3 shows the characteristics of the fungal isolates in the three selected rice mill sites. Colony and cell characteristics of the isolates show that they belong to the three genera of fungi based on characterization. *Aspergillus* and *Penicillum* were the common soil fungi present in rice mill sites and were responsible for the degradation of starch in rice mill.

Table 3

Colony and Cell Characteristics of Fungal Isolates in Three Rice Mill Sites

Isolate	Rice Mill Site	Colony Characteristics	Cell and Spore Characteristics
Isolate No. 1: *Aspergillus spp*.	Rice Mills A and B soils	Teleomorph; Colony pigmentation: Dark olive green with white margin; agar pigmentation white; entire colony and dry texture with spores; Reverse characteristics were white, entire, dry.	Spores 1-celled; Colonies, spores, and other tissues colorless or brightly colored; Spores produced in chains of spores break apart thoroughly when placed in a water mount. A few spores will remain together in a group, that can be assumed that they were originally in chains; Conidiophores with a swollen head or vesicle bearing bottle-shaped phialides.
Isolate No. 2: *Aspergillus spp*.	Rice Mills A and C soils	Teleomorph; Colony pigmentation: black with white margin; agar pigmentation white; entire colony and dry texture with spores; Reverse characteristics were white with yellow margin, entire, dry.	Spores 1-celled; Colonies, spores, and other tissues colorless or brightly colored; Spores produced in chains of spores break apart thoroughly when placed in a water mount. A few spores will remain together in a group, that can be assumed that they were originally in chains; Conidiophores with a swollen head or vesicle bearing bottle-shaped phialides.
Isolate No. 3: *Aspergillus spp*.	Rice Mills A and C soils	Teleomorph; Colony pigmentation: Navajo with white margin; agar pigmentation white; undulate colony and dry texture with spores; Reverse characteristics were White with yellow margin, peru with dark brown spot, undulate, dry.	Spores 1-celled; Colonies, spores, and other tissues colorless or brightly colored; Spores produced in chains of spores break apart thoroughly when placed in a water mount. A few spores will remain together in a group, that can be assumed that they were originally in chains; Conidiophores with a swollen head or vesicle bearing bottle-shaped phialides.
Isolate No. 4: *Penicillium spp*.	Rice Mill A soil only	Teleomorph; Colony pigmentation: white margin; agar pigmentation white; entire colony and dry texture with spores; Reverse characteristics were orange, entire, dry.	Spores 1-celled; spores produced in chains;spores in unbranched chains, borne from clusters of cylindrical to bottle-shaped phialides; colonies usually green; a few spores remain together in a group, that can be assumed that they were originally in chains.
Isolate No. 5: *Alternaria spp*.	Rice Mill A soil only	Teleomorph; Colony pigmentation: Dark olive green with white margin; agar pigmentation white; entire colony and dry texture with spores; Reverse characteristics were white, entire, dry.	Spores 1-celled; Colonies, spores, and other tissues colorless or brightly colored; Spores produced in chains of spores break apart thoroughly when placed in a water mount. A few spores will remain together in a group, that can be assumed that they were originally in chains; Conidiophores with a swollen head or vesicle bearing bottle-shaped phialides.
Isolate No. 6: *Penicillium spp*.	Rice Mill A soil only	Teleomorph; Colony pigmentation: dark green; agar pigmentation white; entire colony and dry texture with spores; Reverse characteristics were white, entire, dry.	Spores 1-celled; spores produced in chains;spores in unbranched chains, borne from clusters of cylindrical to bottle-shaped phialides; colonies usually green; a few spores remain together in a group, that can be assumed that they were originally in chains.
Isolate No. 7: *Penicillium spp*.	Rice Mills A, B and C soils	Teleomorph; Colony pigmentation: orange with white margin; agar pigmentation white; undulate colony and dry texture with spores; Reverse characteristics were orange, undulate, dry.	Spores 1-celled; spores produced in chains;spores in unbranched chains, borne from clusters of cylindrical to bottle-shaped phialides; colonies usually green; a few spores remain together in a group, that can be assumed that they were originally in chains.

