Food Research 7 (Suppl. 1) : 276 - 282 (2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Isolation and identification of gastric acid-tolerant yeast from tapai

[1]Wahyuni, I., [2]Purwandari, U., [3]Subagio, A. and [3,*]Nurhayati, N.

[1]*Graduate School of Biotechnology, University of Jember, Jember 68121, Indonesia*
[2]*Department of Agroindustrial Technology, Faculty of Agriculture, University of Trunojoyo Madura, Madura 69162, Indonesia*
[3]*Department of Agricultural Product Technology, Faculty of Agricultural Technology, University of Jember, Jember 68121, Indonesia*

Article history:
Received: 4 February 2022
Received in revised form: 8 March 2022
Accepted: 11 March 2022
Available Online: 15 August 2023

Keywords:
Fermented food,
Identification,
Isolation,
Probiotic,
Tapai,
Yeast

DOI:
https://doi.org/10.26656/fr.2017.7(S1).13

Abstract

Lactobacilli and *Bifidobacteria* are the most extensively employed bacterial strains in marketable probiotic supplements. However, another probiotic was recently developed from yeast screening based on tolerance against gastric acid. This research aimed to isolate yeasts from traditional Indonesian fermented food (tapai). Screening of probiotic yeasts was based on their survival in gastric acid of pH 2.0. Yeast strains were cultured in malt extract agar, and their phenotype and genotype characteristics were identified. Phenotype characteristics were based on yeast cells' colony, microscopy, and physiology. Meanwhile, genotype characteristics were determined using the PCR-fingerprinting technique to identify the sequence homology compared to the GenBank database and the phylogenetic tree construction. The result showed that SUL and SM isolates have the highest survival on artificial gastric acid of pH 2.0. The SUL isolate from tapai brand "Sumber Madu" has a morphologically wrinkled colony, no pseudo mycelium, white surface colony, and round cell shape. In contrast, the SM isolate from tapai brand "Sari Madu" has a thin wide colony with no pseudo mycelium, turbid white surface, and oval cell shape. After 2 h incubation on gastric acid, SUL and SM isolates grew up to 6.20±0.35 CFU/mL (survival yeast of 82.71%) and 5.75±0.45 CFU/mL (survival yeast of 79.74%), respectively. The SUL isolate was identified as *Kodamaea ohmeri*, while the SM isolate was identified as *Pichia kudriavzevii*.

1. Introduction

From 2004 to 2015, Indonesia was the sixth-largest producer of cassava (*Manihot esculenta*) (Yuliati *et al*., 2019). Up to 32.8 million tons of cassava were produced in 2018, which equates to an average productivity of 21.85 tons/ha. The agricultural sector in Jember regency has a wide range of potential commodities and considers the availability of a large harvest area of cropping cassava to offer resources for generating healthier foods. Fermented foods have increased nutritional bioavailability in the industrialized world by regulating microbiota and altering certain target activities for host health. For example, tapai, a popular fermented cassava meal in Jember, imparts a range of sweet-sour flavours, soft textures, and yeast as a starter culture (Nuraida and Owens, 2014). Yeast in tapai consists of diverse species, including *Trichosporon* sp., *Clamydomucor* sp., *Candida* sp., and *Saccharomyces* sp. (Tamang *et al*., 2016). Currently, microbes such as yeast and bacteria have been investigated to contain probiotic properties on human health (Czerucka *et al*., 2007; de Melo Pereira *et al*., 2018; Xu *et al*., 2018). In 2002, the Food and Agriculture Organization (FAO) and World Health Organization (WHO) defined probiotics as live microorganisms that possess health outcomes and effects when administered in balanced amounts (Halder and Mandal, 2015).

Lactic acid bacteria (LAB) are the most common and well-studied probiotics. However, certain yeast species have recently been promoted as an effective probiotic in many functional clinical studies (Salminen *et al*., 2010; Syal and Vohra, 2013; Xu *et al*., 2018). Some popular commercial yeasts include *Saccharomyces boulardii* (Edwards *et al*., 2007) and *Saccharomyces cerevisiae,* extensively identified due to their significant contribution to the performance of probiotics (Czerucka *et al*., 2007; Didari *et al*., 2014). Widyatmoko *et al*.

