Fermentation of amylolytic yeast and lactic acid bacteria to improve the quality of modified cassava

Ema Damayanti1*, Meylida Ichsyani2, Lusty Istiqomah1, Ayu Septi Anggraeni1 and Muhamad Kurniadi1

1Research Unit for Natural Product Technology, Indonesian Institute of Sciences (BPTBA-LIPI) Jl. Jogja Wonosari KM 31.5, Gunungkidul, D.I.Yogyakarta, 55861, Indonesia
2Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia

*Corresponding author: ema.damayanti@lipi.go.id

Abstract. The amylolytic activity has been studied in yeast and lactic acid bacteria (LAB) which are isolated from various types of traditional fermented cassava. The study aimed to obtain yeast and LAB isolates which high amylolytic activity for inoculum in cassava fermentation. A total of 23 yeasts and 22 LAB isolates were isolated from Gatot, Tiwul, Tapai, and Cassava fermented using chloramphenicol yeast glucose (CYG) media for yeast and de Man Rogosa Sharpe agar (MRSA) + CaCO₃ for LAB. Yeast amylolytic activity was tested using yeast extract peptone starch agar medium, while the amylolytic LAB used MRS + starch medium with Lugol's iodine indicator. Colonies showing clear zone areas were measured as amylase activity on the iodine complex method. Isolates that have the highest amylolytic activity were used as inoculum in cassava fermentation. Yeast Tr7 and LAB G6 isolates showed the highest amylolytic activity (0.0624 U/mL and 0.0627 U/mL). Cassava fermentation products using yeast Tr7 and BAL G6 resulted 52.06% of hydrogen cyanide (HCN) reduction higher than without inoculum (32.32%). According to biochemical identification using API 50 CHL kit, LAB G6 isolate was 99.6% identical with Pediococcus pentosaceus whereas biochemical identification using API 20 C AUX showed that yeast Tr7 was 95.8% identical with Candida tropicalis. The biochemical identification result was similar to the molecular identification result. In molecular identification using 28S rRNA sequence analysis revealed that yeast Tr7 was 99% similar with Candida tropicalis NITCSK13 strain. LAB G6 was 100% similar with Pediococcus pentosaceus BSS1375 strain using 16S rRNA sequence analysis. C. tropicalis Tr7 and P. pentosaceus G6 potentially used for inoculum in cassava fermentation process to improve cassava quality as a functional food.

1. Introduction
Cassava (Manihot esculenta Crantz) is an important source of calories in tropical countries [1]. Cassava is a food crop commodity as a source of carbohydrates and raw material for food and animal feed. Fresh cassava is easily damage, contains high water and contains antinutrient, hydrogen cyanide (HCN) [2]. Various techniques were performed to make cassava safe for consumption, one of which is through the fermentation. Fermentation process was known to increase protein levels and reduce the HCN content of
cassava products [3]. The fermentation involves specific microbes especially lactic acid bacteria (LAB), cellulolytic bacteria, and amylolytic yeast [4]. Yeast and lactic acid bacteria (LAB) are the microbial strains that have been frequently associated with the production of an enzyme for HCN reduction during fermentation of cassava and the development of flavor [5].

Indonesia is the fourth-largest world cassava supply country in the world at 5.8% [6]. In Indonesia, especially the Special Region of Yogyakarta, a variety of traditional processed products from cassava, such as Growol, Tapai, Tiwul, and Gatot, are processed using the natural fermentation without the addition of microbes. The traditional cassava fermentation process is dependent on the presence of natural microorganisms from raw materials and microorganisms from the environment [7]. Naturally in Gatot and Growol (fermented raw cassava) there are strains of LAB which are included in the genus Lactobacillus especially species of *L. plantarum*, *L. pentosus*, *Pediococcus pentosaceus* and *Streptococcus thermophilus* [8]. In tapai which is made traditionally was known contain a microbial consortium consisting of molds, yeasts, and bacteria [7]. In Gatot, homofermentative LAB of *L. manihotivorans*, heterofermentative LAB of *L. fermentum* [9], *L. manihotivorans*, *Bacillus licheniformis*, *Brevibacillus brevis*, and *L. fermentum* [10] were found.

