

# Screening of Amylolytic Activity and Morphological Characterization of Amylase-Producing Bacteria from Rice Mill Soil in Tigbauan, Iloilo

Jayvee J. Jore<sup>1,2</sup>, Mary Rose C. Faderogao<sup>1</sup>, Rey G. Tantiado<sup>1</sup>

<sup>1</sup>West Visayas State University, Luna St., La Paz, Iloilo City, Philippines

<sup>2</sup>Central Philippine University, Jaro, Iloilo City, Philippines

Corresponding author: jayvee.jore@wvsu.edu.ph

## Abstract

This study was conducted to determine and characterize the populations of amylase-producing bacteria from the three rice mill soils in Tigbauan, Iloilo. Soil samples were collected, characterized, and assessed for the presence of amylase-producing bacteria. Serial dilutions, spread plate method of soil samples on starch agar plates, and incubation for 72 hours were done to detect the presence of zone of amylolytic activity. Soil samples in three rice mills in Tigbauan, Iloilo have the presence of amylase-producing bacteria. Bacterial isolates had very active amylolytic activity after 72 hours of incubation and vary in their colony morphology and cell characteristics. It showed no significant difference on the amylolytic activity among the isolates in the three rice mill sites, indicating comparative amylolytic capability. Biochemical tests and molecular characterization of the bacteria should be done to identify the species of the bacterial isolates.

**Keywords:** Amylase, bacteria, rice mill, starch-iodine test

## INTRODUCTION

Microorganisms are known to be the main source of enzyme production. In nature, enzymes are essential for the biodegradation process of compounds (Pandey et al., 2000; Schmidt et al., 2002). In 1894, a microbial enzyme amylase was used as a drug to treat digestive disorders, and the first enzyme to be manufactured in industry (Pandey et al., 2000). Amylase is a group of enzymes used in industries that only cover about 30% of enzymes (Pandey et al., 2000). They have been a great source for commercial microbiology, including pharmaceuticals, liquefaction, or starch saccharification, detergents and textiles (commercially used to make size agents), fibers, paper, foods and even baking, haze clarifying in beer or fruit juice, animal feed processing, corn, and production of chocolate syrup (Leveque et al., 2000; Vijayalakshmi et al., 2012). Their perceived economic and technological importance paved the way for amylase being used as starch degrading enzymes to receive much attention. The biological synthesis of amylases was performed on by-products and agro-industrial waste such as starch to solve problems pertaining to pollution and obtain materials that are low-cost. Unique characteristics such as substrate specificity, activity pattern, optimal temperatures, major reaction products, and pH are industrial reactions that became a baseline for amylase application (Senthilkumar et al., 2012).

Various organisms like humans, plants and even microorganisms produce amylase. Fungi and bacteria secrete amylases outside the cell and carry out extracellular digestion (Cordeiro et al., 2003). When they decompose insoluble starch, maltose and glucose that are soluble products are absorbed into cells (Cordeiro et al., 2003). Although many microorganisms produce this enzyme, *Bacillus amyloliquifaciens*, *Bacillus licheniformis*, and *Bacillus subtilis* are the most used in industrial production (Brook et al., 1969). These bacteria are screened from natural resources such as soil, biogas plants, household waste, and can grow on cheap substrates such as rice shells from rice plants and produce enzymes at a stable rate without toxic substances (Bahadure et al., 2010). Amylase is one of the important enzymes used in microbiology, especially in starch hydrolysis. Although different sources (soils, animals, plants, and microorganisms) can produce amino acids, the productivity and thermostability of microbial amino acids are widely produced and used in industry (Burhan et al., 2003). Based on this concept, this study isolates bacteria that produce amylase from their immediate vicinity and is used for commercial and industrial purposes. Furthermore, *Bacillus subtilis* is widely studied for the development of various forms of amylase, such as

hemicellulose, beta-glucanase, and alpha-amylase (Aiba et al., 1983).

Despite the interest in various soil samples as a promising source of functional enzymes with amylolytic activity, few studies have considered the screening and identification of amylase-producing bacteria from rice mills sites specifically in the target site because it is one of the wide plantations of rice in the province of Iloilo. It is imperative to discover the presence of bacterial isolates with superior and beneficial enzymes with promising biotechnological applications. The present study may contribute to our understanding of rice mills as microbial resources of enzymes with the potential for scientific research and industrial applications. Hence, it is a must to robustly develop the domestic industry. Moreover, the investigation of new amylase-producing microbial resources remains a critical goal. In addition, further screening of bacterial amylases for specific adaptive characteristics in a unique natural environment should be a target (Anbu et al., 2017; Thapa et al., 2019). At present, bacterial amylases with distinctive characteristics isolated from aquatic environment have been well documented (Mesbah & Wiegel, 2014; Wu et al., 2018). On the contrary, soil-derived amylase-producing bacteria with promising enzymatic activities have received relatively little attention.

Thus, this study was conducted to isolate amylase-producing bacteria from rice mill soil in Tigbauan, Iloilo. Specifically, it has the following objectives: (1) to characterize morphologically the populations of amylase-producing bacteria from the three rice mill soils in Tigbauan, Iloilo and (2) determine significant difference on the number of amylase-producing bacteria from rice mill soil samples.

