Isolation and selection of amylase-producing microbes isolated from *ragi* tape and cassava *tape* available on the markets

I B W Gunam¹,², I G A Sujana¹, I M M Wijaya¹, Y Setiyo³, I W W P Putra¹, L Suriati³

¹Department of Agroindustrial Technology, Faculty of Agricultural Technology, Udayana University, Badung 80361, Bali, Indonesia
²Department of Agricultural Engineering and Biosystem, Faculty of Agricultural Technology, Udayana University, Badung 80361, Bali, Indonesia
³Department of Food Science and Technology, Warmadewa University, Jl Terompong 24 Tanjung Bungkak, Denpasar, 80235, Indonesia
⁴Corresponding author: ibwgunam@unud.ac.id

Abstract. Amylase has an important role in biotechnology development and occupies an important position in the world enzyme market, as a biocatalyst in various industrial fields. This study has the goal to find microbial isolates that have the ability to produce amylase enzymes. The study was conducted in two stages, namely: 1) Isolation and selection of microbes that can produce amylase enzymes using starch as substrate, was incubated for 4-7 days at 30°C. Microbial isolates that can produce amylase enzymes are characterized by the presence of clear zones around the colony after the addition of an iodine solution of 1% in the overgrown media of microbes, 2) Test the activity of amylase enzymes using a dinitrosalicylic acid reagent test. The activity of the amylase enzyme is determined by measurement using a spectrophotometer at a wavelength of 540 nm. The sample used comprised of 7 types of *ragi* tape and 2 samples from cassava *tape* that has been fermented for 5-7 days. The results obtained in the first stage were 65 microbial isolates, 16 of which had clear zones, consisting of 7 isolates from *ragi* tape samples and 9 isolates from cassava *tape* samples. In the enzyme activity test, there are several isolates that have the potential to produce amylase enzymes, these include R5I4 (0.897 ± 0.018 U/mL), R2I5.1 (0.814 ± 0.011 U/mL), R5I3 (0.727 ± 0.042 U/mL) (derived from cassava *ragi* tape samples) and T2I2.2 (0.812 ± 0.013 U/mL), T2I6.1 (0.817 ± 0.010 U/mL), T2I2.1 (0.735 ± 0.023 U/mL), T1I4 (0.755 ± 0.020 U/mL) (derived from cassava *tape* samples). The isolate with the highest enzyme activity is the R5I4 which has the value enzyme activity of 0.897 ± 0.018 U/mL and with a fairly high or moderate category, while the lowest enzyme activity is the T1I1.1 isolate of 0.284 ± 0.020 U/mL.

1. Introduction

Enzymes have an important role as biocatalysts in various industrial fields. One of the enzymes that has this important role is the amylase enzyme. Amylase enzyme is an enzyme that can be used to hydrolyze polysaccharides such as starch into simple sugar [1]. Amylase occupies an important position in the world enzyme market by 25% to 33% [2], [3]. While the consumption of amylase enzymes in the country reached 2,500 tons with an import value of 187.5 billion in 2015 [4]. Amylase enzymes are commonly used in the bread making, textile, detergent and pharmaceutical industries [5], [6]. The most common amylase used in the food industry are the α-amylase (EC.3.2.1.1) and β-amylase (EC 3.2.1.2) [6], [7].
Amylase is produced from all sources of life (plants, animals, and microorganisms) and its demand continues to increase due to various industrial applications [8]. One source of amylase enzymes is a fermented product of cassava, namely cassava tape. Apart from cassava tape, of course, the enzyme can also be obtained from the production of microbes used for the production of tape itself. Amylase enzymes can be produced by microorganisms especially amylolytic microbes [9]. However, amylase enzymes for industrial purposes are mostly isolated from microbes. The choice of microbes as a source of enzymes because it has several advantages when compared to plants and animals, such as easy and rapid to grow, easier in the production scale-up, cheaper in the production costs, and easy to control in many production parameters.