Cassava is an energy source food which main components are starch and fibre [11]. Microbial and biochemical composition changes occur traditionally during the fermentation process of cassava [12]. Fermentation process of cassava using microbes has a very important role [13]. Polysaccharide starches are amylose polymers and amyllopectin (branched polymers of glucose molecules that are linked by 1,6-glycoside bonds). Microbes are known to have the ability to break down starch in cassava through the production of amylase enzymes, including molds [14], yeast [15,16] and bacteria [17]. Modified cassava fermentation (Mocaf) now is one of the fermented cassava products that is being developed in Indonesia. The use of an inoculum for Mocaf fermentation is important to ensure the quality products according to the standard. This study aims to select microbes with amylolytic activity as an inoculum in the fermentation process of cassava and potentially reduce the content of cyanide acid in the final product.

2. Material and Method

2.1. Materials
The traditional fermented cassava used for samples in yeast isolation. Tapai, Tiwul, Gatot, and Ragi tape obtained from Demangan market and Wonosari market, Yogyakarta, Indonesia. Twenty-one of LAB isolates which used in this research were microbiology laboratory collection in BPTBA LIPI. LAB isolates were isolated from fermented cassava such as Gatot, Tiwul, Gaplek and modified cassava fermentation (Mocaf) products. LAB isolates were stored in 30% glycerol at -20 °C of temperature and were re-cultured on MRSA medium. Local variety of cassava root obtained from the Wonosari market, Gunungkidul, Yogyakarta, Indonesia.

2.2. Yeast isolation
A total of 5 grams of cassava fermented sample was mixed in 45 mL of chloramphenicol yeast glucose broth (CYG) and incubated for 72 hours at 24 °C of temperature. Samples were homogenized in sterile distilled water and serial dilutions were made from $10^1 - 10^7$. The last three series dilutions were spread on chloramphenicol yeast glucose agar (CYGA) surface. Incubation was carried out aerobically at 24 °C of temperature for 48 hours. The yeast colonies that were grown were calculated, selected, and observed for morphological and biochemical characteristics.
2.3. **Qualitative amylolytic assay**

Twenty-two yeast isolates and 21 LAB isolates were inoculated in a medium containing 2% starch substrate. Yeasts were cultured on yeast extract peptone starch agar medium while LABs were cultured on modified MRS agar by replacing the carbon source into starch [18]. Yeast culture was incubated for 3 days at 24 °C and LAB culture were incubated for 2 days at 37 °C. Two 2 mL of 0.3% Lugol’s iodine solution was dropped on the media surface and left for 10 minutes and then it was washed using 3 mL of physiological saline (0.85% NaCl) for 10 minutes. The clear zone that appears around the colonies indicates the presence of amylolytic activity from the microbes [19].

2.4. **Quantitative amylolytic assay**

Quantitative amylolytic activity assay was carried out to determine degrading starch with α-amylase enzyme using iodine complex method [18]. One hundred μL of 24-hour-old LABs and yeasts were cultured on liquid media containing 2% starch (w/v) and 0.5 g/L CaCl₂, 6.0 of. The experiment was performed in 3 replicate samples. Yeasts were incubated for 24 hours in a shaker incubator (Stuart Orbital Incubator, S1500), 120 rpm of agitation, 24 °C of temperature. LABs were incubated for 20 hours, 120 rpm of agitation, 37 °C of temperature. The culture was centrifuged (Selecta BL II) at 7000 rpm for 5 minutes at 4 °C. The crude enzyme was obtained from the supernatant. One hundred μL of crude enzymes dissolved in starch and 0.1 mol/L citrate-phosphate buffer pH 5.5 and incubated at 55 °C for 30 minutes. One hundred μL of 1 mol/L H₂SO₄ was used to stop the reaction by the homogenizing process for 10 seconds. The absorbance of 100 μL of the solution was measured using a spectrophotometer (Dynamica Holo RB-10) at λ=620 nm with 2.4 mL of Iodine 4% reagent (g/L) and KI : I (30:3 ratio) addition. The standard curve was determined by using 1.2% (w/v) starch solution at 0.0 – 0.3% of concentration. One enzyme unit is equivalent to the amount of enzyme produced from the hydrolysis of 10 mg of starch for 30 minutes at 55 °C. Yeast and LABs isolates with high amylolytic activity were selected, purified, and stored in 30% CYG-glycerol medium for yeast and 30% MRS-glycerol for LAB at -20 °C.