## MATERIALS AND METHODS

**Soil Sample Collection.** Soil samples were collected in containers under sterile conditions from soil in Tigbauan, Iloilo in 3 Rice mill sites (Figure 1). The three rice mill sites were summarized in Table 1 along with the soil characteristics and the number of amylase-producing bacteria present. Soil samples were collected from rice mill soil at 3 to 4 cm depth with the help of sterile spatula in three sites representing the three trials of sampling. Samples were transferred to sterile plastic bags and maintained in aseptic conditions.



**Figure 1.** The Different sampling site for Rice Mill in Tigbauan, Iloilo.

**Table 1.** Characteristics of Three Rice Mill Sites in Tigbauan, Iloilo

Rice Mill	Site and Geographical Coordinates	Elevation	Area (sq.m.)	Sample	Soil Characteristic				n	% Bacterial-isolates
					Texture	Color	pH	Others		
Minerva Rice Mill	Bagumbayan, Tigbauan, Iloilo N 10°42.600 E 122°31.520	34 m	1,500 m <sup>2</sup>	A	Medium Sand	Brownish Black	5	Granular, day, loose, soft	15	26.67% (f=4)
				B	Coarse Sand	Black	5	Granular, day, loose soft		26.67% (f=4)
				C	Clay	Brownish Black	5	Structure: Less massive, solid mass without aggregate, sticky		46.67% (f=7)
Eking Rice Mill	Bitas, Tigbauan, Iloilo N 10°21.683 E 122°3.239	27 m	500 m <sup>2</sup>	A	Very Coarse Sand	Grayish Brown	5	Structure: Less, single grain, very dryable	13	30.76% (f=4)
				B	Very Coarse Sand	Brownish Gray	5	Structure: Less, single grain, dry, loose		30.76% (f=4)
				C	Very Coarse Sand	Light Gray	5	Structure: dry, loose, slightly hard		38.46% (f=5)
Nulada Rice Mill	Cordova Sur, Tigbauan, Iloilo N 10°21.683 E 122°3.239	39 m	2,000 m <sup>2</sup>	A	Medium Sand	Brownish Black	5	Granular: moist, friable	7	28.57% (f=2)
				B	Medium Sand	Brownish Black	5	Structure: less, single grain, sand, moist, very friable		28.57% (f=2)
				C	Medium Sand	Brownish Black	5	Granular: moist		42.85% (f=3)

**Preparation of Starch-Nutrient Agar Plates.** In one liter solution, 23 g/L of nutrient agar and 2.76 g/L of starch (Vaseekaran et al., 2010) were thoroughly mixed. An antibiotic, 50 mg/ml fluconazole was added to inhibit the growth of fungi. These were completely mixed, constantly stirred, and heated until completely dissolved. These were autoclaved at 15 psi, 15

minutes at 121° C. These were then transferred aseptically to sterile petri dishes. Afterwards, these were allowed to cool for further uses.

**Preparation of Starch-Nutrient Agar Slants.** Five (5) ml of liquid sterile starch-nutrient agar solution was aseptically dispensed in a sterile 30 ml sterile vial with rubber cap. It was then immediately covered and allowed to solidify in a slant position. Agar slants in vials were then stored for further use.

**Isolation and Screening of Amylase-Producing Bacteria Using Starch Iodine Test.** A procedure by Alariya et al. (2013) was followed with modification. One gram of soil sample dissolved in 100 ml sterile normal saline solution was serially diluted from  $10^{-1}$  to  $10^{-10}$  dilutions. One hundred (100) microliters of the aliquot sample from even dilution tubes were spread to starch agar plates. After dilution, all plates were incubated at 37°C for 3 days. Later, starch agar in the individual plates were flooded with Gram's iodine for 30 seconds and observed for the presence of starch hydrolysis. Negative results were interpreted through the presence of blue color around the growth of colonies and a positive result showed clear zone of hydrolysis around the growth (Kaur et al., 2012). Isolation and screening of soil samples were done in three trials with three replicates each. The isolates produced clear zones of hydrolysis were considered, counted, isolated, and purified. Quantitative scoring of the individual plate initially screened from the serially diluted soil samples was scored from 1 to 5 wherein 1 = absence of zone of starch hydrolysis; 2 = > 1% but < 33% zone of starch hydrolysis; 3 = >33% but < 66% zone of starch hydrolysis; 4 = <100% but >66% zone of starch hydrolysis; and 5 = 100% zone of starch hydrolysis. This was done in three trials and replicates each.

**Purification of Bacterial Isolates.** After the initial screening of the bacterial isolates showing zone of starch hydrolysis, they were further subcultured to obtain pure culture. A loopful of bacterial isolate was repeatedly streaked in a new starch agar plate. After streaking, they incubated for 24 hours. The procedure of streaking and incubation was done three times to ensure purification of the isolate. Pure isolates on starch agar slants were maintained at 4°C overlaid with sterile mineral oil.