Ragi tape is an inoculum containing microbial cultures which have amylolytic and fermentative activities. The same types of inoculums as ragi in Indonesia are bubod from the Philippines, luk pang from Thailand, peh chu from China, bakkar; muchar; ranu; u-y-iar from India, juipaing from Malaysia and nooruk from Korea. Amylolytic and fermentative microbes that have been successfully isolated by the researchers from various brands of ragi tape from several places and markets in Indonesia are a combination of Amylomyces rouxii, Rhizopus oryzae, Endomycopsis burtonii, Mucor sp., Candida utilis, Saccharomyces fibuligera, Saccharomyces cerevisiae, and some lactic acid bacteria (LAB) Pediococcus pentosaceus, Lactobacillus plantarum, and L. fermentum [10], [11], [12]. The researchers from Philippines, Malaysia, Thailand and Vietnam also found similar microbial species in the inoculums used in their regions. Amylolytic microbes in traditional fermentation have good uses, which will accelerate the process of starch degradation into simple sugars. For example, in the fermentation of cassava tape the resulting simple sugar will be converted into organic acids and alcohols which make a distinctive aroma of tape. Therefore, the amylolytic ability of the enzyme is interesting because it has potential for many practical purposes. This research is a first step in obtaining amylase enzymes produced by amylolytic microbial isolates from ragi and cassava tape so that it can be a source of alternative for amylase enzymes. The purpose of the study was to isolate and select amylolytic microbes found in ragi and cassava tape.

2. Materials and methods

2.1. Materials

Ragi tape samples (ragi 99, ragi Sari Madu, ragi NKL, ragi Gedang, ragi Matahari Cakra, ragi Berlian, and ragi LBC), cassava tape samples (Jember honey cassava tape "Special" and sweet cassava tape "Sari Madu"), starch, peptone, beef extract, yeast extract, NaCl, agar powder, iodine, potassium iodide, CH₃COOH, C₂H₃NaO₂, 3.5-dinitrosalicylic acid (DNS), distilled water, and alcohol.

2.2. Isolation and selection of microbes

Microbe isolation was done by spread plate method and isolate purification. The insulation media used starch agar medium (peptone 0.5 g; beef extract 0.15 g; yeast extract0.15 g; NaCl 0.5 g; starch,1 g; agar 2 g; distilled water 100 mL) [13], [14]. Before isolation, the sample was first diluted to 10⁻⁷ in NaCl solution, then the spread plate stage was carried out and incubated for 5–7 days at a temperature of 30 °C. Then the purification of isolate was conducted using starch agar medium (SAM).

The pure isolate was then tested for a clear zone test by adding 1% iodine solution (iodine 0.2 mL; potassium iodide 0.4 mL; distilled water 100 mL) [13], [14]. The isolate showed a clear zone with the addition of iodine solution, then the isolate can be confirmed to be able to produce amylase enzymes. The data obtained were then calculated the diameter of clear zone and the amylolytic index value. Amylolytic microbial isolates were tested on the amylolytic index based on the formula [15]:

\[ \text{Amylolytic Index} = \frac{\text{Diameter of Clear Zone}}{\text{Volume of Culture}} \]
2.3. Test of amylase enzymes activity

The activity of amylase enzymes was measured using a combined method from Naguib and Qureshi [16], [17]. Substrates containing 1 mL of amylase enzymes were mixed into a reaction containing 1 mL of 0.5% soluble starch in an acetate buffer (pH 5.6) then incubated at 30°C for 30 minutes [16], [14]. Then the reaction was stopped by adding 2 mL of reagent DNS solution, then heated for 5 minutes. The tubes were cooled to room temperature and absorbance was measured at 540 nm against empty substrates and enzymes [17]. Furthermore, the obtained data calculated the value of enzyme activity and glucose levels produced by comparing with the standard glucose curve.

2.4. Morphological test of amylase enzyme-producing microbe

Selected yeast cultures were inoculated on the medium yeast extract peptone glucose agar (YEPGA) in Petri dish by a scratch method, incubated at room temperature for 3–7 days. Colonies that grow separately (single colonies) were observed morphologically include form, elevation, margin, and color. Microscopic observations were made of the shape of the cell. Cell were observed by staining using methylene blue [18].

2.5. High glucose tolerance test

The isolate was added into the medium yeast extract peptone glucose broth (YEPGB). Each tube contains 5 mL of medium with glucose concentrations of 10% (m/v), 20% (m/v), 30% (m/v), 40% (m/v), and 50% (m/v). The density value of the cell was calculated by absorbance at a wavelength of 600 nm using the UV-Vis Spectrophotometer at an incubation of 30°C for 2–5 days [19], [20], [21].

