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FULL PAPER

The isolation and characterization of indigenous microbes with probiotic potential from *gatotan*

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Abstract

Indigenous probiotics, which are more adaptive to ethnoecology such as human gut health are found in local food. Therefore, this study aimed to isolate, select, and characterize indigenous microbes as probiotic candidates namely *gatotan*, which is an Indonesian fermented food from cassava. The potential probiotic was dissolved in artificial gastric acid at pH 2.0 and incubated for 2 hrs, then the De Man Rogosa Sharpe and yeast in Malt Extract medium were used for selective isolation of lactic acid bacteria (LAB). The phenotype including morphological and physiological characteristics as well as the genotype such as PCR-fingerprinting was identified. The results showed that two of the three isolates have the potential to be developed as probiotics possibly growing in an acidic environment. The BGP (LAB) and YGK (yeast) isolates have the highest survival artificial gastric acid of pH 2.0. Furthermore, the BGP from cassava var. white (*Malang 2*) had a milk-white colony, rod-shaped, undulate colony edges, gram-positive bacteria, non-motile, negative catalase, and heterofermentative. The YGK from cassava var. yellow (*Malang 4*) also had white-colony characteristics, irregular shape, rough surface, opaque transparency, spheroidal cell morphology, and amylolytic index of 1.06±0.12 mm. Conclusively, the genotype characterization identified both isolates as *Lactobacillus brevis* and *Pichia kudriavzevii,* respectively.

1. Introduction

Over the years, probiotic foods have attracted many scientists because of their health benefits. The WHO has defined probiotics as a microorganism that provide health benefits to the host in appropriate amounts. They are mostly found in fermented local food one of which is the Indonesian *gatotan*. Previous studies identified microbes that have the potential as probiotic candidates (Emmawati *et al.*, 2016). Astriani *et al*. (2018) isolated *gatotan* microbes as a starter to produce *gatotan* and those identified were *Rhizopus oligosporus*, *Aspergillus niger*, *Bacillus licheniformis, Trichoderma* sp., *Lactobacillus manihotivorans*, *L. ferment* and *Bacillus brevis*. Furthermore, the fermentation of *gatotan* occurs spontaneously, hence, it has the potential to support the growth of several microbes. The microorganisms present during fermentation affect the quality and safety of the final product. In the case of *gatotan*, it causes a change in black colour and soft texture.

Many superior probiotic LAB strains are found in local food sources such as spontaneously fermented products. The LAB exhibit functional and probiotic properties that are resistant to low pH, therefore they are classified as GRAS (Generally Recognized as Safe) bacteria by the US FDA or QPS (Qualified Presumption of Safety by EFSA) (Won *et al.*, 2021). Another local food that has the potential as a candidate source of probiotics is "Mandai". Additionally, fermented products from *dami* or straw cimpedak fruit contain the probiotic microbe *Lactobacillus plantarum* which is resistant to bile salts and gastric acid (Emmawati *et al.*, 2016). LAB probiotic isolates have long been developed from foods, and their functional properties stimulate the immune system (Emmawati *et al.,* 2016). *Lactobacilli* and *Bifidobacteria* survive in the intestine and benefit health (Barakat *et al.*, 2011). Probiotic microbes may provide enhanced growth, immune responses, nutrients, produce enzymes, and inhibit pathogens (Manhar *et al.*, 2011). Meanwhile, the potential probiotic characteristics of

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indigenous microbes from *gatotan* remain unknown. Therefore, this study attempted to isolate and identify the phenotype and genotype characteristics of probiotic candidates, which can survive in pH 2.0 of artificial gastric acid.

2. Materials and methods

2.1 Gatotan preparation

Fresh cassava was peeled to separate the outermost cassava peel, which was then washed and dried in the sun. Dried cassava which is an ingredient for *gatotan* was mixed with sterile distilled water 1:3 (w/v) and spontaneously fermented anaerobically in a sterile jar for 5 days (28°C). During fermentation, the texture of the substrate changes to become soft, and the slurry was used as sample isolation of microbe from indigenous *gatotan*. The main ingredients used were cassava var. yellow (*Malang 2*) and cassava var. white (*Malang 4*).