**Characterization of Amylase-producing Bacteria.** The isolates were observed by the naked eye to obtain the colony morphology i.e., color, shape, size, nature of colony and pigmentation (Harley, 2005). After incubation, single colonies of bacteria showed different morphological characteristics

such as size, shape, color, elevation, and margin were characterized from the different plates. The bacterial isolates were gram stained and observed under a high-power objective. The purified colonies were described and noted based on colony characteristics on agar media as seen with the naked eye.

**Gram Staining of Amylase-Producing Bacteria.** A procedure by Moyes et al. (2009) was followed. In a clean glass slide, a smear of culture was placed, air-dried and gently heated over a flame. After passing the heat, the smear was covered with crystal violet for one minute and washed gently with tap water. Gram's iodine was added to the smear for 1 min and then washed with tap water. Ninety-five percent ethanol was added to decolorize and remove the first stain, crystal violet. After complete decolorization, it was counterstained with safranin and washed with tap water, air dried, and viewed under high power objective. Gram-positive cells retained the violet color while pink to red color indicates a gram-negative organism.

**Statistical Data Analysis.** Frequency was used to determine the number of isolates which are amylase producers and the capacity to degrade starch. The mean  $\pm$  SD was used to assess the zone of starch hydrolysis. A scale was used to evaluate the mean zone of starch hydrolysis in the three-rice mill soils of purified bacterial isolates screen for amylolytic activity (Table 2) modified from Skrabanja et al. (2001).

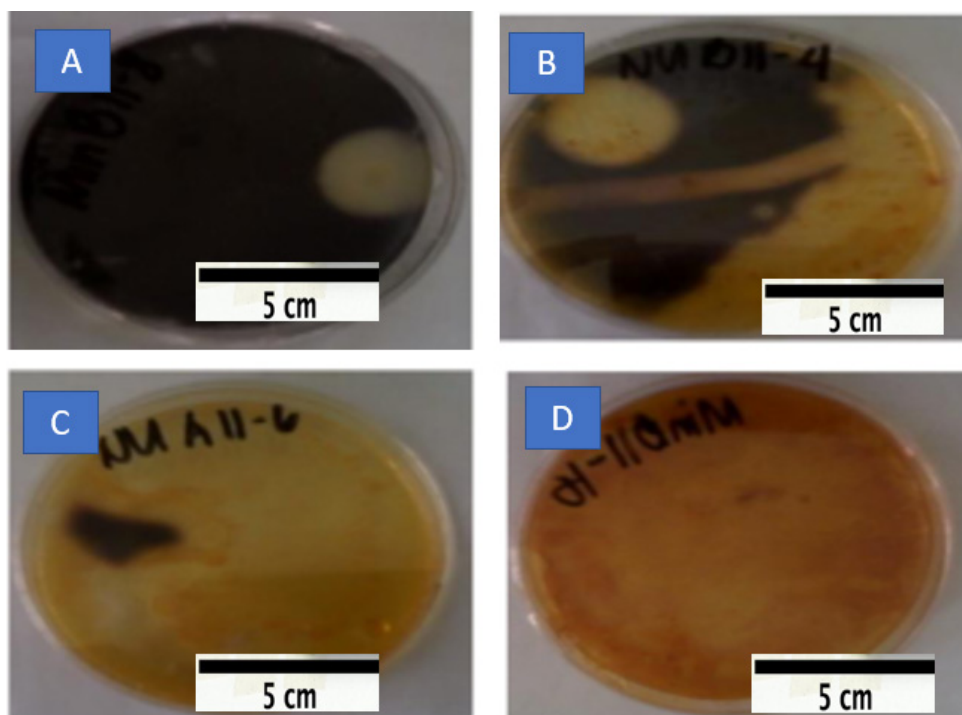
**Table 2.** Scale for the Average Zone of Starch Hydrolysis

Mean Score	Description
0-1.22	Inactive
1.23-2.48	Slightly Active
2.49-3.74	Moderately Active
3.75-5.0	Very Active

One-Way Analysis of Variance was used to determine any significant difference among the three-rice mill soil Tigbauan, Iloilo of amylase-producing bacterial isolates for starch degradation set at  $\alpha=0.05$ .

## RESULTS

The populations of amylase-producing bacteria from the three rice mill soils in Tigbauan, Iloilo and their amylytic capability shows that the three different rice mills were described as very active in their amylytic activity (Table 3). Bacterial isolates from Minerva Rice Mill show the highest frequency. Figure 2 shows the different sizes of the zones of amylytic activity. The One-Way Analysis of Variance on the number of amylase-producing bacteria from rice mill soil in Tigbauan, Iloilo shows no significant difference on the population of amylase-producing bacteria from the three rice mill soils in Tigbauan, Iloilo,  $F(2,34) = 0.081$ ,  $p(0.922) > 0.05$ . This means that isolates from the three rice mills may differ in the soil characteristics and the number of isolates, the bacterial isolates may have the same amylytic capability. The eta squared = 0.005 is very small. A small effect size can be attributed to the other factors that can affect the rate of amylase production among the isolates. These factors may pH, temperature, and type of substrates. The condition of the environment may also affect the growth and physiology of the bacteria isolates present in the production of amylase to degrade rice hulls and other complex carbohydrates in the environment.