2.6. Effect of incubation temperature on amylase enzyme activity

Substrates containing the enzyme amylase as much as 1 mL were mixed into a reaction containing 1 mL of soluble starch 0.5% in an acetate buffer (pH 5.6) and incubated at 30°C, 35°C, and 40°C for 30 minutes [16], [14]. Then the reaction was stopped by adding 2 mL of reagent DNS solution, subsequently it was heated for 5 minutes. The tubes are cooled to room temperature and the absorbance was measured at 540 nm against empty substrates and enzymes [17].

2.7. Effect of pH on amylase enzyme activity

Substrates containing the enzyme amylase as much as 1 mL were mixed into a reaction containing 1 mL of soluble starch 0.5% in acetate buffers (pH 4, 5, and 6) and incubated at 30°C for 30 minutes [16], [14]. The reaction was stopped by adding 2 mL of reagent DNS solution, after that it was heated for 5 minutes. The tubes were cooled to room temperature and the absorbance was measured at 540 nm against empty substrates and enzymes [17].

3. Results and discussions
3.1 Microbial isolation and selection

A total of 65 isolates were isolated from *ragi* and cassava *tape*, only 16 isolates showed a clear zone after the addition of iodine solution. Table 1 shows the result of isolate selection that has a clear zone diameter of ≥ 1 mm and a list of amylolytic index values.

| Isolates | Clear zone diameter (mm) | Amylolytic Index |
|----------|--------------------------|------------------|
| R2I5.1   | 10.25 ± 2.54             | 2.69 ± 0.84      |
| R6I4     | 6.25 ± 3.00              | 1.38 ± 0.35      |
| R5I3     | 15.08 ± 0.83             | 1.78 ± 0.34      |
| R6I1     | 7.42 ± 0.83              | 1.32 ± 0.05      |
| R1I2     | 3.92 ± 0.69              | 1.42 ± 0.15      |
| R5I4     | 12.33 ± 3.14             | 3.36 ± 1.01      |
| R3I9     | 4.67 ± 1.91              | 1.23 ± 0.14      |
| T1I4     | 13.25 ± 2.08             | 2.18 ± 0.38      |
| T2I2.1   | 16.75 ± 1.48             | 2.18 ± 0.23      |
| T1I1.1   | 9.83 ± 2.15              | 1.45 ± 0.21      |
| T1I1.1   | 8.25 ± 1.75              | 1.31 ± 0.09      |
| T1I5     | 8.92 ± 0.42              | 1.38 ± 0.05      |
| T2I6.2   | 6.92 ± 1.93              | 1.36 ± 0.12      |
| T2I6.1   | 18.42 ± 3.49             | 2.22 ± 0.39      |
| T2I2.2   | 18.17 ± 1.69             | 2.25 ± 0.23      |
| T2I5     | 4.92 ± 0.57              | 1.15 ± 0.02      |

Note: The number after ± indicates the deviation value of a treatment

Table 1 shows that the resulting clear zone is 3.92 – 18.42 mm and has an amylolytic index value of 1.15 – 3.36. Here are some examples of clear zone images produced when the addition of iodine solution is done.

![Figure 1](image-url)

**Figure 1.** Isolates that produce the highest clear zone: (a) T2I2.2, (b) T2I6.1, (c) T2I2.1, (d) T1I4, (e) R5I4, (f) R2I5.1, (g) R5I3. The clear zone was tested by adding 1% iodine solution.