2.2 Isolation of lactic acid bacteria and yeast

A total of 1 mL slurry was diluted using 0.85% physiological solution. Subsequently, the isolation of LAB was carried out aseptically in MRSA (Himedia, India) + $CACO_3$ and MEA (Himedia, India) yeast medium. Incubation was carried out aerobically for LAB and yeast at 37°C and 30°C, respectively for 48 hrs. The microbe colonies grown were selected and calculated, and then the LAB and yeast were purified using the quadrant method. Pure cultures were then streaked into slants of medium agar and stored at 4°C for further characterization.

2.3 Probiotic screening of the artificial simulated gastric acid pH 2

The LAB and yeast isolates can tolerate simulated gastrointestinal (GI) tract conditions (Zhai *et al.*, 2015). Furthermore, the screening of probiotic candidates used two types of *gatotan* from cassava namely *Malang 4* and *2*. Gastric acid juice with the following contents (g/L): 8 NaCl; 0.2 KCL; 8.25 $Na_2HPO_4.2H_2O$; 14.35 Na_2HPO_4; 0.1 $CaCl_2.2H_2O$; 0.18 $MgCl_2.6H_2O$ is used as hydrochloric acid buffer. A volume of 1 mL slurry was added with 5 mL artificial gastric acid at pH 2 and suspended in a medium to measure the survival microbe. After 2 hours of incubation, the culture with the treatment of 1 mL was added to 9 mL of artificial simulated gastric acid at pH 2. The cultures were then incubated at 37 and 30°C (48 hrs). The percentage survival of strains was calculated as follows (Luang-In *et al.*, 2021):

$$\text{Survival rate} = \frac{\text{cell number at final time } (\log_{10} \text{CFU/mL})}{\text{cell number at initial time } (\log_{10} \text{CFU/mL})} \times 100\%$$

2.4 Characterization of gatotan lactic acid bacteria of tolerance gastric acid

Gatotan LAB isolates of gastric acid tolerance were characterized based on their phenotype (morphological and physiological) and genotype (PCR-fingerprinting). The results from probiotic screening obtained one LAB (BGP) strain from two gatot variants, which was isolated from cassava var. white. Furthermore, the morphological characteristics of LAB indigenous *gatotan* include macroscopic and microscopic. The macroscopic observations were made with a magnification of 400× and 1,000× and it included colony edges, shape, and colour, as well as their cell form. A gram-positive and gram-negative bacterium is indicated by purple and red colour, respectively. LAB's physiological properties include catalase test, motility, and type of fermentation. The catalase aims to determine the ability of bacteria to degrade H_2O_2; when this test shows the presence of oxygen bubbles, it is a catalase-positive (aerobic) bacterial isolate and catalase-negative (anaerobic) did not show bubbles. The formation of oxygen bubbles indicates the production of catalase enzyme by the test bacterium. Additionally, motility aims to determine the growth of bacteria, which is indicated by spreading growth and non-motile for positive and negative, respectively. The fermentation type test is used to determine the group of bacteria. Heterofermentative bacteria produce oxygen bubbles in the Durham tube and homofermentative will not.

The molecular analysis of LAB was performed using the 16S ribosomal rRNA encoding gene. The PCR master mix consists of dd H_2O 9.5 mL, MyTaq Red Mix (2×) 12.5 mL, 10 µM 27F primer, 10 µM 1492R primer, and 1 mL DNA template. Furthermore, the genomic DNA was extracted using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) and PCR amplification was carried out using (2x) MyTaq HS Red Mix (BIO-25048). The PCR amplification uses primer 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-AGAGTTTGATCMTGGCTCAG-3' from PT. Genetika Science, Indonesia. LAB PCR conditions include initial denaturation at 95°C for 60 s (1 cycle), at 96°C for 15 s (35 cycles), annealing at 52°C for 30 s (35 cycles), extension at 72°C for 45 s (35 cycles), and held at 4°C (1 cycle).