**Figure 2.** Different zones of amylytic activity from three sampling sites. A indicates inactive amylytic activity. B. Slightly active amylytic activity. C. Moderately active amylytic activity. D. Very active amylytic activity.

**Table 3.** Populations of Amylase-Producing Bacteria from the Three Rice Mill Soil in Tigbauan, Iloilo and their Amylolytic Capacity.

Rice Mill Site	Number of Bacterial Present	Mean Zone of Amylolytic Activity $\pm$ SD	Description
Minerva Rice Mill	15	4.200 <sup>a</sup> $\pm$ 1.082	Very Active
Eking Rice Mill	13	4.077 <sup>a</sup> $\pm$ 1.187	Very Active
Nulada Rice Mill	7	4.000 <sup>a</sup> $\pm$ 1.290	Very Active

<sup>a</sup>P >0.05 is not significant.

The characteristics of amylase-producing bacteria from Minerva Rice Mill soil in Tigbauan, Iloilo shows that all bacteria isolate in the three sites and composite samples of the rice mill soil show variation in their colonial morphology (Table 4). All isolates in the different samples show unique characteristics among the other isolates. Microscopic cell characteristics of bacterial isolates is shown in Figure 3.

**Table 4.** Characteristics of Amylase-Producing Bacteria from Minerva Rice Mill in Tigbauan, Iloilo.

Isolate: Rice Mill	F	E	M	A	OP	P	T	GR	CS	CA
Min A2	Irregular	Flat	Lobate	Dull	Translucent	Non-pigmented	Smooth	+	Cocci	Cluster
Min C2-1	Punctiform	Flat	Entire	Dull	Translucent	Non-pigmented	Smooth	+	Cocci	Cluster
Min C2-2	Irregular	Flat	Lobate	Dull	Translucent	Non-pigmented	Smooth	+	Cocci	Streptococci
Min B8	Circular	Flat	Entire	Dull	Translucent	Non-pigmented	Smooth	+	Bacilli	Single
Min C10	Rhizoid	Flat	Lobate	Dull	Translucent	Non-pigmented	Smooth	+	Cocci	Single
Min B2-1	Irregular	Flat	Lobate	Dull	Translucent	Non-pigmented	Smooth	-	Cocci	Chain
Min B2-2	Circular	Flat	Entire	Dull	Translucent	Non-pigmented	Smooth	+	Cocci-bacilli	Single
Min C4	Circular	Raised	Entire	Shiny	Opaque	Non-pigmented	Smooth	+	Bacilli	Single
Min C6	Punctiform	Flat	Entire	Dull	Translucent	Non-pigmented	Smooth	+	Bacilli	Single
Min C8	Circular	Raised	Curled	Dull	Opaque	Non-pigmented	Smooth	+	Bacilli	Single

Min A6-1	Rhizoid	Flat	Filamentous	Dull	Translucent	Non-pigmented	Smooth +	Bacilli	Single
Min A6-2	Irregular	Flat	Undulated	Dull	Opaque	Non-pigmented	Smooth +	Bacilli	Single

Legend: F = Form; E = Elevation; M = Margin; A = Appearance; OP = Optical Property; P = Pigmentation; T = Texture; GR = Gram Reaction; CS = Cell Shape; CA = Cell Arrangement

The characteristics of amylase-producing bacteria from Nulada Rice Mill soil in Tigbauan, Iloilo show that the bacterial isolates in the three sites and composite samples of the rice mill soil show variation in their colonial morphology (Table 6). All isolates in the different samples show unique characteristics among the other isolates. Microscopic cell characteristics of bacterial isolates are shown in Figure 3.

**Table 5.** Characteristics of Amylase-Producing Bacteria from Eking Rice Mill in Tigbauan, Iloilo.

Isolate:	F	E	M	A	OP	P	T	GR	CS	CA
Rice Mill										
Min A2	Irregular	Flat	Lobate	Dull	Translucent	Non-pigmented	Smooth +	Cocci	Cluster	
Min C2-1	Punctiform	Flat	Entire	Dull	Translucent	Non-pigmented	Smooth +	Cocci	Cluster	
Min C2-2	Irregular	Flat	Lobate	Dull	Translucent	Non-pigmented	Smooth +	Cocci	Streptococci	
Min B8	Circular	Flat	Entire	Dull	Translucent	Non-pigmented	Smooth +	Bacilli	Single	
Min C10	Rhizoid	Flat	Lobate	Dull	Translucent	Non-pigmented	Smooth +	Cocci	Single	
Min B2-1	Irregular	Flat	Lobate	Dull	Translucent	Non-pigmented	Smooth -	Cocci	Chain	
Min B2-2	Circular	Flat	Entire	Dull	Translucent	Non-pigmented	Smooth +	Cocci	Single	
Min C4	Circular	Raised	Entire	Shiny	Opaque	Non-pigmented	Smooth +	Bacilli	Single	
Min C6	Punctiform	Flat	Entire	Dull	Translucent	Non-pigmented	Smooth +	Bacilli	Single	
Min C8	Circular	Raised	Curled	Dull	Opaque	Non-pigmented	Smooth +	Bacilli	Single	