Figure 1 shows that the clear zone formed around the colony indicates that the isolate is capable of hydrolyzing the starch. The starch will form a deep blue complex with iodine reagents. The iodine-starch reaction is caused by the presence of helical amylose and iodine in forming a deep blue complex which fills the helical nucleus. Active hydrolysis of starch by amylase enzymes will cause the starch-iodine complex to decompose thus forming a clear zone. The absence of a clear zone around the colony indicates a reaction between iodine reagents and non-hydrolyzed starches in starch-containing mediums [22]. Gana *et al.* [23], reported that the ability or the power to produce the enzyme amylase of a microbe is characterized by the formation of clear zones in the medium containing starch.
Sixteen isolates of microbes that have the ability to produce the enzyme amylase were tested for their ability based on the amylolytic index (AI). Based on the amylolytic index (Table 1), various AI values were obtained. Total of 3 isolates from *ragi* samples and 4 isolates from *tape* samples were potential or capable of producing AI≥1.5 or moderate to high category in producing amylase compared to other isolates, namely isolates R2I5.1, R5I4, R5I3, T1I4, T2I2.1, T2I6.1, and T2I2.2 with AI values of 2.69 ± 0.84, 3.36 ± 1.01, 1.78 ± 0.34, 2.18 ± 0.38, 2.18 ± 0.23, 2.22 ± 0.29, and 2.25 ± 0.23. Meanwhile isolates R3I9 and T2I5 had the lowest AI values, namely 1.23 ± 0.14 and 1.15 ± 0.02. This is in accordance with the research of Bansal et al. [24] screened fungi capable of producing amylase with an AI of 2.95. Ouedraogo et al. [25], who succeeded in isolating yeast and screening potential amylase-producing yeasts from potatoes with an AI value of 2.35. Desai et al. [26] succeeded in isolating yeast and obtained an AI value of 2.60.

### 3.2 Test of amylase enzymes activity

Amylase enzyme activity testing was conducted to determine the activity of amylase enzymes against 16 potential isolates in previous tests. The measurement of amylase enzyme activity was done using microbial isolate culture in suspense form in a 40% glycerol solution. The isolate suspension was grown in starch medium broth with a peptone composition, yeast extract, and starch. Peptone, and yeast extract are sources of organic nitrogen that serve as important macronutrients in cell growth. In addition, the starch being an important macronutrient also acts as a source of carbon inducing amylase enzymes [27]. Here are the results of the amylase enzyme activity test as well as the amount of glucose content produced.

| Isolates | Absorbance value | Total glucose (mg/mL) | Enzyme activity (U/mL) |
|----------|------------------|-----------------------|------------------------|
| R2I5.1   | 1.327 ± 0.017    | 1.466 ± 0.019         | 0.814 ± 0.011          |
| R1I2     | 0.857 ± 0.035    | 0.935 ± 0.039         | 0.520 ± 0.022          |
| R5I4     | 1.458 ± 0.028    | 1.614 ± 0.032         | 0.897 ± 0.018          |
| R3I9     | 1.095 ± 0.045    | 1.204 ± 0.050         | 0.670 ± 0.028          |
| R5I3     | 1.187 ± 0.066    | 1.308 ± 0.075         | 0.727 ± 0.042          |
| R6I1     | 0.822 ± 0.051    | 0.897 ± 0.057         | 0.498 ± 0.032          |
| R6I4     | 0.960 ± 0.050    | 1.052 ± 0.056         | 0.585 ± 0.031          |
| T2I2.1   | 1.199 ± 0.036    | 1.322 ± 0.041         | 0.735 ± 0.023          |
| T1I1.1   | 0.481 ± 0.032    | 0.511 ± 0.036         | 0.284 ± 0.020          |
| T1I5     | 1.156 ± 0.024    | 1.274 ± 0.027         | 0.708 ± 0.015          |
| T1I4     | 1.231 ± 0.031    | 1.358 ± 0.035         | 0.755 ± 0.020          |
| T2I2.2   | 1.322 ± 0.021    | 1.461 ± 0.024         | 0.812 ± 0.013          |
| T2I5     | 1.036 ± 0.034    | 1.138 ± 0.038         | 0.632 ± 0.021          |
| T2I6.1   | 1.329 ± 0.017    | 1.47 ± 0.019          | 0.817 ± 0.010          |
| T2I6.2   | 1.154 ± 0.050    | 1.271 ± 0.056         | 0.706 ± 0.031          |
| T1I3.1   | 1.081 ± 0.138    | 1.189 ± 0.156         | 0.660 ± 0.086          |

Note: The number after ± indicates the deviation value of a treatment

Based on the measurements of amylase enzyme activity (Table 2), it can be seen that some potential isolates produce the highest amylase enzyme activity. These include R5I4 (0.897 ± 0.18 U/mL), R2I5.1 (0.814 ± 0.11 U/mL), R5I3 (0.727 ± 0.042 U/mL) (derived from cassava *ragi* samples) and T2I2.2 (0.812 ± 0.013 U/mL), T2I6.1 (0.817 ± 0.010 U/mL), T2I2.1 (0.735 ± 0.023 U/mL), T1I4 (0.755 ± 0.020 U/mL) (derived from cassava *tape* samples). The isolate that has the highest enzyme activity is the R5I4.
isolate with enzyme activity of $0.897 \pm 0.018$ U/mL with a fairly high or moderate category, while the lowest enzyme activity in T1I1 isolate is $0.284 \pm 0.020$ U/mL.