2.5. **Cassava fermentation**

The modified cassava (Mocaf) fermentation process is carried out by the submerged fermentation method. Cassava was prepared, washed, and made chips using a slicer machine. Almost 1000 g of cassava chips were added with 1500 mL of distilled water in a plastic container. The fermentation process was carried out with 2 treatments, with inoculum addition (A) and without inoculum (B), 3 replicates of each. The inoculum consisted of 25 mL CYG broth containing 5% yeast (10⁵ CFU/mL) and 25 mL MRS broth containing 5% LAB (10⁷ CFU/mL). Fermentation was carried out for 48 hours with observed parameters including pH, temperature, total LAB, and total coliform at 0, 12, 24, 36 and, 48 of fermentation time. The pH value of 15 mL of fermentation liquid was measured using a pH meter. The LAB and coliform total of fermentation liquid was calculated using the total plate count (TPC) method MRS agar + CaCO₃ medium for LAB and EMB medium for coliform. The media was incubated at 37 °C for 24 – 48 hours. At the final fermentation process, cassava chips were separated from water and then it washed two times. Cassava chips were dried using a drying oven at 50 – 55 °C. Mocaf flour was obtained by grinding the chip using a grinder machine and was sieved to result in 120 mesh of particle size. HCN levels were determined in fresh cassava, before and after fermentation. HCN analysis was conducted in the Laboratory of Agriculture Technology and Food Product, Faculty of Agriculture Technology, Universitas Gadjah Mada, Yogyakarta. The picrate paper kit method was used for the semiquantitative determination of HCN cassava [20].

2.6. **Molecular and biochemical identification**

Molecular identification of lactic acid bacteria was performed using 16S rRNA sequences analysis. PCR amplification on 16 S rDNA using Primer 27 F: 5’-AGA GTT TGA TCC TGG CTC AG-3’ and Primer
1492 R: 5' - GGT TAC CTT GTT ACG ACT T-3' GTT. Isolation of bacterial genomes, amplification, and sequencing of 16S rRNA gene sequences was carried out at InaCC, Research Center for Biology, Indonesian Institute of Sciences, Cibinong, Indonesia. Whereas molecular identification of yeast was performed using 28S rRNA sequence analysis. Isolation of yeast genomes, amplification, and sequencing of 28S rRNA gene sequences was carried out at Biotechnology Laboratory, Agency for Assessment and Application of Technology, Serpong, Indonesia. The sequenced data were trimmed and assembled with the BioEdit program and then converted in FASTA format. The DNA sequencing results in the following FASTA format at Basic Local Alignment Search Tool – Nucleotide (BLASTN) to locate the homology online at the DNA database center at National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/).

Biochemical identification procedures were carried out using the API 50 CHL kit (BioMerieux) for LAB isolates and API 20 C AUX kit (BioMerieux) according to manual instructions. Analysis of test results is carried out using the Web API.

2.7. Morphological analysis
Morphological characteristics of LAB and yeast were observed using a scanning electron microscope (SEM) after incubation at 37°C for 18 h on MRSA medium for LAB and 30 °C for 24 h on CYGA medium for yeast. After incubation, one loop of a single colony was added evenly on the surface of the carbon-tape covered stub. The sample was air-dried and coated with Au (using Au ion sputter-Hitachi MC1000) with a setting of 10 mA for 60 s and observed using SEM Hitachi SU3500. The SEM setting of high vacuum mode, accelerating voltage of 5 kV, spot intensity of 30%, and magnification of 10,000x.

2.8. Data Analysis
Quantitative data were analyzed statistically to find out the significance of the mean obtained. The analysis used was a one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT). Statistical analysis was performed with SPSS Statistics 16.0 software.

3. Result and Discussion
Twenty yeast isolates were obtained from Tapai, Tiwul, Gatot and Ragi Tapai samples. Qualitative assay of amylolytic activities on yeast extract peptone – starch medium selected 13 yeast isolates. Whereas 21 LAB isolates were selected on MRS – starch medium. The quantitative assay with iodine complex method of selected yeast and LAB isolates resulted several yeast and LABs isolates with amylolytic activities (table 1). Amylolytic activity of yeast were higher than amylolytic activity of LAB. Yeast Tr7 which was isolated from Gatot dan LAB G6 which was isolated from Ragi Tapai resulted the highest amylolytic enzyme (0.0624 dan 0.0267 U/ml). Both of selected isolates used for inoculum in cassava fermentation.