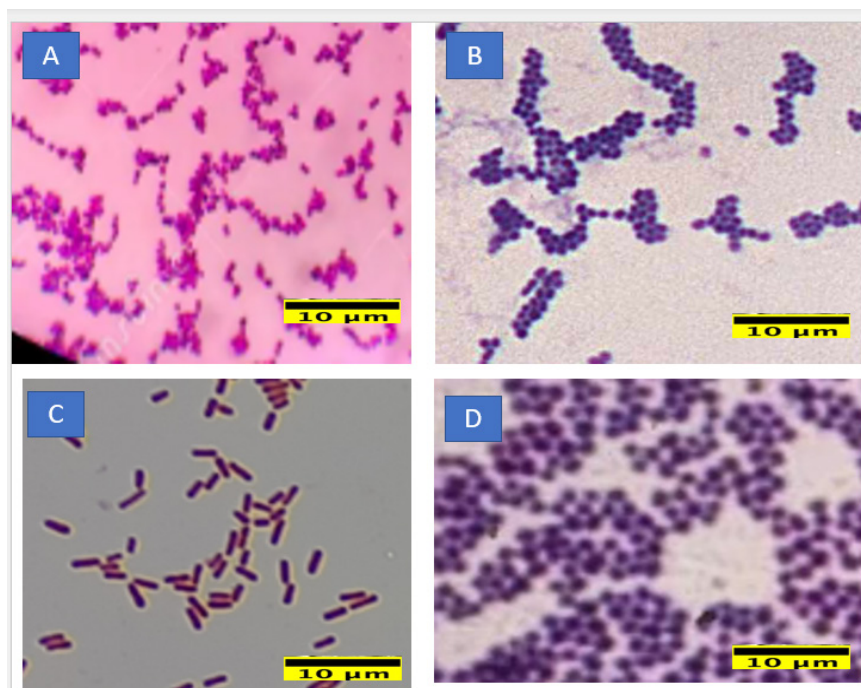
Legend: F = Form; E = Elevation; M = Margin; A = Appearance; OP = Optical Property; P = Pigmentation; T = Texture; GR = Gram Reaction; CS = Cell Shape; CA = Cell Arrangement

**Table 6.** Characteristics of Amylase-Producing Bacteria from Nulada Rice Mill in Tigbauan, Iloilo.

Isolate: Rice Mill	F	E	M	A	OP	P	T	GR	CS	CA
Min A2	Irregular	Flat	Lobate	Dull	Translu- cent	Non- pigmented	Smooth	+	Cocci	Cluster
Min C2-1	Puncti- form	Flat	Entire	Dull	Translu- cent	Non- pigmented	Smooth	+	Cocci	Cluster
Min C2-2	Irregular	Flat	Lobate	Dull	Translu- cent	Non- pigmented	Smooth	+	Cocci	Strepto- cocci
Min B8	Circular	Flat	Entire	Dull	Translu- cent	Non- pigmented	Smooth	+	Bacilli	Single
Min C10	Rhizoid	Flat	Lobate	Dull	Translu- cent	Non- pigmented	Smooth	+	Cocci	Single
Min B2-1	Irregular	Flat	Lobate	Dull	Translu- cent	Non- pigmented	Smooth	-	Cocci	Chain
Min B2-2	Circular	Flat	Entire	Dull	Translu- cent	Non- pigmented	Smooth	+	Cocco- bacilli	Single
Min C4	Circular	Raised	Entire	Shiny	Opaque	Non- pigmented	Smooth	+	Bacilli	Single
Min C6	Puncti- form	Flat	Entire	Dull	Translu- cent	Non- pigmented	Smooth	+	Bacilli	Single
Min C8	Circular	Raised	Curled	Dull	Opaque	Non- pigmented	Smooth	+	Bacilli	Single

Legend: F = Form; E = Elevation; M = Margin; A = Appearance; OP = Optical Property;

P = Pigmentation; T = Texture; GR = Gram Reaction; CS = Cell Shape; CA = Cell Arrangement



**Figure 3.** Different cellular characteristics of bacterial isolates screened with amylolytic activity. A. Gram (+) cocci in clusters. B. Gram (+) cocci in chains. C. Gram (+) bacilli. D. Gram (+) cocci occur singly.

### DISCUSSION

In the present study, bacterial isolates were obtained from the rice plant soil in Tigbauan, Iloilo. They showed variations in morphology and colony characteristics (Figure 3). The rice mill soil consists mainly of starch material, and it has been found that isolated bacteria from these locations can produce amino acid enzymes under adverse conditions. According to the study by Akpomie et al. (2012), the identification of bacteria isolates can be done by morphological characteristics and reactions to various biochemical tests. However, in this study, no identification of the isolates was made but only characterization of the colonies and cell characteristics. The study only assumed the possibilities of the genera based on related studies of bacterial isolates present in soil samples. Based on literature, it is composed of *Bacillus*, *Mycobacterium*, *Streptococcus*, and *Lactobacillus*. According to a study by Dunca et al. (2004), molecular analysis of bacterial isolates in cultivated soil shows that they belong to the genus *Bacillus* and *Staphylococcus*. They showed the characteristics of shortened ends or rounded and parallel edges. Sporulated rods are usually present, and a few