Some studies that are able to produce enzyme activity are quite high, including, Ouédraogo et al. [25], successfully measured the activity of amylase enzymes in the potential yeast of amylase-producing results from potatoes of $0.774$ U/mL. Oliveira et al. [28] successfully measured the activity of amylase enzyme in yeast *Saccharomyces cerevisiae* enzyme activity of $0.734$ U/mL.

### 3.3. Morphological test of amylase-producing microbes

After testing the activity of enzymes, 7 suspected isolates were found to be potential in the production of amylase enzymes. Based on the morphological character of yeast isolates that have been done, the following results are obtained:

| Isolates | Form | Elevation | Margin | Color       | Observation on a microscope Form a colony |
|----------|------|-----------|--------|-------------|------------------------------------------|
| T2I2.1   | Irregular | Raised   | Undulate | Snuff-colored | Oval                                      |
| T2I6.1   | Irregular | Umbo     | Undulate | Reddish-yellow | Tapering                                 |
| T2I2.2   | Irregular | Convex   | Erose   | Cream        | Oval                                     |
| T1I4     | Circular | Convex   | Entire  | Cream        | Oval                                     |
| R5I4     | Irregular | Raised   | Undulate | Cream        | Oval                                     |
| R2I5.1   | Irregular | Convex   | Lobate  | Cream        | Oval                                     |
| R5I3     | Irregular | Flat     | Lobate  | Cream        | Round                                    |

Potential yeast isolates are microscopically round to oval and the presence of buds as a form of breeding. According to Buckle [29] yeast can grow in liquid and solid media in the same way as bacteria. Cell division occurs asexually with the formation of buds, a process that is a typical property of yeast.
Among the yeast isolates observed, some had capsules in their cells. According to Limoli et al. [30] some yeasts are covered by slimy extracellular components and are called capsules. The capsule covers the outside of the cell wall and consists mainly of polysaccharides, a starch-like polymer, and heteropolysaccharides that contain more than one type of sugar unit such as pentose, hexose, and glucuronic acid.

3.4. High glucose tolerance test

The test of microbial growth at high glucose concentrations was to determine whether microbial isolates could grow at high glucose concentrations. The value of isolate density at different glucose concentrations can be seen in Figure 2.

![Figure 2. The absorbance isolate values at some glucose concentrations](image)

The high glucose tolerance level (high osmotic pressure) possessed by isolated yeast isolates is at a glucose concentration of 50%. At that concentration, the optical density value measured by the UV-Vis spectrophotometer (λ 600 nm) has the smallest value compared to other glucose concentrations. The isolates of R2I5.1 and R5I3 were able to survive and thrive at high glucose concentrations because they could still grow well at a concentration of 50%, while the largest optical density values R5I3 isolates are produced at a concentration of 30% which indicates their optimum glucose concentration at 30%. The same thing happened in a study conducted by Nasir [21]. R5I4 isolates cannot tolerate high glucose concentrations because R5I4 isolates is an isolate that produces amylase enzyme.

3.5. Effect of incubation temperature on amylase enzyme activity

After obtaining microbes that have the potential to produce amylase enzymes, the influence of temperature on the activity of the enzyme produced. Here are the values of enzyme activity at temperatures of 30°C, 35°C, and 40°C, can be seen in Figure 3.
Figure 3. Amylase enzyme activity at different temperatures

In Figure 3 it can be seen that all enzymes are able to survive up to 40°C. However, some enzymes decrease the value of activity at 35°C to 40°C. While isolated R5I4 and isolated R5I3 tend to increase to a temperature of 40°C and isolate R5I4 has the highest amylase enzyme activity compared to other isolates. So, it can be interpreted that each enzyme obtained from a different isolate has different enzyme activity. The best isolate that has resistance to temperature and enzyme activity is isolate R5I4. Then the research by Vidyalakshmi et al. [31] reported that, amylase activity decreased significantly with an increase in temperature beyond 37°C. Divakaran et al. [32] also reported that amylase enzyme activity decreased after incubation above 40°C.