Table 4 shows the amylolytic activity of the fungal isolates from the three selected rice mill sites. Rice mill A has seven fungal isolates, rice mill B has two fungal isolates, and rice mill C has three fungal isolates. Fungal isolates amylolytic activity ranged from slightly effective to very effective. Isolate 7 (M=77.56 mm) has the highest zone of amylolytic activity which is present among the three rice mill sites while fungal isolate 3 (M=32.89 mm) has the lowest zone of amylolytic activity and present in rice mill A and rice mill C sites.

Table 4

Zone of Amylolytic Activity of Fungal Isolates in Selected Rice Mill Sites

Group	Fungi	SD	Mean Zone of Amylolytic Activity (mm)	Description
Positive Controls	*T. harzianum*	10.187	42.56	Moderately effective
	A. niger	2.538	41.78	Moderately effective
	A. oryzae	6.245	34.67	Slightly effective
Negative Control	Agar disc only	0.000	0.00	Not effective
Experimental	Isolate 1[A]	13.525	64.22	Very effective
	Isolate 2[B]	3.723	43.11	Moderately effective
	Isolate 3[B]	11.515	32.89	Slightly effective
	Isolate 4[C]	12.845	54.67	Moderately effective
	Isolate 5[C]	10.416	48.33	Moderately effective
	Isolate 6[C]	9.340	70.33	Very effective
	Isolate 7[D]	5.703	77.56	Very effective

Note: [A]From Rice Mills A & B; [B]From rice mills A & C; [C]From rice mill A only; [D]From all rice mills

Table 5 shows the One-Way ANOVA result on the zone of amylolytic activity of fungi isolates in the three rice mill sites. There is a significant difference on the zone of amylolytic activity among the isolates, $F(10,88)=50.564$, $p=0.00<0.05$, partial eta squared=0.851. This may imply as indicated in Table 2, a significant variation on the amylolytic activity among the soil fungi isolates. The effect size is large indicating the effect on the hydrolysis of starch is due to the amylase produced among the fungi isolates from rice mill sites. Based on the Least Significant Difference (LSD) for pair wise comparison, there is a significant difference among the isolates (Isolates 1,2,3,4,5,6 and 7 and *Aspergillus oryzae*; Isolates 1,2,3,4,6 and 7 and *Trichoderma harzianum* and *Aspergillus oryzae*) the positive control groups and negative control group, $p < 0.05$.

Table 5

One-Way Analysis of Variance on the Zone of Amylolytic Activity

Group	Sum of Squares	df	Mean Square	F	Sig.	Eta Squared Value
Between Groups	40079.838	10	4007.984	50.564*	.000	0.851
Within Groups	6975.333	88	79.265			
Total	47055.172	98				

Note: $*p < 0.05$.

It has been demonstrated in the result of the study that filamentous fungi isolated from rice mill sites secrete enzymes that can degrade starch and cellulose naturally. The ability of filamentous fungi to secrete large amounts of extracellular protein has made them well suited for the industrial enzyme production (Abdullah, 2005). It is also parallel to the result of the identified fungi are starch degraders such as *Trichoderma sp*., *Penicillium griseoreseum*, and *Alternaria sp*. (Arnesen, Eriksen, Olsen & Jensen, 1998; Ray 2001; Poornima *et al*., 2008). Many species of *Aspergillus* such as *A. niger*, *A. tamarii, A. awamori* and *A. oryzae* have received most attention to obtain many kinds of hydrolytic enzymes like alpha amylase (Abdullah, 2005).

Related studies on fungal amylase focused on amylolytic activity of fungi from agricultural wastes by Adeniran and Abiose (2009), amylolytic activities of fungi species on the screening medium adjusted to different ph by Balkan, Aydoðdu, Balkan and Ertan (2012) and earlier studies focused on rice bran, cassava peels, and sugar cane straw (Ali, Mahmood, Alam, & Hossain, 1989; Sani et al., 1992; Ortega et al., 1993; Akinyosoye, Adeniran, & Oboh, 2004).