2.5 Characterization of gatotan yeast of tolerance gastric acid

The candidates for probiotic yeast were obtained from two *gatotan* variants and the characterization used YGK samples with the highest survival rate. The YGK strain was isolated from cassava var. yellow and the macroscopic observations were carried out by observing

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yeast colonies growing directly on the surface of the media, including their surface, colour, transparency, and cell morphology (Nurhayati *et al.,* 2018). Furthermore, the microscopic observations were carried out at 400× magnification with a microscope by staining with crystal violet solution and the cells fixed on a glass object. Yeast was cultured on extract agar medium with starch (yeast 0.3%; malt 0.3%; starch 2%; and agar 1.5%) and incubated for 2 days at 30°C. Afterwards, Lugol's iodine solution (1 g I_2, 2 g KI in 300 mL of distilled water) was added to the culture (Nurhartadi and Rahayu, 2011). The clear zone that appears around the colonies indicates amylolytic activity from the yeast and was calculated as follows (N1: clear zone diameter that appears around the colonies; N2: colonies diameter):

$$\text{Amylolytic activity (mm)}: \frac{N1 - N2}{N2}$$

The molecular analysis of yeast was performed using the 28S ribosomal rRNA, the genomic DNA was extracted using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) and PCR amplification was carried out with (2×) MyTaq HS Red Mix (BIO-25048). The PCR master mix consists of dd H_2O 9.5 mL, myTaq HS Red Mix (2×) 12.5 mL, 20 µM NL-1 primer, 20 µM NL-4 primer, and 1 mL DNA template. PCR amplification of yeast using primer NL1: 5'-GCATATCAATAAGCGGAGGAA AG3-' and primer NL4: 5'-GGTCCGTGTTTCAAGACGAG-3' from PT. Genetika Science, Indonesia. Also, the PCR conditions of yeast included initial denaturation at 95°C for 3 minutes (1 cycle) and 10s (35 cycles), annealing at 52°C for 30s (35 cycles), extension at 72°C for 45s (35 cycles), and held at 4°C (1 cycle).

2.6 Phylogenetic analysis

The amplification of the gene was carried out using primer and sequencing of the obtained DNA fragments. Subsequently, DNA sequencing was performed from purified PCR products using Bi-directional sequencing. Isolates characterization aims to determine the genus and strain (Suryani *et al.*, 2021). The assembly of PCR-16S rRNA and 28S rRNA was then carried out on May 28, 2021, and analyzed using BLASTN (Basic Local Alignment Search Tool Nucleotides) (http://www.ncbi.nlm.nlh.gov/), which was also used to present the DNA sequencing results in the FASTA. Sequence similarity was estimated by homology and the phylogenetic tree was constructed at National Center for Biotechnology Information (NCBI) database.

3. Results and discussion

3.1 Gatotan microbial tolerance to artificial gastric acid

The probiotic must have resistance to gastric acid pH using a bile salt solution test (1%) and low pH (2) (Jafari -Nasab *et al.*, 2021). The results of the *gatotan* microbial resistance test against artificial gastric acid can be seen in Table 1. Furthermore, the surviving *gatotan* microbes were characterized as shown in Table 2.

The results showed differences in the type of growing microbes. *Gatotan* var. white indicates the growth of LAB and yeast, while *gatotan* var. yellow only shows yeast. This is also due to the composition of the cassava content of the white variety which is different from the yellow variety. Ayetigbo *et al*. (2018) reported that yellow cassava has a yellower colour and is sweeter than white.

According to Astriani *et al.* (2018), the types of microbes identified in *gatotan* are molds including *R. oligosporus*, *A. niger*, and *Trichoderma* sp. as well as LAB namely *L. manihotivorans*, *L. ferment*, *B. licheniformis*, and *B. brevis*. Figure 1 shows the difference in the appearance of the whole cassava, peeled cassava, dried *gatotan* and submerged *gatotan*. The slurry *gatotan* has pH of 5.3 in both variants.