*Corresponding author.
Email: *nurhayati.ftp@unej.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

(2018) isolated and identified the indigenous yeast from Jember's favourite tapai products. The screening results from using amylolytic yeast to improve the cassava starch fermentation process. Indigenous yeasts used as probiotics are expected to resist gastric acidity and bile salts, anti-microbial activity against pathogens, presence of gastrointestinal enzymes, and body temperature at 37° C (Gil-Rodríguez *et al*., 2015; Johansen *et al*., 2019). However, the presence of indigenous tapai yeast (ITY) as a potential probiotic candidate remains unknown. This study isolates and identifies indigenous yeast's phenotype and genotype characteristics from tapai. It evaluates the potential of probiotic sources with tolerance to artificial gastric acid of pH 2 to develop the probiotic from yeast rather than lactic acid bacteria.

2. Materials and methods

2.1 Screening the yeast from favorite tapai products

Four types of favourite tapai products were collected from Jember and Bondowoso regions in Indonesia. Tapai Sumber Madu and Sari Madu were obtained from Jember, while Handayani and Tapai Manis BWS were obtained from the Bondowoso district of East Java, Indonesia. The artificial gastric acid and 5 M buffered hydrochloric acid (pH 2) were used with the following contents: NaCl 8 g; KCL 0.2 g; $Na_2HPO_4.2H_2O$ 8.25 g; $NaHPO_4$ 14.35 g; $CaCl_2.2H_2O$ 0.1 g; $MgCl_2.6H_2O$ 0.18 g. A total of 10 g of tapai sample was homogenized in 90 mL of sterile distilled water after three days of incubation. Approximately 1 mL of the solution was diluted in a 9 mL physiological solution of 0.85% NaCl (w/v). A total of 1 mL was transferred to a malt extract agar (MEA) medium using the pour plate method. The samples were incubated for 48 hrs at 30°C until growth was achieved (Ebabhi *et al*., 2013). After the incubation, 5 mL of gastric acid was dropped in the plate and incubated for 24 and 48 hrs at 30°C. Then, 2 mL of solution from the plate was pipetted, inoculated separately in MEA medium, and incubated at 30°C for 48 hrs.

2.2 Determination of macroscopic and microscopic morphological of yeast

Macroscopic characters were recorded based on colony colour, convexity, shape, surface, and elevation (Meyer *et al*., 1984; Widiastutik and Alami, 2014). The appearance of the colony was achieved using streaking methods of the colony on MEA medium after 48 hrs incubation at 30°C. The microscopic character was conducted using crystal violet dye, staining yeast cells fixed on a glass object. Yeast cells were seen using a microscope with a magnification of 400×.

2.3 Determination of growth temperature of tapai yeast isolates

Two loopful of yeast isolates were transferred to 1 mL of sterile physiological solution. Additionally, a 10 µL liquid sample was diluted in a 1 mL malt extract broth (MEB) medium and homogenized using a vortex. The growth temperature of each ITY isolate was incubated at 10°C, 28°C , 37°C , 40°C , and 45°C for 48 hrs. The results were assessed based on the turbidity of the MEB medium in the cell culture tube.

2.4 Analysis of survival yeast on artificial gastric acid

The pre-selected yeasts from the previous analysis were further characterized for their resistance to gastric acid of pH 2 using the method of Rajkowska and Kunicka-Styczynska (2010) with some modifications. Samples were collected at 0 resemble, as the initial population and after 2 hrs of incubation in 5 M buffered hydrochloric acid (pH 2) as the final population of ITY. Two loopful of ITY were dissolved in 5 mL sterile MEB broth and incubated at 30°C overnight. A 1 mL solution was transferred to a 9 mL physiological solution of 0.85% NaCl (w/v) to determine initial yeast survival. The solution (1 mL) was diluted in 9 mL buffered hydrochloric acid (pH 2) and incubated for 2 hrs at room temperature to measure final yeast survival. For initial and final yeast survival, seven serial dilutions of yeast isolates were collected in 9 mL saline water (0.85% NaCl). Then, 1 mL of the dilutions were pour-plated into MEA at 30°C for 48 hrs. The percentage survival of yeast strains was calculated using the equation given by the following formula:

$$\text{Survival (\%)} = \frac{\text{CFU/mL Final}}{\text{CFU/mL Initial}} \times 100\%$$