The observed parameters of cassava fermentation using yeast Tr7 and LAB G6 as inoculum shown in table 2. Cassava fermentation process by inoculum resulted in higher on the LAB population, lower on coliform population, lower on pH value, and higher on temperature than without inoculum. The population of LAB at the beginning of fermentation, was 6.9 log_{10} CFU/mL in fermentation with inoculum while in fermentation without inoculum has not been detected even though at 12 hours after fermentation the population reached 7.89 log_{10} CFU/mL. On fermentation cassava using the inoculum, the LAB population increased until the end of fermentation at 48 hours. While on the fermentation of cassava without inoculum, the amount of LAB decreased at the end of fermentation. The coliform population in cassava fermentation by inoculum was less than the cassava without inoculum. A high of total LAB in the fermentation medium indicated that LAB was able to use cassava as a substrate for growth. During the fermentation process, the LAB converted the organic acids into lactic acid and resulted in a decrease in the pH of media. Acidic pH conditions made a selective environment for microbes that are intolerant of acidic conditions such as bacterial coliform pathogens [21].
Table 1. Amylolytic activity of lactic acid bacteria and yeast isolates.

| Lactic acid bacteria | Yeast | | | Isolates | Source | Amylolytic activity (U/mL) | Isolates | Source | Amylolytic activity (U/mL) |
|----------------------|-------|---|---|----------|--------|---------------------------|----------|--------|---------------------------|
| T1                   | Tiwul | 0.0210±0.0016bcd | Ta1        | Tapai   | 0.0507±0.0056b             |
| T2                   | Tiwul | 0.0178±0.0041d   | Ta3        | Tapai   | 0.0561±0.0072ab            |
| T6                   | Tiwul | 0.0188±0.0014bcd | Ta4        | Tapai   | 0.0499±0.0081b             |
| T10                  | Tiwul | 0.0237±0.0023ab  | Ta3        | Tapai   | 0.0531±0.0024ab            |
| G6                   | Gatot | 0.0267±0.0016a   | Tc2        | Tiwul   | 0.0562±0.0013ab            |
| G7                   | Gatot | 0.0212±0.0030bcd | Tc3        | Gatot   | 0.0536±0.0009ab            |
| G8                   | Gatot | 0.0167±0.0007d   | Tc4        | Tiwul   | 0.0494±0.0065b             |
| G9                   | Gatot | 0.0202±0.0046bcd | Tc5        | Tiwul   | 0.0571±0.0052ab            |
| G10                  | Gatot | 0.0182±0.0027cd  | Tr2        | Ragi tapai | 0.0569±0.0021ab          |
| M1                   | Mocaf | 0.0206±0.0024bcd | Tr4        | Ragi tapai | 0.0571±0.0053ab          |
| M6                   | Mocaf | 0.0231±0.0023abc | Tr7        | Ragi tapai | 0.0580±0.0017ab          |

Means in the same column with different superscript differ significantly P<0.05.

However, at the end of fermentation, the coliform did not completely disappear due to coliform still grow at pH = 4. In this study, the pH of media at the end of fermentation in all treatments were 4.25 and 4.30 (table 2). The best condition for the inactivation of coliform was pH 3.7 [22]. In another study showed that Escherichia coli which is one type of coliform bacteria were still detected in populations of 10^4 CFU/mL at pH 3 for 24 hours incubation time [23].

Table 2. The parameters of cassava fermentation using yeast Tr7 and lactic acid bacteria G6 inoculum.

| Fermentation time (hours) | Total of lactic acid bacteria (log10 CFU/mL) | Total of coliform (log10 CFU/mL) | pH | Temperature (°C) |
|---------------------------|--------------------------------------------|---------------------------------|----|-----------------|
|                           | A                           | B                           | A  | B               | A                      | B               |
| 0                         | 6.90±0.02b                   | 0.00±0.00a                   | 4.75±0.05a                   | 4.84±0.06b                   | 6.49                      | 7.02                      | 28.00                      | 28.00                      |
| 12                        | 8.34±0.02d                   | 7.87±0.56c                   | 6.53±0.14d                   | 6.80±0.05d                   | 4.42                      | 4.45                      | 28.33                      | 28.00                      |
| 24                        | 8.79±0.12f                   | 8.53±0.06b                   | 7.19±0.05f                   | 8.16±0.05f                   | 4.36                      | 4.39                      | 29.00                      | 28.33                      |
| 36                        | 8.97±0.06f                   | 8.25±0.17e                   | 6.93±0.03c                   | 7.18±0.22f                   | 4.28                      | 4.34                      | 29.33                      | 29.00                      |
| 48                        | 8.55±0.39de                  | 6.93±0.24b                   | 5.76±0.11b                   | 6.28±0.05c                   | 4.25                      | 4.30                      | 29.67                      | 29.00                      |

Means in the same column with different superscript differ significantly P<0.05. Fermentation experiments using 1000 g fresh cassava with 2.5% inoculum (v/w). Inoculum composition was 1:1 (yeast : LAB) A: with inoculum and B: without inoculum.