Figure 1. Characteristics of cassava var. white (A) and cassava var. yellow (B) that the whole cassava (i), peeled cassava (ii), dried *gatotan* (iii), and submerged *gatotan* (iv)

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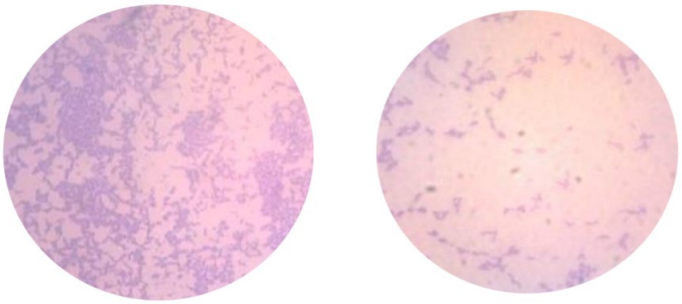
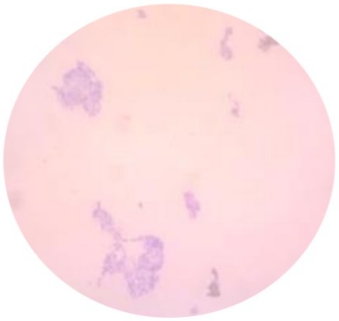
Table 1. The microbial survival on the artificial gastric acid at pH 2.0

Gatotan variant	Microbial type	Population 0 hr (log_{10} CFU/mL)	Population 2 hr + pH 2 (log_{10} CFU/mL)	Survival (%)
White	Yeast (YGP)	8.82±0.43	7.75±0.41	85.88±0.45
	Bacteria (BGP)	8.59±0.33	7.76±0.53	90.29±2.68
Yellow	Yeast (YGK)	8.67±0.55	7.68±0.59	88.51±1.27

Table 2. The characteristics of BGP and YGK isolates.

Microbial	Characteristics	Description
BGP	Physiological	A form of convex colonies, milk-white with undulated edges, rod-shaped, gram-positive, catalase-negative (anaerobic), clear zone is formed, heterofermentative, and non-motile
	Morphological	Macroscopic:  Microscopic (400x and 1000x): 
YGK	Physiological	Irregular colony, rough surface, white, opaque transparency, spheroidal cell morphology, and amylolytic index of 1.06±0.12 mm
	Morphological	Macroscopic: Microscopic (400x):  

Three isolates were obtained from slurry *gatotan* (yellow and white var. of cassava). Furthermore, the high survival rate of microbe, which includes BGP (90.29%) and YGK (88.51%) indicates that they can live at low pH. The lowest was 85.88% (YGP). The results showed that probiotic bacteria and yeast only grow on var. white indicating that cassava varieties affect their growth. The microbial population decreased in time intervals (0-2 hrs) proving that all isolates had the same acidity tolerance, and an increasing acidic condition affects their viability. It showed that the indigenous *gatotan* microbial has potential as probiotic candidate.

3.2 Characteristics of gatotan microbial resistance to artificial gastric acid

Gatotan LAB isolation results of tolerance gastric acid were obtained from the *gatotan* var. white. Meanwhile, LAB is known as a commercial species and an important group of bacteria in the food industry such as dairy products (Elzeini *et al.*, 2021). Various LABs are considered safe in food or probiotics and improve nutrition for human health (Aplevicz *et al.,* 2014). Those found in the cassava var. white can survive at pH 2, hence, they have the potential as probiotics. Table 1 showed the survival of According to Mulaw *et al.* (2019), fermented food contains probiotic microorganisms that can degrade carbohydrates, it was

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used as a sample to obtain LAB isolates that have the potential as probiotics. The main LAB species in fermented foods is the genus *Lactobacillus* (8 log CFU/g) (Aplevicz *et al*., 2014).

The results of LAB morphology are consistent with the reports of Aplevicz *et al*. (2014). The morphology of LAB was able to produce lactic acid as the end product, which was gram-positive, negative catalase, non-spore forming rods, and non-motile. Negative catalase indicates that they are anaerobic, hence cannot grow in the presence of O_2 or acidic substances due to the formation of H_2O_2 in the atmosphere, which is toxic to bacteria. In spontaneously fermented amaranth samples, 15 gram-positive and catalase-negative, eight cocci-shaped, and seven rod-shaped isolates were identified. They included *L. plantarum*, *Lactococcus garvieae*, and *Weissella cibaria* (Iruene *et al.,* 2021). LAB isolated from *gatotan* is homofermentative bacteria and can only produce lactic acid as the main product of fermentation (Anjum *et al.*, 2014).