The result of the study may indicate that amylase can be obtained from several fungi species isolated from rice mill sites. However, fungi have gained much attention because of their availability and high productivity which are also amenable to genetic manipulation (Sidkey et al., 2011; Balkan, et. al., 2012). Many fungi had been found to be good source of amylolytic enzymes. The literature indicates that amylases of fungal origin are more stable than those of bacterial origin (Sanghvi et al., 2011). Filamentous fungi have been well known for the starch and cellulose degrading enzymes in which they naturally secrete (Abdullah, 2005). The ability of filamentous fungi to secrete large amounts of extracellular protein has made them well suited for the industrial enzyme production (Arnesen et al., 1998; Ray 2001; Poornima et al., 2008).

Soil fungal population was observed to vary among different sites. The result of the study is consistent with the result of Jena , Tayung, Rath and Parida (2015) that *Aspergillus* species were found to be the most dominant species among the community in all sampling sites. The dominant species of *Aspergillus* could be in any of the following species of *Aspergillus* such as *A. alliceus*, *A. flavus*, *A. fornscecous*, *A. humicola*, *A. luchuensis*, *A. niger*, *A. awamori*, *A. tamarine*, *A. terrus*, *A. wentii*, *A. ustus,*and *A. panamensis*. In terrestrial ecosystem, fungi are important componentsof microbial communities and play vital role in maintaining soil health and productivity. Studies suggest that soil fungal diversity and composition is governed by wide range of biotic and abiotic factors (Lauber *et al.*, 2008; Barcenas-Moreno et al., 2009; Hawkes et al., 2011). According to Das and Dkhar (2011) microbial flora is dependent on moisture content, pH, temperature, nitrogen, and carbon sources. Fungi in soil demonstrate a variety of life styles, which is an indicative of the variation and dynamism in the soil habitat (Dighton, White, & Oudemans, 2005). Groups of different fungi utilize different carbon resources, with the result that very little organic carbon remains in soil, or passes through the soil in water.

A significant variation of the amylolytic activity among the fungi isolates may be due to efficiency of enzyme systems that microbes thrive well in inhospitable habitats (Cooke, 1979). With mechanisms for adapting to environmental extremes and for the utilization of their trophic niche, the ability of microorganisms to produce extracellular enzymes is of great survival value (Gopinath, Anbu, & Hilda, 2005).

In this study, the isolation, screening and identification methods were quick and efficient to assess several good amylase-producing fungi from rice mill sites. The isolates displaying the greatest starch-degrading activity were distinguished. Finding naturally occurring starch-degrading fungi from the environment is important in the field of bio-refining to help overcome costly hurdles in the bio-refining process. The amylase positive isolates could be an integral part of future work to develop good amylases or produce efficient amylase producing systems such as microbial consortia which can be used for industry.

Conclusion and Recommendation

Seven isolates of amylase producing fungi were isolated in the three rice mill sites in Tigbauan, Iloilo. Minerva Rice Mill has the highest fungi isolates (f=7) while Eking Rice Mill (f=2) has least amylolytic fungi isolates. There were three genera of amylolytic fungi recovered from the area under the genera of *Aspergillus, Penicillum,* and *Alternaria.* Isolate 1 (*Aspergillus*) and isolate 7 (*Penicillum*) have the highest amyloytic activity, M=64.22 mm and M=77.56 mm which were described as 'very effective' starch degraders, respectively. There is also a significant difference on the zone of amyloytic activity among the fungi isolates after 72 hours of incubation and Iodine's test, p (0.000) < 0.05.

The use of these starch-degrading fungi as bio-inoculants can be incorporated to enhance organic matter decomposition in soil to increase soil fertility and to minimize the fertilizer application. They can also be applied to reduce environmental pollution and to promotes sustainable agriculture. Identification was done at the cellular and up to the genus level. Thus, it is recommended to do molecular characterization and analysis of the fungal isolates to identify the species of the amylase-producing fungi. Other method of amylolytic assays such as spectrophotometric analysis on the amount of enzyme produced by each isolate is recommended. Isolation and purification of amylase should also be done. The enzyme activity and its specific activity should be determined as influenced by various physico-chemical factors. Thus, optimum parameters required for the stability and better activity of enzyme should also be studied. Further improvement in the performance of starch-degrading fungi can be imparted by mutagenesis and protein engineering techniques for the better industrial applications.