2.5 Isolation of yeast DNA

DNA extraction was conducted using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) and amplification PCR used (2×) MyTaq HS Red Mix (Bioline, BIO-25048). PCR master mix consists of dd H_2O 9.5 mL, 2× myTaq HS Red Mix 12.5 mL, 20 µM NL-1 primer, 20 µM NL-4 primer, and 1 mL DNA template. The DNA strands NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGTTTCAAGACGG-3') of the D1/D2 28S rRNA gene were read using the PCR primers from PT. Genetika Science, Indonesia. The PCR reaction is under the following condition: 3 mins of initial denaturation at 95°C (1 cycle), denaturation at 95°C for 10 s (35 cycles), annealing at 52°C for the 30 s (35 cycles), extension at 72°C for 45 s (35 cycles) and held at 4°C (1 cycle).

https://doi.org/10.26656/fr.2017.7(S1).13

2.6 Sequencing analysis of tapai yeast isolates

The genes were amplified using primers, and DNA fragments produced were sequenced. DNA sequencing was performed from purified PCR products, and Bi-directional sequencing was used. The results were then compared to the sequences included in the GenBank database using BLASTN (Basic Local Alignment Search Tool) at www.ncbi.nlm.nih.gov/BLAST (Altschul *et al*., 1990). The NCBI Blast Tree Method was used to generate the phylogenetic tree using Neighbor-Joining (Unrooted Tree).

3. Results and discussion

3.1 Microscopic and macroscopic morphological of tapai yeast isolates

The five yeast isolates were cultured using an MEA medium incubated at 30^{o}C for 48 hrs. The morphological characteristic of ITY is represented in Table 1. SUL and SUP isolates were generated from the tapai Sumber Madu brand, while SM isolates were from the tapai Sari Madu brand. TM isolate was isolated from Tapai Manis BWS and H isolate from tapai Handayani brand. The microscopic and macroscopic appearance is represented in Figure 1 and Figure 2.

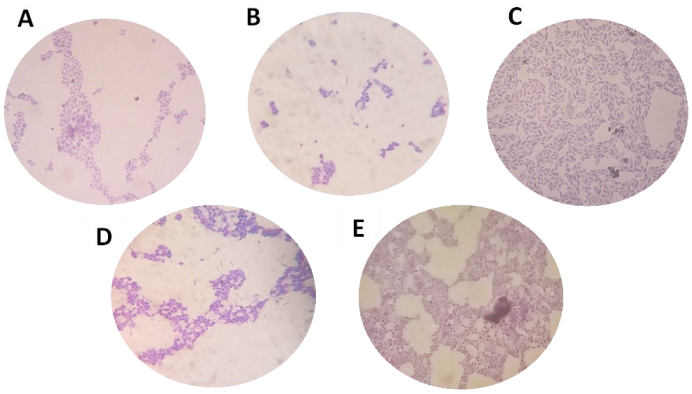

Figure 1. The cell of tapai yeast isolates under microscopic observation at 400x magnification using crystal violet staining: A: SUL, B: SUP, C: SM, D: TM, E: H.

The macroscopic morphology of yeast has been reported with white colour, cream-grained texture, opaque colony surface, and convex elevation (Widiastutik and Alami 2014). According to Moon *et al*. (2014), yeasts also identified the morphology characteristics with white colour, circular shape, entire colony edge, convex elevation, growing at 37°C, with an elongated cell having pseudo mycelium. A similar result in morphological characteristics of yeast has been observed in the previous study, where *Candida tropicalis* has a white-cream colour, round colony shape, convex elevation, flat edge, smooth surface, and oval cell shape (Suryaningsih *et al*., 2018).