The BGP isolate was identified as *L. brevis* which had 99.93% similarity (Figure 2). The phylogenetic tree construction of *L. brevis* contained two groups; the first consists of *L. brevis* strain TMW 1.2112 (CP016797.1) and 1.2113 (CP019750.1)*, L. brevis* F2a (KX010095.1), Lb13H (KP793173.1), Lb2H (KP793167.1), gp71 (KM495920.1), gp33 (KM495905.1). The second group consists of *Levilactobacillus brevis* strain TMW 1.2113 (CP019750.1); *L. brevis* 7872 (MT464157.1), 8466 (MT464328.1), and 4116 (MT544691.1). Nucleotide BLAST homology of LAB was carried out using assembly sequences 1428bp and according to Yakabe *et al*. (2009), *L. brevis* has been found in fermented food. The strain *L. brevis* GRL1 from fermented olives is found in the human guts and can tolerate by GI (Rönkä *et al.,* 2003).

3.3 Characteristics of gatotan yeast of tolerance gastric acid

Yeast is a safe eukaryotic microorganism widely used in fermented foods (Wulan *et al.,* 2021). Previous studies have identified probiotic yeasts in fermented foods such as kefir, cocoa, cheese, and other products. According to Hsiung *et al.* (2021), yeast can grow at 37° C, survive at the pH of gastric acid and bile salts, and aggregate with various pathogenic microbes. In this study, YGK was isolated from *gatotan* var. yellow which can survive at pH 2, hence it has the potential as a probiotic candidate. Generally, several species of yeast are probiotics, an example is the *Saccharomyces boulardii*, which is used to treat gastrointestinal (GI) (Zanello *et al.,* 2009). The reasons for the exploration of probiotic yeast include (1) even though yeast cell size is roughly 10 times greater than bacterial, it can only contribute about <0.1% of microbiota in the GI tract, suggesting that it can represent stearate inhibition which is significant for bacteria; (2) differences in the composition of the cell wall of yeast and bacteria, the response is called immunobiotic (Huffnagle and Noverr, 2013). Subsequently, the morphological characteristics of yeast include colonies, surface, colour, transparency, and cell morphology. In a study by (Moon *et al.*, 2014), yeast was isolated and identified in kimchi that had irregular or circular colonies; rough or smooth surface; ivory, white, and pink colours; translucent or opaque transparency; oval or spheroidal cell morphology. They include *Rhodotorula mucilaginosa*, *Kazachstania bulderi*, *K. exigua*, *K. servazzii* and *Pichia kudriavzevii*.

The fermentation of *gatotan* involves various types of microorganisms such as molds, LAB, and yeasts. Furthermore, the raw material for *gatotan* is cassava which contains starch causing changes in components during fermentation. Microorganisms such as fungi, yeast, and bacteria found in cassava can decompose starch through the production of an enzyme called amylase (Damayanti *et al.*, 2021). Cassava starch is a polysaccharide that includes amylose and amylopectin polymers and it is a branched polymer of glucose molecules having 1.6 glycoside bonds. The results showed that the amylolytic index of YGK was 1.06±0.12 mm with a microbial and clear zone diameter of 5.61 mm and 11.50 mm, respectively. This result is consistent with the reports of Nurhartadi and Rahayu (2011) that tape contains two amylolytic yeast isolates namely *Saccharomycopsis fibuligera* and *Pichia burtonii*.

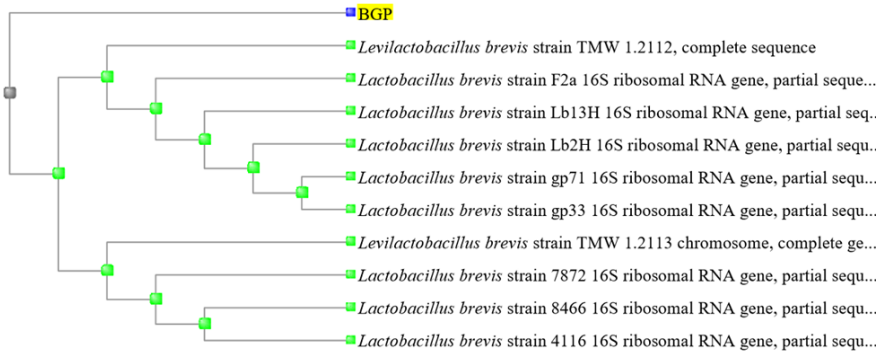

Figure 2. Neighbour-joining phylogeny (NJP) tree analysis of BGP isolate (*Lactobacillus brevis*).