Future works should include the enzyme efficiency increase to work under extremely adverse industrial conditions by genetically engineering the microbes or their products in order to achieve high production potentials and wide industrial applications. It is recommended to try other sources aside from the rice hulls since most of the rice waste are converted to organic fertilizers. Since rice mill A has the most number of fungal isolates with amylolytic activity, it is recommended to fully utilize this site for its promising amylase producing fungi.

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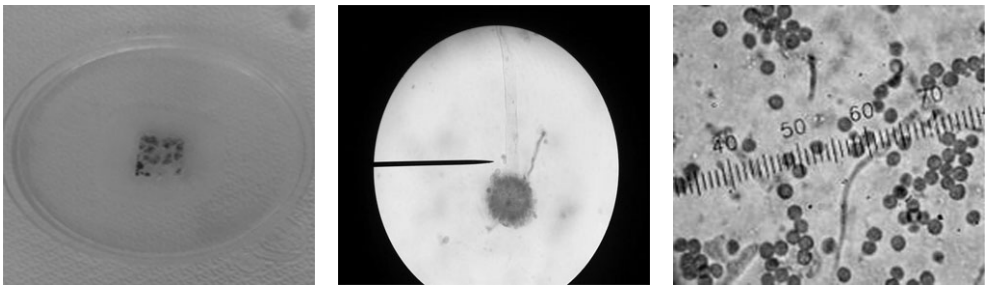
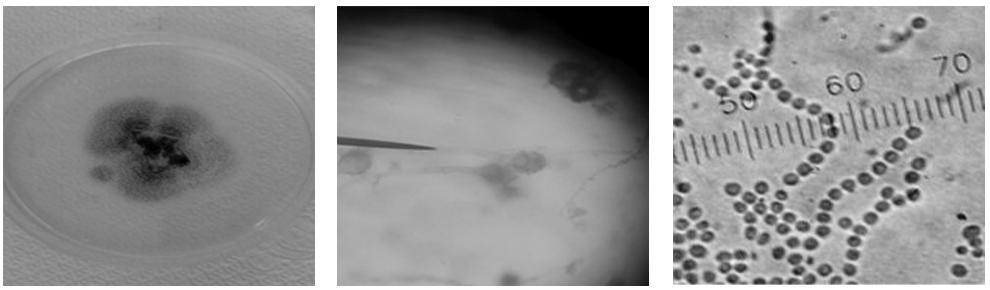
Appendix A

Photographs of Colonial and Cellular Characteristics of Amylolytic Fungal Isolates in Rice Mill Sites



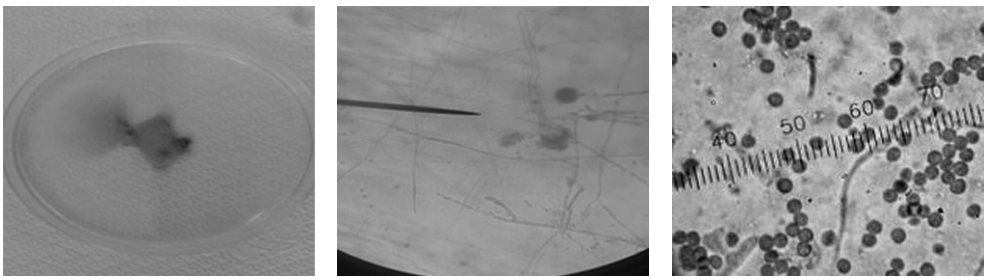
Top View of Colony Fungal Vegetative Parts Fungal Spores

Figure 1. *Aspergillus spp*. in Rice Mills A and B soils.