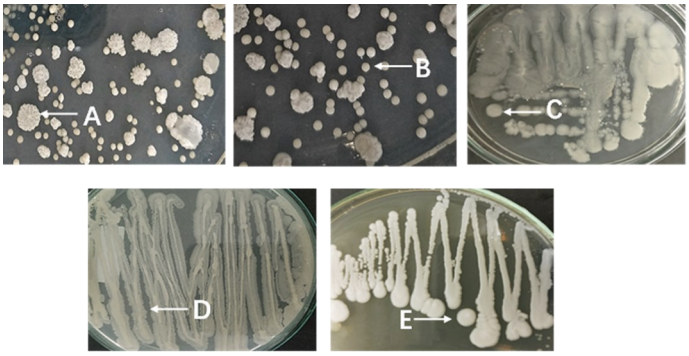

Figure 2. Macroscopic morphological observation of tapai yeast isolates, A: SUL, B: SUP, C: SM, D: TM, E: H.

3.2 Growth temperature of tapai yeast isolates

The yeast isolates were cultured in MRSB (Man Ragosa Sharpe Broth) medium for 24 and 48 hrs incubation at various temperatures. When selecting a yeast strain starter for industrial fermentation, stress resistance to pH and temperature is required. Suriasih *et al*. (2012) proved that higher yeast counts increased with higher incubation temperature (28±2°C). However, potential probiotic yeast should resist viability at the body temperature (37°C) (Gil-Rodríguez *et al*., 2015; Johansen *et al*., 2019). In this study, all strains showed specific growth in the presence of temperature stress at 37-40°C. It provides information regarding resistance to the fermentation temperature of tapai. During the fermentation process, heat production was caused by the exothermic reaction to increase the optimal temperature of tapai by 35-40°C (Kanino, 2019).

3.3 Yeast survival on the artificial gastric acid

The best-demonstrated survival percentage of indigenous tapai yeast as probiotic agents under stressful

Table 1. Morphological characteristic of tapai yeast isolates.

Code isolate	Colony shape	Color	Morphological characteristics				
			Elevation	Colony edge	Surface	Cell form	Pseudohyphae
SUL	Wrinkle round	White milk	Raised	Undulate (Wavy)	Rough	Spherical oval	-
SUP	Small round	White milk	Convex	Entire	Smooth	Spherical oval	-
SM	Thin wide	White turbid	Flat	Entire	Smooth	Elongated cell	-
TM	Thin wide	White turbid	Flat	Lobate	Rough	Spherical oval	-
H	Small round	White milk	Convex	Entire	Smooth	Spherical oval	-

Note: SUL, SUP, and SM were isolated from tapai favorite products of Jember; TM and H were isolated from tapai favorite products of Bondowoso

https://doi.org/10.26656/fr.2017.7(S1).13

pH 2 were SUL (82.71%) and SM isolates (79.74%). At the end of 2 hrs incubation at artificial gastric acid, these isolates had a growth capacity of 6.20±0.35 CFU/mL and 5.75±0.45 CFU/mL. In artificial gastric acid, the probiotic survival percentage for the other three presented only a slight variation of 52.68% (SUP), 54.81% (TM), and 54.40% (H). However, the growth rate of the yeasts equal 3.98±0.03 CFU/mL for SUP, 3.81±0.31 CFU/mL for TM, and 3.99±0.07 CFU/mL for H. All isolates have the same acidity tolerance, with increasing acidity affecting probiotic viability. This pattern is evident at all time intervals (0 and 2 hrs), as tests showed a drop in viable yeast counts as the acidity level of the sample increased.

Wickerhamomyces anomalus LV-6, the yeast strain isolated from the fermented excreta of broilers, has been investigated with a survival percentage of 98.30% and viability of yeast of 7.50±0.11 CFU/mL under gastric acid of pH 2 for 3 hrs incubation (García-Hernández *et al*., 2012). *Pichia kudriavzevii* OM11 had the highest survival of 93.46% at pH 2 after an exposure period of 3 hrs at 37°C. The crucial characteristic of a good probiotic source is tolerating high acid levels (pH) in the stomach ranging from 2-5. Each yeast isolate is significant in low pH tolerance because the probiotic source can survive at 37°C, colonize the gastrointestinal system, and the presence of bile salt (Karasu-Yalcin *et al*., 2019). Sahadeva *et al*. (2011) demonstrated that microorganisms from cultured milk drinks in the Malaysian marketplace survive at pH 3.0 and make them good probiotic sources. Brands A, B, and C had a growth capacity of 6.94 CFU/mL, 6.60 CFU/mL, and 9.40 CFU/mL at 37°C for 3 hrs incubations.