Cassava used in this study was fresh cassava contained HCN content of 289.57 ppm. HCN content in this study was measured before and after fermentation. HCN content of cassava produced from fermentation with the addition of inoculum and without inoculum significantly were lower (p<0.05) than fresh cassava (table 3). In other study reported that the free cyanide (FC) reduction was recorded in cassava flour fermentation by L. plantarum, L. mesenteroides, and Saccharomyces cerevisiae and the FC content of all fermented sample at three fermentation time (24, 36 and 48 h) and inoculums level (0.25 ml, 0.50 ml, and 0.75 ml) was lower than the unfermented samples [24].
Table 3. Cyanide content (HCN) in fermented cassava process.

| Fermentation type | Average of HCN content (ppm) | HCN reduction (%) |
|-------------------|------------------------------|-------------------|
|                   | Before fermentation | After fermentation |                   |
| A                 | 118.07±16.63         | 75.57±4.17        | 52.19             |
| B                 | 115.49±28.57         | 78.09±7.39        | 32.38             |
| Average of HCN content (ppm) | 136.78±31.48 | 76.83±5.87<br>b |

Means with different superscript differ significantly P<0.01). Fresh cassava contains 289.57 ppm of hydrogen cyanide (HCN) content. A: with inoculum and B: without inoculum.

In this study also showed that the use of inoculums produced higher HCN formation than without inoculums. Table 3 showed that HCN residue and HCN reduction on cassava with inoculum addition was 75.57 ppm and 52.06%, these results were lower (P<0.05) than without inoculum addition which was 78.16 ppm and 32.32% (table 3). These differences occurred due to an increase in the population of bacteria, which accelerate cyanogenic glycoside damage [25]. Total HCN content in cassava chips can be substantially reduced by drying at temperature of 70 °C and air velocity of 2.75 m/s for TMS30572. The reduction in total HCN content of the cassava chips by this treatment might be due to enhanced hydrolysis process of cyanogenic glucosides by the enzyme linamarase. The higher the moisture content of the cassava the greater the loss in the cyanide content during drying [26]. HCN content in cassava could be removed using natural submerged fermentation methods. HCN is soluble in water and has a boiling point of 29 °C.

Table 4. Biochemical identification of lactic acid bacteria G6 using API 50 CHL kit.

| No. | Substrate                  | Result | No. | Substrate                  | Result |
|-----|----------------------------|--------|-----|----------------------------|--------|
| 0   | Control                    | -      | 25  | Esculin                    | +      |
| 1   | Glycerol                   | -      | 26  | Salicin                    | +      |
| 2   | Erythritol                 | -      | 27  | D-Cellobiose               | +      |
| 3   | D-ARabinose                | -      | 28  | D-Maltose                  | +      |
| 4   | L-ARabinose                | +      | 29  | D-Lactose (bovine origin)  | -      |
| 5   | D-Ribose                   | +      | 30  | D-Melibiose                | -      |
| 6   | D-Xylose                   | +      | 31  | D-Saccharose (sucrose)     | -      |
| 7   | L-Xylose                   | -      | 32  | D-Trehalose                | +      |
| 8   | D-Adonitol                 | -      | 33  | Inulin                     | -      |
| 9   | Methyl-βD-Xylopyranoside   | -      | 34  | D-Melezitrose              | -      |
| 10  | D-Galactose                | +      | 35  | D-Raffinose                | -      |
| 11  | D-Glucose                  | +      | 36  | Amidon (starch)            | -      |
| 12  | D-Fructose                 | +      | 37  | Glycogen                   | -      |
| 13  | D-Mannose                  | +      | 38  | Xylitol                    | -      |
| 14  | L-Sorbose                  | -      | 39  | Gentiose                   | -      |
| 15  | L-Rhamnose                 | -      | 40  | D-Turanose                 | -      |
| 16  | Dulcitol                   | -      | 41  | D-Lyxose                   | -      |
| 17  | Insitol                    | -      | 42  | D-Tagatose                 | +      |
| 18  | D-Mannitol                 | -      | 43  | D-Fucose                   | -      |
| 19  | D-Sorbitol                 | -      | 44  | L-Fucose                   | -      |
| 20  | Methyl-αD-Mannopyranoside  | -      | 45  | D-Arabinol                 | -      |
| 21  | Methyl-αD-Glucopyranoside  | -      | 46  | L-Arabinol                 | -      |
| 22  | N-Acetylglucosamine        | +      | 47  | Potassium Gluconate        | -      |
| 23  | Amygdalin                  | +      | 48  | Potassium 2-Ketogluconate  | -      |
| 24  | Arbutin                    | +      | 49  | Potassium 5- ketogluconate | -      |