3.6. Effect of pH on amylase enzyme activity

After obtaining microbes that have the potential to produce amylase enzymes, then experiments on the effect of pH on the activity of the enzyme amylase produced. Here are the values of enzyme activity at pH 4, 5, and 6, can be seen in Figure 4.
Figure 4 indicates that it is still against the activity of enzymes at pH 4, 5, and 6, which indicates that the enzyme can survive at that pH. However, most enzymes of some isolates decrease activity at pH 6 and most have optimum pH conditions at pH 5. In testing the effect of pH on enzyme activity produces the best enzyme obtained from R5I4 isolates. This is in line with the results of the study of Todoro et al. [33] which states that a further increase in pH results in a decrease in the activity of the enzyme amylase. Divakaran et al. [32] also stated that the higher the pH, the value of amylase enzyme activity decreased.

4. Conclusion
From the results of this study, we can conclude that there are 16 isolates from 65 isolates that have clear zones after a clear zone test with the addition of iodine solution. While the isolate that has the highest enzyme activity is R5I4 isolates with enzyme activity of 0.897 ± 0.018 U/mL with a fairly high or moderate category. Furthermore, there are 7 isolates that have the potential to produce amylase enzymes which are able to survive at varying incubation temperatures and pH. The most potential to produce amylase enzymes is R5I4 isolates.

Acknowledgments
The authors express their gratitude to Udayana University which has provided funding through the Udayana Innovation Research Grant with contract no: B/96-85/UN14.4.A/PT.01.05/2021 and laboratory facilities.

References
[1] Yalçın HT and Çorbacı C 2013 Isolation and characterization of amylase producing yeasts and improvement of amylase production Turk. J. Biotech 38(2):101–108
[2] Ashwini K, Gaurav K, Karthik L, and Bhaskara R K V 2011 Optimization, production and partial purification of extracellular α-amylase from Bacillus sp. marini Arch Appl Sci Res 3(1):33–42
[3] Kumar SS, Sangeeta R, Soumya S, Ranjan R P, Bidyut B, and Kumar D M P 2014 Characterizing novel thermophilic amylase producing bacteria from Taptapani hot spring, Odisha, India Jundishapur J Microbiol 7(12):1–7.

[4] Ministry of Trade of the Republic of Indonesia 2015 Development of Indonesia's commodity import exports Kemendag RI, Jakarta.

[5] Pandey A, Nigam P, Soccol C R, Soccol V T, Singh D, and Mohan R 2000 Advances in microbial amylases J. Biotech. App. Biochem 31(2):133–152

[6] Souza PM, and Magalhães P O 2010 Application of microbial α-amylase in industry - a review Braz. J. Microbiol. 41:850-861

[7] Singh H, Saharan R, and Sharma KP 2014 Isolation and characterization of amylase producing bacteria from diverse environmental samples J. Microbiol Biotech Res 4(4):8-18.

[8] Qureshi US, and Dahot M U 2009 Production of proteases by Staphylococcus epidermidis EFRL 12 using cost effective substrate (molasses) as a carbon source Sir. J. Biotechnol 6:55–60.

[9] Abu EA, Ado S A, and James D B 2005 Raw starch degrading amylase production by mixed culture of Aspergillus niger and Saccharomyces cerevisiae grown on Sorghum pomace Afr. J. Biotechnol 4:785 – 790.

[10] Gandjar I 2003 Tapai from Cassava and Cereals Paper presented at the First International Symposium and Workshop on Insight to the World of Indigenous Fermented Foods for Technology Development and Food Safety, Kasetsart University, August 13-17, 2003.

[11] Ko SD 1972 Tape fermentation J. Applied Microbiol 23:976-978.

[12] Steinkraus KH 1996 Handbook of Indigenous Fermented Foods Marcel Dekker, Inc., New York.

[13] Suganthi R, Benazir J F, R. Santhi, Ramesh V, Kumar, Hari A, Meenakshi N, Nidhiya K A, Kavitha G, and Lakshmi R 2011 Amylase production by Aspergillus niger under solid state fermentation using agricultural wastes Int. J. Eng. Sci. Technol 3(2):1756–1763

[14] Saleem A, and Ebrahim M K H 2013 Production of amylase by fungi isolated from legume seeds collected in Almadinah Almunawwarah, Saudi Arabia Journal of Taibah University for Science 8(2014):90–97.