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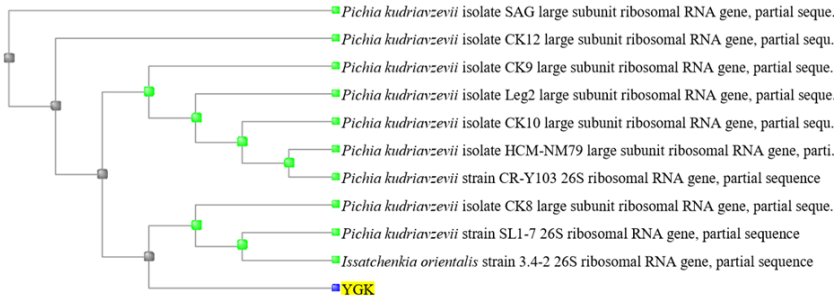

Figure 3. Neighbour-joining phylogeny (NJP) tree analysis of isolated YGK (*Pichia kudriavzevii*).

Additionally, the amount of amylolytic activity indicated that yeast plays a major role in starch hydrolysis.

The YGK isolate was identified as *P. kudriavzevii*, which had 100% similarity and the phylogenetic tree construction of *P. kudriavzevii* contained four groups (Figure 3). The first and second consist of *P. kudriavzevii* SAG (MN595038.1) and CK12 (MN712334.1), respectively. The third group includes *P. kudriavzevii* CK9 (MN710524.1), Leg2 (MH472647.1), CK10 (MN710525.1), HCM-NM79 (MK101219.1), CK8 (MN710523.1), and strain CR-Y103 (KX118628.1), and the last include *P. kudriavzevii* SL1-7 (HM123747.1), and 3.4-2 (FJ455113.1). Nucleotide BLAST homology of yeast was determined using assembly sequences 578 bp. This result is consistent with the report of Kurtzman (2011) that *P. kudriavzevii* is distributed in spontaneous fermentation and is proven in this study using *gatotan*. According to Rodriguez *et al.* (2021), *P. kudriavzevii* can be a probiotic candidate from a food environment.

4. Conclusion

Lactobacillus brevis and *P. kudriavzevii* were microbes isolated from *gatotan* that survived in artificial simulated gastric acid, with a survival rate of 88.51% and 90.29%, respectively. The characteristics of *L. brevis* from the isolation of *gatotan* var. white include milk-white colony, rod-shaped, undulate edges, gram-positive bacteria, non-motile, heterofermentative, and negative catalase. Similarly, *P. kudriavzevii* characteristics from the isolation of *gatotan* var. yellow include white-colony and irregular colony, rough surface, opaque transparency, and spheroidal cell morphology with an amylolytic index of 1.06±0.12 mm. Subsequently, the indigenous microbe from *gatotan* obtained LAB and yeast with promising probiotic properties for further evaluation, hence in vitro testing is recommended.

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References

Anjum, N., Maqsood, S., Masud, T., Ahmad, A., Sohail, A. and Momin, A. (2014). *Lactobacillus acidophilus*: characterization of the species and application in food production. *Critical Reviews in Food Science and Nutrition*, 54(9), 1241-1251. https://doi.org/10.1080/10408398.2011.621169

Aplevicz, K.S., Mazo, J.Z., Ilha, E.C. and Dinon, A.Z. (2014). Isolation and characterization of lactic acid bacteria and yeasts from the Brazilian grape sourdough. *Brazilian Journal of Pharmaceutical Sciences*, 50(2), 321-327. https://doi.org/10.1590/S1984-82502014000200011

Astriani, A., Diniyah, N., Jayus, J. and Nurhayati, N. (2018). Phenotypic identification of indigenous fungi and lactic acid bacteria isolated from 'Gatot' an Indonesian fermented food. *Biodiversitas Journal of Biological Diversity*, 19(3), 947-954. https://doi.org/10.13057/biodiv/d190325