bacteria that are not sporulated (Dunca et al., 2004). The spores have a round or oval shape, central or sub-terminal shape, and are undistorted. There is also a difference in the grouping of rods in the slide. However, the dominating group are isolated long chains or isolated chains. In color, Gram-positive are most isolated strains, with Gram-negative as a few exceptions. The results of the study were also consistent with those of the work of Ghasemi et al. (2010) wherein gram-positive and gram-negative bacterial isolates were characterized. Gram-negative strains may be found in *Rheinheimera* and *Aeromonas* (Ghasemi et al., 2010). *Rheinheimera* is a Gram-negative cocci or rod, not form and move by a flagellum in a single pole (Ghasemi et al., 2010).

*Bacillus* can be commonly found in soil (Ghasemi et al., 2010). Endospore formation is a mode of persistence in the soil environment in *Bacillus*, widely believed by Ghasemi et al., (2010). The presence of *Bacillus* in most places studied are being associated to the aerodynamic distribution of dormant spores (Olajugbe et al., 2005; Ghasemi et al., 2010). These observations were consistent with the results of the study, showing that *Bacillus* is widespread in soil habitats. The results of a screening of the amylase production of the isolate clearly show that all isolates produce amylase. The work of Akpomie et al. (2012) revealed that *Bacillus* spp., *Corynebacterium*, *E. coli*, *M. luteus*, and *Lactobacillus* can produce amylase. Thus, it can be applied to the production of amylase for biotechnology applications in the food, pharmaceutical and medical industries.

In this study, the number of amylase producers of bacterial isolates varies from three rice mill soil samples. In this study, it showed that amylase-producing bacteria are predominantly present in soil samples characterized to be sandy, coarse, brownish black, with pH=5, soft in texture and loose. Distinct difference in bacterial composition and diversity among the three rice mill sites soil samples may indicate a possible relationship between soil properties and bacterial diversity. Liu et al. (2020) explained the variation in the composition of the microbial community could be due to the physiochemical properties of the soil. These may include the soil pH, total soil elements (P, N, and organic C) as the factors. Future study may include soil physiochemical properties to be analyzed in determining the relationship with bacterial diversity. Moreover, some studies have expounded that soil physiochemical properties are regulated by various factors, such as altitude and agricultural practices which could result to differences in bacterial composition and diversity (Kumar et al., 2019).

The dominating factors for the enzyme production in microbes are physiological parameters such as optimal temperatures, substrate

concentrations, and pH ranges (Bose et al., 1996; Gupta et al., 2003). The composition and concentration of the medium have a major impact on the production and growth of extracellular amylases in bacteria (Chanda et al., 1980; Srivastava et al., 1986). Starch is naturally everywhere and a source of easy-to use energy (Ryan et al., 2006). The production of enzymes by microorganisms is congruent to the incubation time (Smitt et al., 1996). The present study showed that enzyme activity was enhanced as incubation time increased. Some *Bacillus* being observed, these results come differently, where increasing the time of incubation reduces enzyme activity (Aiyer, 2004). Temperature effect on amylase production is related to microbial growth (Kathiresan & Manivannan, 2006) such as in *Bacillus licheniformis* (Saito & Yamamoto, 1975). Catabolite suppression occurs in the early stages of high-temperature culture, but with the progression of growth, inhibitors can be active and induce more enzyme secretion (Saito & Yamamoto, 1975). However, according to Bose and Das (1996), the activity of amylases in starch degrading bacteria is not related to growth. This may explain the optimal growth rate of currently reported strains at 37°C degrees, while the highest enzyme activity is observed at 70°C degrees. Amylase is divided into two categories: endoamylase and exoamylase (Naidu Saranraj, 2013). Endoamylases randomly catalyze hydrolysis in the inside of starch molecules (Naidu & Saranraj, 2013). This action causes the formation of linear oligosaccharides with different lengths of chains and branches. Exoamylases are hydrolyzed from the non-reduction end and are produced in succession into short end products (Naidu & Saranraj, 2013). Today, many enzymes that have hydrolyzed starch molecules into different products are known, and the combined action of different enzymes is required to completely hydrolyze starch (Naidu & Saranraj, 2013).

## CONCLUSIONS

The three rice mill soils contain a unique environment favorable to the presence of amylase-producing bacteria. In this study, 35 amylase-producing bacteria were isolated from the three rice mill soil samples in Tigbauan, Iloilo. These were dominated by gram positive bacilli and cocci. Based on the number of amylase-producing isolates, there is a variation in the diversity of the bacterial community, which is based on the soil physico-chemical characteristics. It may imply that the soil sample may contain substances or factors that affect the growth of the bacteria. The amount of starch for example in which bacteria grows best vary from the three sampling sites giving different number of isolates. The bacterial isolates have different colonial morphology and cell characteristics. It may

imply that there are variations in species or genus of bacterial isolates capable of amylase production. Hence, the bacterial isolates in this study are likely to have potential to be further utilized for the amylase industry.