The probiotics should be present in sufficient quantities to benefit the intestinal epithelium until they are adhered to and colonized by the intestinal epithelium for a fermented food designated as probiotics. Probiotics' positive effect has generated tremendous interest due to the proteins, vitamins, minerals, and different immune-stimulating chemicals (proteases, β-glucans, and mannan oligosaccharides) found in yeast (Gil-Rodríguez *et al*., 2015; Azhar *et al*., 2019; Wulan *et al*., 2021). During sugar fermentation, microbiota as probiotics reduce the environment's pH and inhibit the growth of undesired microorganisms. They also contribute to food preservation by generating secondary metabolites such as lactic acid, fatty acid, and bacteriocin (Evivie *et al*., 2017). According to Fernández *et al*. (2003), probiotic sources should be able to withstand a pH of at least 3.0 and greater than 1.5 while fasting with high amounts of acid. Yeast ability of SUL and SM isolate has a good tolerance of the artificial gastric acid of pH 2, closely related to their strain specification (Lin *et al*., 2006).

3.4 Genotypic characteristics of tapai yeast

The divergent D1/D2 domain of the 28S rRNA of SUL and SM for the highest survival as the probiotic source was amplified. The sequences were compared to the nucleotide database using the BLAST program from the National Centre for Biotechnology Information (NCBI). The % identity from BLAST shows that SUL was classified as cluster *Kodamaea ohmeri* (99-100%), while SM shows (99-100%) identity with *Pichia kudriavzevii* (Figures 3 and 4). The PCR fragments of SUL and SM prove a distinctive band of approximately 500-600 bp.

Based on pioneering studies, *P. kudriavzevii* was found in fruits, soil, and miscellaneous fermented food and beverages, capable of producing ethanol and growing at temperatures of 45°C (Yuangsaard *et al*., 2013; Mbuk *et al*., 2016). *Pichia* sp. isolated from traditional Indian fermented foods (idli and jalebi batter), has beneficial properties for present viable probiotic agents and can be widely employed as food and feed supplements (Syal and Vohra, 2013). Meanwhile, *K. ohmeri* is a yeast isolated from fermented glutinous rice and can produce ethanol (Sumerta and Kanti, 2017). Experiments by Azhar *et al*. (2019) have shown that *Kodamaea sp* is used as a probiotic source and consumed as a fermented beverage by the Malaysian people. In a previous study, *K. ohmeri* and *P. kudriavzevii* also showed good tolerance to temperature stress after 24 hrs of incubation at 30°C and 37°C (Amoikon *et al*., 2018). The yeast strains were associated with various biotechnological applications, including probiotic

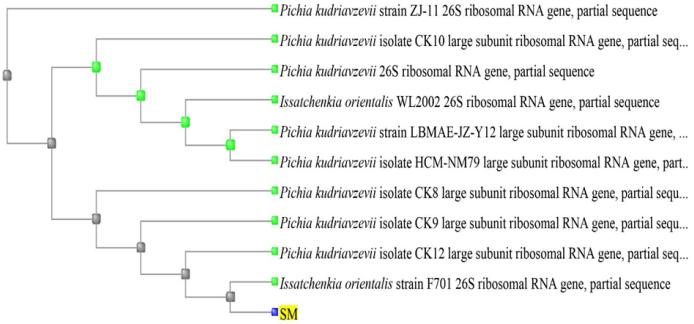

Figure 3. Midpoint neighbor-joining phylogeny tree analysis of isolated yeast SM (*Pichia kudriavzevii*).