[15] Goldbeck R, Andrade C C P, Pereira G A G, and Filho F M 2012 Screening and identification of cellulase producing yeast-like microorganisms from Brazilian biomes Afr. J. Biotechnol 11(53):11595–11603.

[16] Naguib MI 1964 Effect of sevin on the carbohydrate and nitrogen metabolism during the germination of cotton seeds Indian J. Exp. Biol 2:149–152.

[17] Qureshi AS, Bhutto M A, Khushk I, and Dahot M U 2013 Optimization of cultural conditions for protease production by Bacillus subtilis EFRL 01 Afr. J. Biotechnol 10:5173–5181.

[18] Yarrow D 1998 Method for the Isolation Maintenance and Identification of yeasts in: The Yeast, A taxonomic Study, Kurtzman CP, and Fell J W (Eds) 4th ed Elsevier Science B.V. Amsterdam 69 – 100.

[19] Kurtzman C P, Fell J W, and Boekhout T 2011 The Yeast: A Taxonomic Study Fifth Edition Elsevier, USA.

[20] Ali M N, and Khan M M. 2014. Screening, Identification and Characterization of Alcohol Tolerant Potential Bioethanol Producing Yeast. Current Research in Microbiology and Biotechnology. 2(1):316-324.

[21] Nasir A, Rahman S S, Hossain M M, and Choudhury N 2017 Isolation of Saccharomyces cerevisiae from Pineapple and Orange and Study of Metal's Effectiveness of Ethanol Production European Journal of Microbiology and Immunology 1(7):76-91.

[22] Cappuccino JG, and Sherman N 2002 Microbiology: A laboratory manual, 9th ed Addison-Wilsey California.
[23] Gana NHT, Mendoza B C, and Monsalud R G 2014 Isolation, screening, and characterization of yeasts with amylolytic, lipolytic, and proteolytic activities from the surface of Philippine bananas (Musa spp.) Philipp. J. Sci 143:81–87

[24] Bansal N, Tewari R, Gupta JK, Soni R, and Soni SK 2011 A novel strain of Aspergillus niger producing a cocktail of hydrolytic depolymerizing enzymes for the production of second-generation biofuels Peer-Reviewed Article 6:552–569.

[25] Ouëdraogo N, Savadogo A, Zongo C, Somda K, and Traoré A 2014 High performance amylolytic yeast strains isolation and identification for valorization of potatoes waste available in Burkina Faso Int. Food Res. J 19(4):1463–1469.

[26] Desai MV, Dubey K V, Vakil B V, and Ranade V V 2012 Isolation, identification and screening of the yeast flora from Indian cashew apple for sugar and ethanol tolerance Int. J. Biotechnol Well. Indus. 1:259–265.

[27] Gupta R, Gigras P, Mohapatra H, Goswami V K, and Chauhan B 2003 Microbial α-amylases: a biotechnological prospective Process. Biochem. 38:1599–1616.

[28] Oliveira APA, De Silvestre M A, Alves-prado H F, Rodrigues A, Fossa M, Fonseca G G, and Leite R S R 2015 Bioprospecting of yeasts for amylase production in solid state fermentation and evaluation of the catalytic properties of enzymatic extracts Afr. J. Biotechnol 14(14):1215–1223.

[29] Buckle K A, Edwards R A, Fleet G H, and Wotton M 1987 food science Translator: Purnomo H, and Adiono, UI – Press, Jakarta.

[30] Limoli D H, Jones C J, and Wozniak D J 2015 Bacterial extracellular polysaccharides in biofilm formation and function Microbiol Spectr. 3(3):1–30

[31] Vidyalakshmi R, Paranthaman R, and Indhumathi J 2009 Amylase Production on Submerged Fermentation by Bacillus spp World Journal of Chemistry 4(1):89-91

[32] Divakaran D, Chandran A, and Chandran R P 2011 Comparative study on production of α-amylase from Bacillus licheniformis strains Brazilian Journal of Microbiology 42:1397-1404

[33] Teodoro CE, De S, Martins M L L 2000 Culture conditions for the production of thermostable amylase by Bacillus sp. Braz. J. Microbiology 31:298-302.