Ayetigbo, O., Latif, S., Abass, A. and Müller, J. (2018). Comparing characteristics of root, flour and starch of biofortified yellow-flesh and white-flesh cassava variants, and sustainability considerations: A Review. *Sustainability*, 10(9), 89. https://doi.org/10.3390/su10093089

Barakat, O.S., Ibrahim, G.A., Tawfik, N.F., El-Kholy, W.I. and El-Rab, G.D. (2011). Identification and probiotic characteristics of *Lactobacillus* strains isolated from traditional domiati cheese. *International Journal of Microbiology Research*, 3 (1), 59-66. https://doi.org/10.9735/0975-5276.3.1.59-66

Damayanti, E., Ichsyani, M., Istiqomah, L., Anggraeni, A.S. and Kurniadi, M. (2021). Fermentation of amylolytic yeast and lactic acid bacteria to improve the quality of modified cassava. *IOP Conference Series: Materials Science and Engineering*, 1011(1), 012004. https://doi.org/10.1088/1757-899X/1011/1/012004

Elzeini, H.M., Ali, A.R.A.A., Nasr, N.F., Elenany, Y.E. and Hassan, A.A.M. (2021). Isolation and

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identification of lactic acid bacteria from the intestinal tracts of honey bees, *Apis mellifera* l., in Egypt. *Journal of Apicultural Research*, 60(2), 49-57. https://doi.org/10.1080/00218839.2020.1746019

Emmawati, A., Jenie, B.S.L., Nuraida, L. and Syah, D. (2016). Aggregation and adhesion abilities to enterocyte-like HCT-116 cells of probiotic candidates *Lactobacillus plantarum* strains isolated from 'mandai', Indonesian fermented food against enteropathogens. *International Food Research Journal*, 23(5), 34-40.

Hsiung, R.T., Fang, W.T., Lepage, B.A., Hsu, S.A., Hsu, C.H. and Chou, J.Y. (2021). In vitro properties of potential probiotic indigenous yeasts originating from fermented food and beverages in Taiwan. *Probiotics and Antimicrobial Proteins*, 13(1), 113-124. https://doi.org/10.1007/s12602-020-09661-8

Huffnagle, G.B. and Mairi, C.N. (2013). The emerging world of the fungal microbiome. *Trends in Microbiology*, 21(7), 34-41. https://doi.org/10.1016/j.tim.2013.04.002

Iruene, I.T., Wafula, E.N., Kuja, J. and Mathara, J.M. (2021). Phenotypic and genotypic characterization of lactic acid bacteria isolated from spontaneously fermented vegetable amaranth. *African Journal of Food Science*, 15(6), 54-61. https://doi.org/10.5897/AJFS2021.2107

Kurtzman, C.P. (2011). Discussion of teleomorphic and anamorphic ascomycetous yeasts and yeast-like taxa. In Kurtzman, C.P., Fell, J.W. and Boekhout, T. (Ed.) The yeasts. 5th ed., p. 293-307. Netherlands: Elsevier. https://doi.org/10.1016/B978-0-444-52149-1.00013-6

Luang-In, V., Saengha, W., Karirat, T., Deeseenthum, S., Buranrat, B., Thanoi, S.N., Ma, N.L. and Narbad, A. (2021). Probiotic attributes, GABA-producing capacity and cytotoxic effects of microbes isolated from Thai fermented foods. *International Journal of Agriculture and Biology*, 25(2), 409-419. https://doi.org/10.17957/IJAB/15.1682

Jafari-Nasab, T., Khaleghi, M., Farsinejad, A. and Khorrami, S. (2021). Probiotic potential and anticancer properties of *Pediococcus* sp. isolated from traditional dairy products. *Biotechnology Reports*, 29, e00593. https://doi.org/10.1016/j.btre.2021.e00593

Manhar, A.K., Bashir, Y., Saikia, D., Nath, D., Gupta, K., Konwar, B.K., Kumar, R., Namsa, N.D. and Mandal, M. (2016). Cellulolytic potential of probiotic *Bacillus subtilis* AMS6 isolated from traditional fermented soybean (Churpi): An in-vitro study with regards to application as an animal feed additive. *Microbiological Research*, 186(1), 62-70. https://doi.org/10.1016/j.micres.2016.03.004