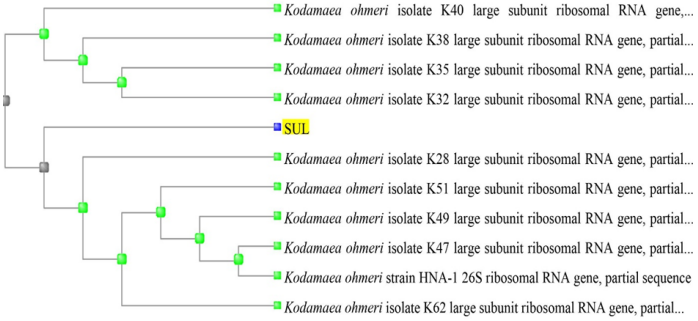

Figure 4. Midpoint neighbor-joining phylogeny tree analysis isolated yeast SUL (*Kodamaea ohmeri*).

https://doi.org/10.26656/fr.2017.7(S1).13

sources (Fleet, 2007; García-Hernández *et al*., 2012).

The D1/D2 domain of the 28S rRNA sequences is used to construct the ITY phylogenetic tree. SM isolates are divided into A and B clusters (Figure 3) and *P. kudriavzevii* strain ZJ-11 represents cluster A with *P. kudriavzevii* isolate CK10, *Issatchenkia orientalis* strain WL2002, *P. kudriavzevii* strain LBMAE-JZ-Y12, and *P. kudriavzevii* isolate HCM-NM79. Therefore, cluster B was performed as *P. kudriavzevii* isolate CK8, CK9, CK12, *I. orientalis* strain F701, and SM isolate. The construction of the phylogenetic tree shows two SUL isolates, clusters A and B (Figure 4). Meanwhile, cluster A involved K40, K38, K35, and K32 isolates from *K. ohmeri*. Cluster B contained K28, K51, K49, K47, strain HNA-1, and K62 SUL isolates from *K. ohmeri*.

4. Conclusion

The approaches for identifying yeast as a probiotic source were explored using genotype and phenotype features extracted from tapai as fermented cassava. Survival percentage under artificial gastric acid, the SUL isolate identified as *K. ohmeri*, exhibited the highest survival percentage of 82.71%, with a growth capacity of 6.20 CFU/mL. The SM isolate was identified as *P. kudriavzevii* with a survival percentage of 79.74%and growth capacity of 5.75 CFU/mL after 2 hrs incubation in gastric acid of pH 2. The yeast strains *P. kudriavzevii* and *K. ohmeri* isolated from the fermented food of tapai have shown promising properties to be further evaluated as probiotic candidates through in vitro methods.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors are thankful to the University of Jember, Ministry Education, Culture, Research, and Technology, Republic of Indonesia for funding this research No: 2901/UN25.3.1/LT/2021 and 5450/UN25.3.1/LT/2023. In addition, they are grateful to the technician for collecting the data from instrumentals and PT. Genetika Science Indonesia for analysis of genotypic analysis.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Amoikon, T., Aké, F., Bakayoko, M., Djéni, T., Mougo, E. and Djè, M. (2018). Physiological profiles of indigenous yeasts isolated from raffia wine originated of côte d'ivoire. *Journal of Global Biosciences*, 7(7), 5459–5474.

Azhar, M.A., Sakinah, M. and Munaim, A. (2019). Isolation and molecular identification of potential probiotic yeast strains found in Malaysian kefir drinks samples. *International Journal of Pharma Medicine Biological Sciences,* 8(4), 128–131. https://doi.org/10.18178/ijpmbs.8.4.128-131

Czerucka, D., Piche, T. and Rampal, P. (2007). Review article: yeast as probiotics-*Saccharomyces boulardii*. *Alimentary Pharmacology and Therapeutics*, 26(6), 767–778. https://doi.org/10.1111/j.1365-2036.2007.03442.x

de Melo Pereira, G.V., de Oliveira Coelho, B., Magalhães Júnior, A.I., Thomaz-Soccol, V. and Soccol, C.R. (2018). How to select a probiotic? A review and update of methods and criteria. *Biotechnology Advances*, 36(8), 2060–2076. https://doi.org/10.1016/j.biotechadv.2018.09.003

Didari, T., Solki, S., Mozaffari, S., Nikfar, S. and Abdollahi, M. (2014). A systematic review of the safety of probiotics. *Expert Opinion on Drug Safety*, 13(2), 227–239. https://doi.org/10.1517/14740338.2014.872627

Ebabhi, A.M., Adekunle, A.A., Okunowo, W.O. and Osuntoki, A.A. (2013). Isolation and characterization of yeast strains from local food crops. *Journal of Yeast and Fungal Research*, 4(4), 38–43.