## RECOMMENDATIONS

Biochemical tests and molecular characterization of the bacteria should be done to reveal the real identity and mechanism of the isolates. Widening the range of the study by conducting the experiment with other target isolates like amylase-producing fungi. Commercial production of amylase could be done in submerged fermentation and solid-state fermentation as potential tools for its production, especially applying agro-based waste residues such soil mixed with rice hull as substrate.

## References

- Aiba, S., Kitai, K., & Imanaka, T. (1983). Cloning and expression of thermostable alpha amylase gene from *Bacillus stearothermophilus* and *Bacillus subtilis*. *Applied and Environmental Microbiology Journal*, 46(5), 1059- 1065. <https://doi.org/10.1128/aem.46.5.1059-1065.1983>
- Aiyer, P.V. (2004). Effect of C:N ratio on alpha amylase production by *Bacillus licheniformis* SPT 27. *African Journal of Biotechnology*, 3 (10), 519-522. <https://doi.org/10.5897/AJB2004.000-2103>
- Akpomie, O.O., Akponah, E., & Okorawhe, P. (2012). Amylase production potentials of bacterial isolates obtained from cassava root peels. *International Research Journals Agricultural Science Research Journals*, 2(2), 95-99.
- Alariya, S. S., Sethi, S., Gupta, S., & Gupta, B. L. (2013). Amylase activity of a starch degrading bacteria isolated from soil. *Archives of Applied Science Research*, 5(1), 15-24.
- Anbu, P., Chaulagain, B. P., & Lakshmipriya, T. (2017). Microbial enzymes and their applications in industries and medicine. *BioMed Research International*, 17. <https://doi.org/10.1155/2017/2195808.2195808>
- Bahadure, R.B., Agnihotri, U.S., & Akarte, S.R. (2010). Assay of population density of amylase producing bacteria from different soil samples contaminated with flowing effluents. *International Journal for Parasitology Research*, 2, 09- 13. <https://doi.org/10.9735/0975-3702.2.1.9-13>

- Bose, K. & Das, D. (1996). Thermostable  $\alpha$ -amylase production using *B. licheniformis* NRRL B1438. *Indian Journal of Experimental Biology*, 34, 1279-1282.
- Brook, E.J., Stanton, W.R., & Wall-Bridge, A. (1969). Fermentation Methods for protein enrichment of cassava. *Biotechnology & Bioengineering*, 11, 1271-1284. <https://doi.org/10.1002/bit.260110620>
- Burhan, A., Nisa, U., Gokhan, C., Omer, C., Ashabil, A., & Osman, G. (2003). Enzymatic properties of a novel thermophilic, alkaline and chelator resistant amylase from an alkaliphilic *Bacillus* sp. isolate ANT-6. *Journal Process Biochemistry*, 38, 1397-1403. [https://doi.org/10.1016/S0032-9592\(03\)00037-2](https://doi.org/10.1016/S0032-9592(03)00037-2)
- Cordeiro, C.A.M., Martinas, M.L.L., & Luciano, A. (2003). Production and Properties of alpha amylase from thermophilic *Bacillus* species. *Brazilian Journal of Microbiology*, 33, 1-3. <https://doi.org/10.1590/S1517-83822002000100012>
- Dunca, S., Ailiesei, O., Nimitan, E. & Stefan, M. (2004). Microbiologie aplicata. Ed. Technopress, Iasi. *Commagene Journal of Biology-Plant Biology*, 49- 50, 31-39.
- Ghasemi, Y., Rasoul-Amini, S., Ebrahiminezhad, A., Zarrini, G., Kazemi, A., Kousavi-Khordisi, S., Ghoshoon, M. B., & Raei, M.J. (2010). Halotolerant amylase production by a novel bacterial strain, *Rheinheimera aquimaris*. *Research Journal of Microbiology*, 5(2), 144-149.
- Gupta, R., Gigras, P., Mohapatra, H., Goswami, V.K., & Chauhan, B. (2003). Microbial  $\alpha$ -amylases: a biotechnological prospective. *Process Biochemistry*, 38, 1599-1616. [https://doi.org/10.1016/S0032-9592\(03\)00053-0](https://doi.org/10.1016/S0032-9592(03)00053-0)
- Harley, J.P. (2005). *Laboratory Exercises in Microbiology*, 6th ed. Boston: McGraw Hill Higher Education.
- Kathiresan, K. & Manivannan, S. (2006). Amylase production by *Penicillium fellutanum* isolated from mangrove rhizospheric soil. *African Journal of Biotechnology*, 5, 829-832. <https://doi.org/10.5897/AJB>
- Kaur, A., Kaur, M., Samyal, M. L., & Ahmed, Z. (2012). Isolation, characterization, and identification of bacterial strain producing amylase. *Journal of Microbiology and Biotechnology*, 2(4), 573-579.