- = negative, + = positive
Statistical analysis results showed that fermentation process and the type of fermentation could significantly reduce the HCN levels. Additionally, submerged fermentation can dissolve linamarin and lotaustralin compounds, and stimulate the growth of microorganisms that can break down toxins into organic acids. The cassava fermentation method also inactivated the linamarase enzyme and catalyzed the formation of HCN [2].

The biochemical identification of LAB isolates using the API 50 CHL kit based on the ability of bacteria to hydrolyze carbohydrates. The results of identification using the API 50 CHL kit showed that the G6 isolate had 99.6% of similarity with *Pediococcus pentosaceus*. Positive results detected on several monosaccharide, disaccharide, and oligosaccharide (table 4). This result similar with other study that show that *Pediococcus pentosaceus* JCM 5890\(^1\) was able to ferment arabinose, ribose, xylose, glucose, fructose, galactose, mannose, cellobiose and maltose [27].

Biochemical identification of Tr7 yeast isolates using the API 20 C AUX kit (table 5). Test results using API 20 C AUX kit showed that the Tr7 yeast isolate had a 95.8% similarity with *Candida tropicalis*. The positive results were obtained in glucose, gluconate, xylose, adonitol, galactose, sorbitol, glucopyranoside, glucosamine, maltose, saccharose, trehalose, and melezitose. This result was similar with other study that *Candida tropicalis* has positive result in fermentation of glucose, maltose, galactose and xylose [28].

### Table 5. Biochemical identification of yeast Tr7 using API 20 C AUX kit.

| Tests | Substrate       | Results | Tests | Substrate       | Results |
|-------|-----------------|---------|-------|-----------------|---------|
| 0     | Aucun           | -       | SOR   | D-Sorbitol      | +       |
| GLU   | D-Glucose       | +       | MDG   | Mélthyl-DD-Glucopyranoside | + |
| GLY   | Glycérol        | -       | NAG   | N-Acétyl-glucosamine | + |
| 2KG   | Calcium 2-céto-Gluconate | + | CEL   | D-Celobiose   | - |
| ARA   | L-Arabinose     | -       | LAC   | D-Lactose      | -       |
| XYL   | D-Xylose        | +       | MAL   | D-Maltose      | +       |
| ADO   | Adonitol        | +       | SAC   | D-Saccharose   | +       |
| XLT   | Xylitol         | -       | TRE   | D-Trehalose    | +       |
| GAL   | D-Galactose     | +       | MLZ   | D-Mélézitose   | +       |
| INO   | Inositol        | -       | RAF   | D-Rafinose     | -       |

- = negative, + = positive

Confirmation of identification of the two selected isolates was carried out by molecular methods using rRNA sequence analysis. The 16S rRNA amplification results of LAB G6 isolates obtained sample PCR products visualized using a gel documentation system with 1500 bp of gene size. G6 isolate was identified as *P. pentosaceus*. Homology BLAST nucleotide of 16S rDNA sequence with 1420 bp of length in the DNA bank database (http://www.ncbi.nlm.nih.gov/) accessed January 17, 2016) has a 100% max identity against the nearest bacterial taxon *P. pentosaceus* strain BSS1375 (KT351726). While the PCR results from the 28S rRNA sequence after assembled and BLAST nucleotide in DNA bank database (http://www.ncbi.nlm.nih.gov/) accessed May 15, 2017) showed that Tr7 yeast isolate was *Candida tropicalis* strain NITCSK1 with 99% of identity (table 6).