Edwards-ingram, L., Gitsham, P., Burton, N., Warhurst, G., Clarke, I., Hoyle, D., Oliver, S.G. and Stateva, L. (2007). Genotypic and physiological characterization of *Saccharomyces boulardii,* the probiotic strain of *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, 73(8), 2458–2467. https://doi.org/10.1128/AEM.02201-06

Evivie, S.E., Huo, G.C., Igene, J.O. and Bian, X. (2017). Some current applications, limitations and future perspectives of lactic acid bacteria as probiotics. *Food and Nutrition Research*, 61, 1318034. https://doi.org/10.1080/16546628.2017.1318034

Fernández, M.F., Boris, S. and Barbes, C. (2003). Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *Journal of applied microbiology*, 94(3), 449-455. https://doi.org/10.1046/j.1365-2672.2003.01850.x

Fleet, G.H. (2007). Yeasts in foods and beverages: impact on product quality and safety. *Current Opinion in Biotechnology*, 18(2), 170–175. https://doi.org/10.1016/j.copbio.2007.01.010

García-Hernández, Y., Rodríguez, Z., Brandão, L.R., Rosa, C.A., Nicoli, J.R., Elías Iglesias, A., Peréz-Sanchez, T., Salabarría, R.B. and Halaihel, N.

(2012). Identification and *in vitro* screening of avian yeasts for use as probiotic. *Research in Veterinary Science*, 93(2), 798–802. https://doi.org/10.1016/j.rvsc.2011.09.005

Gil-Rodríguez, A.M., Carrascosa, A.V. and Requena, T. (2015). Yeasts in foods and beverages: In vitro characterisation of probiotic traits. *Lwt - Food Science and Technology*, 64(2), 1156–1162. https://doi.org/10.1016/j.lwt.2015.07.042

Halder, D. and Mandal, S. (2015). Curd lactobacilli with probiotic potentiality. *Translational Biomedicine*, 6, 1. https://doi.org/10.21767/2172-0479.100008

Johansen, P.G., Owusu-Kwarteng, J., Parkouda, C., Padonou, S.W. and Jespersen, L. (2019). Occurrence and importance of yeasts in indigenous fermented food and beverages produced in sub-saharan Africa. *Frontiers in Microbiology*, 10, 1789. https://doi.org/10.3389/fmicb.2019.01789

Kanino, D. (2019). The effect of yeast concentration on making tape ketan. *Jurnal Penelitian dan Pengembangan Agrokompleks*, 2(1), 64–74.

Karasu-Yalcin, S., Senses-Ergul, S. and Ozbas, Z.Y. (2019). Yeast strains with technological and probiotic traits isolated from Mihalic cheese. *International Food Research Journal*, 26(4), 1359–1370.

Lin, W.H., Hwang, C.F., Chen, L.W. and Tsen, H.Y. (2006). Viable counts, characteristic evaluation for commercial lactic acid bacteria products. *Food Microbiology*, 23(1), 74–81. https://doi.org/10.1016/j.fm.2005.01.013

Mbuk, E.U., Kwaga, J.K.P., Bale, J.O.O. and Umoh, J.U. (2016). Molecular identification of yeasts associated with raw cow milk from peri-urban farms in Kaduna. *Journal of Yeast and Fungal Research*, 7(5), 39–46. https://doi.org/10.5897/JYFR2016.0172

Meyer, S.A., Ahearn, D.G., Yarrow, D. and Kreger-van Rij, N.J.W. (1984). The yeasts: a taxonomic study. 3rd ed. Amsterdam, Netherlands: Elsevier Science Publisher.

Moon, S.H., Chang, M., 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), 489–497. https://doi.org/10.1007/s10068-014-0067-7

Nuraida, L. and Owens, J.D. (2014). Sweet, sour, alcoholic solid substrate fungal fermentations. *Indigenous Fermented Foods of Southeast Asia*, 137 (2), 56-66.