Moon, S.H., Chang, M.I., Kim, H.Y. and Chang, H.C. (2014). *Pichia kudriavzevii* is the major yeast involved in film-formation, off-odor production, and texture-softening in over-ripened kimchi. *Food Science and Biotechnology*, 23(2), 89-97. https://doi.org/10.1007/s10068-014-0067-7

Mulaw, G., Tessema, T.S., Muleta, D. and Tesfaye, A. (2019). In vitro evaluation of probiotic properties of lactic acid bacteria isolated from some traditionally fermented Ethiopian food products. *International Journal of Microbiology*, 2019, 7179514. https://doi.org/10.1155/2019/7179514

Nurhartadi, E. and Rahayu, E.S. (2011). Isolasi dan karakterisasi yeast amilolitik dari ragi tape isolation and characterization of amylolytic yeast from ragi tape. *Jurnal Teknologi Hasil Pertanian*, 4(1), 66-73. [In Bahasa Indonesia].

Nurhayati, N., Bambang, S., Ika, F. and Jayus, J. (2018). Isolation and identification of osmophilic yeasts isolated from molasses sugarcane as bioethanol starter. *Atlantis Press*, 172(1), 223-228. https://doi.org/10.2991/fanres-18.2018.46

Rodriguez, P.F.P., Villena, M.A., Rosa, I.Z. and Perez, A.B. (2018). Selection of potential non-*Sacharomyces* probiotic yeasts from food origin by a step-by-step approach. *Food Research International*, 112(1), 143-151. https://doi.org/10.1016/j.foodres.2018.06.008

Rönkä, E., Malinen, E., Saarela, M., Koski, R.M., Aarnikunnas, J. and Palva, A. (2003). Probiotic and milk technological properties of *Lactobacillus brevis*. *International Journal of Food Microbiology*, 83(1), 63-74. https://doi.org/10.1016/S0168-1605(02)00315-X

Suryani, A.E., Anggraeni, A.S., Istiqomah, L., Damayanti, E. and Karimy, M.F. (2021). Isolation and identification of phytate-degrading yeast from traditional fermented food. *Biodiversitas Journal of Biological Diversity*, 22(2), 66-73. https://doi.org/10.13057/biodiv/d220241

Won, G., Choi, S.I., Park, N., Kim, J.E., Kang, C.H. and Kim, G.H. (2021). In vitro antidiabetic, antioxidant activity, and probiotic activities of *Lactiplantibacillus plantarum* and *Lacticaseibacillus paracasei* strains. *Current Microbiology*, 78(8), 81-91. https://doi.org/10.1007/s00284-021-02588-5

Wulan, R., Astuti, R.I., Rukayadi, Y. and Meryandini, A. (2021). Evaluation of indigenous *Pichia kudriavzevii* from cocoa fermentation for a probiotic candidate. *Biodiversitas Journal of Biological Diversity*, 22(3), 17-25. https://doi.org/10.13057/biodiv/d220331

https://doi.org/10.26656/fr.2017.7(S1).9

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Yakabe, T., Moore, E.L., Yokota, S., Sui, H., Nobuta, Y., Fukao, M., Palmer, H. and Yajima, N. (2009). Safety assessment of *Lactobacillus brevis* KB290 as a probiotic strain. *Food and Chemical Toxicology*, 47(10), 2450-2453. https://doi.org/10.1016/j.fct.2009.07.001

Zanello, G., Meurens, F., Berri, M. and Salmon, H. (2009). *Saccharomyces boulardii* effects on gastrointestinal diseases. *Current Issues in Molecular Biology*, 11(33), 47-58.

Zhai, Q., Yin, R., Yu, L., Wang, G., Tian, F., Yu, R., Zhao, J., Liu, X., Chen, Y.Q., Zhang, H. and Chen, W. (2015). Screening of lactic acid bacteria with potential protective effects against cadmium toxicity. *Food Control*, 54(1), 23-30. https://doi.org/10.1016/j.foodcont.2015.01.037

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