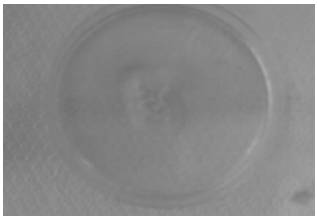
Top View of Colony Fungal Vegetative Parts Fungal Spores

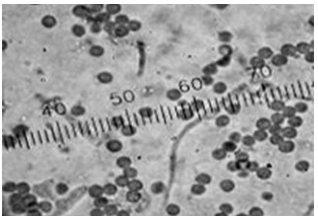
Figure 2. *Aspergillus spp*. in Rice Mills A and C soils.



Top View of Colony Fungal Vegetative Parts Fungal Spores

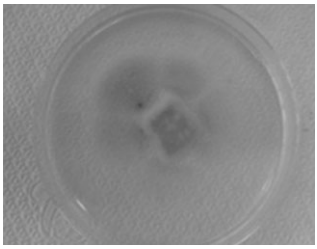
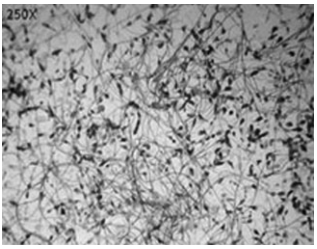
Figure 3. *Aspergillus spp*. in Rice Mills A and C soils.

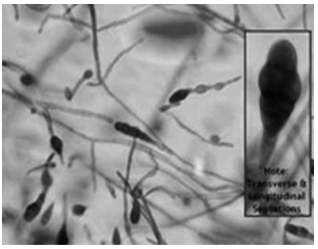




Top View of Colony Fungal Vegetative Parts Fungal Spores

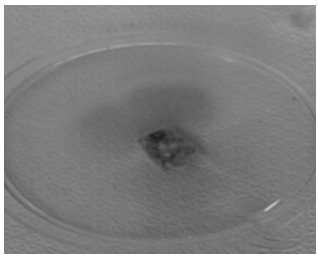
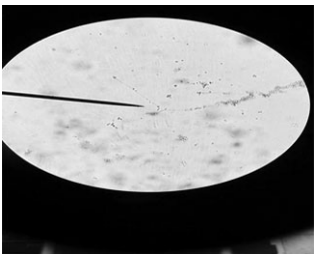
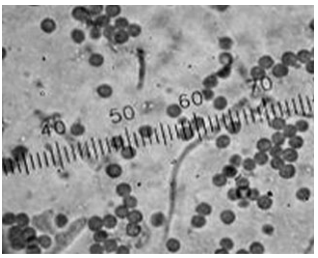
Figure 4. *Penicillium spp*. in Rice Mill A soil.



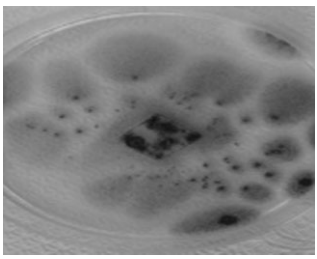
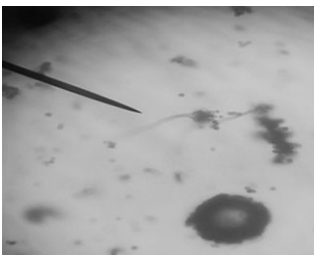
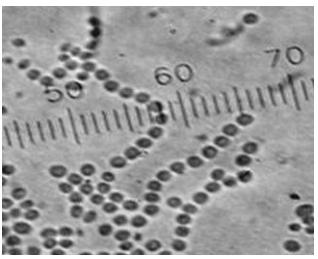
Top View of Colony Fungal Vegetative Parts Fungal Spores

Figure 5. *Alternaria spp*. in Rice Mill A soil.

Top View of Colony Fungal Vegetative Parts Fungal Spores

Figure 6. *Penicillium spp*. in Rice Mill A soil.

Top View of Colony Fungal Vegetative Parts Fungal Spores

Figure 7. *Penicillium spp*. in Rice Mills A, B and C soils.