Rajkowska, K. and Kunicka-Styczyńska, A. (2010). Probiotic properties of yeasts isolated from chicken feces and kefirs. *Polish Journal of Microbiology*, 59 (4), 257-263. https://doi.org/10.33073/pjm-2010-039

Sahadeva, R.P.K., Leong, S.F., Chua, K.H., Tan, C.H., Chan, H.Y., Tong, E.V., Wong, S.Y.W. and Chan, H.K. (2011). Survival of commercial probiotic strains to pH and bile. *International Food Research Journal*, 18(4), 1515–1522.

Salminen, S., Nybom, S., Meriluoto, J., Collado, M.C., Vesterlund, S. and El-Nezami, H. (2010). Interaction of probiotics and pathogens-benefits to human health? *Current Opinion in Biotechnology*, 21(2), 157–167. https://doi.org/10.1016/j.copbio.2010.03.016

Sumerta, N.I. and Kanti, A. (2017). Diversity of ethanol producing yeast isolated from fermented foods in Riau islands. *Jurnal Biologi Indonesia*, 13(1), 61–69. https://doi.org/10.47349/jbi/13012017/61

Suriasih, K., Sucipta, I.N., Putri, W.C.W.S. and Wirawan, I.P.S. (2012). Chemical characteristics and microbiological kefir beverages from Bali cattle during storage. *International Journal of Scientific and Technology Research*, 9(8), 133-138.

Suryaningsih, V., Ferniah, R.S. and Kusdiyantini, E. (2018). Isolat khamir IK-2 hrsasil isolasi dari jus buah sirsak (*Annona muricata* L.). *Jurnal Biologi*, 7 (1), 18–25. [In Bahasa Indonesia].

Syal, P. and Vohra, A. (2013). Probiotic potential of yeasts isolated from traditional Indian fermented foods. *International Journal of Microbiology Research*, 5(2), 390–398. https://doi.org/10.9735/0975-5276.5.2.390-398

Tamang, J.P., Watanabe, K. and Holzapfel, W.H. (2016). Review: Diversity of microorganisms in global fermented foods and beverages. *Frontiers in Microbiology*, 7, 377. https://doi.org/10.3389/fmicb.2016.00377

Widiastutik, N. and Alami, N.H. (2014). Isolation and identification of yeast from *Rhizosphere Rhizophora mucronata* Wonorejo. *Jurnal Sains dan Seni ITS*, 3 (1), 11–16.

Widyatmoko, H., Subagio, A. and Nurhayati, N. (2018). Physicochemical properties of cassava starch fermented by indigenous-tapai yeast. *Agritech-Jurnal Teknologi Pertanian*, 38(2), 140-150. https://doi.org/10.22146/agritech.26323

Wulan, R., Astuti, R.I., Rukayadi, Y. and Meryandini, A. (2021). Evaluation of indigenous *Pichia kudriavzevii* from cocoa fermentation for a probiotic candidate. *Biodiversitas*, 22(3), 1317–1325. https://doi.org/10.13057/biodiv/d220331

Xu, J., Li, Y., Yang, Z., Li, C., Liang, H., Wu, Z. and Pu, W. (2018). Yeast probiotics shape the gut

microbiome and improve the health of early-weaned piglets. *Frontiers in Microbiology*, 9, 2011. https://doi.org/10.3389/fmicb.2018.02011

Yuangsaard, N., Yongmanitchai, W., Yamada, M. and Limtong, S. (2013). Selection and characterization of a newly isolated thermotolerant *Pichia kudriavzevii* strain for ethanol production at high temperature from cassava starch hydrolysate. *Antonie van Leeuwenhoek*, 103(3), 577–588. https://doi.org/10.1007/s10482-012-9842-8

Yuliati, L., Nasir, M.A. and Subagiarta, I.W. (2019). Analisis daya saing komoditas singkong kabupaten Jember di Jawa Timur, presented at National Seminar on Research and Community Service, Jember, 2019. Jember, Indonesia: Proceeding of the National Seminar on Research and Community Service.

https://doi.org/10.26656/fr.2017.7(S1).13