### Table 6. Molecular identification of lactic acid bacteria G6 and yeast Tr7.

| Molecular identification | Reference organism | % identity |
|-------------------------|--------------------|------------|
| TR7                     | 28S ribosomal DNA  | Candida tropicalis strain NITCSK1 | 99 |
| G6                      | 16S ribosomal DNA  | *Pediococcus pentosaceus* strain BSS1375 | 100 |
| Biochemical identification |                     | Pediococcus pentosaceus | % identity |
| TR7                     | API 20 C AUX       | Candida tropicalis | 95.8 |
| G6                      | API 50 CHL         | *Pediococcus pentosaceus* | 99.6 |
The morphological features of the two species are shown in figure 1. *Pediococcus pentosaceus* G6 isolate is a Gram-positive bacterium that has a cell size of 0.5 - 1.2 x 1.0 - 10 μm, coccus cells, and appears in pairs (diplococcus). Whereas yeast *C. tropicalis* Tr7 has ovoid cell and branching like the genus *Candida* in general. *Pediococcus pentosaceus* is commonly found in fermented food products such as rice fermentation, tempe, cassava fermentation and vegetable fermentation [29], corn, millet and sorghum based fermentation products [30], African pearl millet slurries [31], alkaline fermentations of cassava (Manihot esculenta Crantz) leaves, roselle (*Hibiscus sabdariffa*) and African locust bean (*Parkia biglobosa*) seeds [32], Tanzanian fermented food from sorghum, maize, millet and maize-sorghum [34], traditional sorghum fermentation [34]. According to Chahrour [35] study, the genera *Lactobacillus*, *Pediococcus* and *Enterococcus* were the amylolytic LABs that have founded on sorghum fermentation.

![Figure 1](image1.png)

**Figure 1.** A. Cell morphology of *Pediococcus pentosaceus* G6 isolated from gatot (400x of magnification); B. Cell morphology of *Candida albicans* Tr7 isolated from ragi tapai 1000x of magnification) with methylene blue staining; C. Scanning electron micrograph of G6 (10,000x magnification) and D. Scanning electron micrograph of Tr7 (5,000x of magnification).

*Candida* is one of the amylolytic yeast that was founded in various types of fermented products. Oyewole [36] revealed that amylolytic microbial including *Candida krusei*, *Candida tropicalis*, *Pichia saitoi*, *Saccharomyces cerevisiae*, and *Zygosaccharomyces bailii* were found in the fermentation of 'fufu'. Some studies reported that *Candida tropicalis* is commonly found and used in the fermentation process of various types of food because it has certain abilities. *C. tropicalis* BCC7755 is thermostolerant and is used in the saccharification process of ethanol fermentation from cassava pulp [37]. *C. tropicalis* is one of the dominant yeasts found in Brazilian fermented foods' cauim 'based on several substrates are used in its production, including cassava, rice, corn, maize and peanuts [38]. Tanzanian fermented food' togwa 'from sorghum, maize, millet and maize sorghum [33]. *C. tropicalis* with amylolytic activity was found in cassava-
fermenting water in the early part of the fermentation [36]. Other studies related to the ability of yeast *C. tropicalis* in the cassava process. *C. tropicalis* was isolated from Agbelima, fermented cassava meal from Ghana, Togo and Benin having cellulase, polygalacturonase and linamarase processes that can break down cyanogenic glucoside in cassava [39]. *C. tropicalis* grows on soluble starch, corn, and cassava powders. That property has been used to develop a fermentation process whereby *C. tropicalis* can be grown directly on corn or cassava powders. The biomass and residual corn or cassava contains about 20% protein, which represents a balanced diet for either animal fodder or human food. The fact that no extra enzymes are required to hydrolyze starch results in a particularly efficient step of improving the nutritional value of products, through a single-step fermentation process [40].

4. Conclusion
Yeast Tr7 and LAB G6 isolates performed the highest amylolytic activity (0.0624 U/mL and 0.0627 U/mL). Cassava fermentation products using these microbes resulted in higher hydrogen cyanide (HCN) reduction (52.06%) than without inoculum (32.32%). Biochemical and molecular identification result showed that lactic acid bacteria G6 has closest similarity with *P. pentosaceus* and yeast Tr7 has closest identity with *Candida tropicalis*. *C. tropicalis* Tr7 and *P. pentosaceus* G6 potentially used for inoculum in cassava fermentation process to improve cassava quality as functional food.

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