- Kumar, S., Suyal, D. C., Yadav, A., Shouche, Y., & Goel, R. (2019). Microbial diversity and soil physiochemical characteristic of higher altitude. *PLOS One*, *14*(3). <https://doi.org/10.1371/journal.pone.0213844.e0213844>
- Leveque, E., Janecek, S., Belarbi, A., & Haye, B. (2000). Thermophilic archaeal amylolytic enzymes catalytic mechanism, substrate specificity and stability. *Enzyme & Microbial Technology*, *26*, 3-14. [https://doi.org/10.1016/S0141-0229\(99\)00142-8](https://doi.org/10.1016/S0141-0229(99)00142-8)
- Liu, C., Li, L., Xie, J., Coulter, J. A., Zhang, R., & Luo, Z. (2020). Soil bacterial diversity and potential functions are regulated by long-term conservation tillage and straw mulching. *Microorganisms*, *8*(6), 836. <https://doi.org/10.3390/microorganisms8060836>.
- Mesbah, N. M., & Wiegel, J. (2014). Halophilic alkali- and thermostable amylase from a novel polyextremophilic *Amphibacillus* sp. NM-Ra2. *International Journal of Biological Macromolecules*, *70*, 222–229. <https://doi.org/10.1016/j.ijbiomac.2014.06.053>.
- Moyes, R. B., Reynolds, J., & Breakwell, D. P. (2009). Differential staining of bacteria: Gram stain. *Current Protocols in Microbiology*, *15*(1), A-3C. <https://doi.org/10.1002/9780471729259.mca03cs15>
- Naidu, M.A., and Saranraj, P. (2013). Bacterial amylase: A review. *International Journal of Pharmaceutical & Biological Archives*, *4*(2), 274-287.
- Olajugbe, F. & Ajele, J. (2005). Production dynamics of extracellular protease from *Bacillus* species. *African Journal of Biotechnology*, *4*, 776-779.
- Pandey, A., Nigam, P., Soccol, C.R., Soccol, V.T., Singh, D., & Mohan, R. (2000). Advances in microbial amylases. *Biotechnology and Applied Biochemistry*, *31*, 135-52. <https://doi.org/10.1042/ba19990073>
- Ryan, S.M., Fitzgerald, G.F., & Van Sinderen, D. (2006). Screening for and Identification of starch-, amylopectin-, and pullulan degrading activities in bifidobacterial strains. *Applied and Environmental Microbiology*, *72*, 5289- 5296. <https://doi.org/10.1128/AEM.00257-06>
- Saito, N. & Yamamoto, K. (1975). Regulatory factors affecting amylase production in *B. licheniformis*. *Journal of Bacteriology*, *121*, 848- 856.
- Senthilkumar, P.K., Uma, C. & Saranraj, P. (2012). Amylase Production by *Bacillus* sp. Using Cassava as Substrate. *International Journal of Pharmaceutical and Biological Science Archive*, *3*(2), 300-306.

- Schmidt, M., Bowers, B., Varma, A., Roh, D.-H., & Cabib, E. (2002). In budding yeast, contraction of the actomyosin ring and formation of the primary septum at cytokinesis depend on each other. *Journal of Cell Science*, *115*, 293–302. <https://doi.org/10.1016/j.semcdb.2016.01.043>
- Skrabanja, V., Liljeberg Elmståhl, H. G., Kreft, I., & Björck, I. M. (2001). Nutritional properties of starch in buckwheat products: Studies *in vitro* and *in vivo*. *Journal of Agricultural and Food Chemistry*, *49*(1), 490–496. <https://doi.org/10.1021/jf000779w>
- Smitt, J.P., Rinzema, J., Tramper, H., Van, M., & Knol, W. (1996). Solid state fermentation of wheat bran by *Trichoderma reesei* QMQ414. *Applied Microbiology and Biotechnology*, *46*, 489–496. <https://doi.org/10.1007/s002530050849>
- Srivastava, R.K.A. & Baruah, J.N. (1986). Culture conditions for production of thermostable amylase by *Bacillus stearothermophilus*. *Applied and Environmental Microbiology*. *52*, 179–184. <https://doi.org/10.1128/aem.52.1.179-184.1986>
- Thapa, S., Li, H., Ohair, J., Bhatti, S., Chen, F. C., & Nasr, K. A. (2019). Biochemical characteristics of microbial enzymes and their significance from industrial perspectives. *Molecular Biotechnology*, *61*(8), 579–601. <https://doi.org/10.1007/s12033-019-00187-1>.
- Vaseekaran, S., Balakumar, S., & Arasaratnam, V. (2010). Isolation and identification of a bacterial strain producing thermostable  $\alpha$ - amylase. *Tropical Agricultural Research*, *22*(1), 1 – 11. <https://doi.org/10.4038/tar.v22i1.2603>
- Vijayalakshmi, R., Sushma, S., Abha, S., & Chander, P. (2012). Isolation and Characterization of *Bacillus subtilis* KC3 for Amyolytic Activity. *International Journal of Bioscience, Biochemistry and Bioinformatics*, *2* (5), 17- 20. <https://doi.org/10.7763/IJBBB.2012.V2.128>
- Wu, X., Wang, Y., Tong, B., Chen, X., & Chen, J. (2018). Purification and biochemical characterization of a thermostable and acid-stable alpha-amylase from *Bacillus licheniformis* B4-423. *International Journal of Biological Macromolecules*, *109*, 329–337. <https://doi.org/10.1016/j.ijbiomac.2017.12.004>.