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SELECTION AND IN-VITRO POTENTIALS OF AMYLOLYTIC YEAST FROM SEVERAL *RAGI TAPAI* IN WEST SUMATRA PROVINCE

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ABSTRACT: *Ragi Tapai* is a starter used for the fermentation of cassava and glutinous rice to produce conventional fermented products, such as *Tapai* in Indonesia. *Ragi Tapai* contains several enzymes and microbes that help the *Tapai* fermentation process. The purpose of this study was to identify the most potential amylolytic yeast and in-vitro potential (amylolytic, cellulolytic, and fermentative). GPA, YEA, APB, CMCA, and GPACaCO$_3$ were used as a medium for selection and characterization. The result showed that in the seven samples of *Ragi Tapai* West Sumatra (Padang, Padang Pariaman, Batusangkar, Padang Panjang, Payakumbuh, Solok, dan Pesisir Selatan), there was three amylolytic yeast including genera *Schizosaccharomyces* (1 dan 2) and 1 genus *Trichosporon*. In-vitro potential, showed the genera *Schizosaccharomyces* and *Trichosporon* have amylolytic, cellulolytic, and fermentative potential. Genera *Schizosaccharomyces* 1 has greater amylolytic potential than genera *Schizosaccharomyces* 2 and *Trichosporon*.

Keywords: *Ragi Tapai*, Yeast Amylolytic, In-Vitro Potential, Selection.

INTRODUCTION

Ragi Tapai is a starter for making *Tapai*. *Tapai* is a fermented product that is commonly consumed by people in Indonesia and other countries in Asia. *Tapai* is normally consumed as adessert. In Japan, *Ragi Tapai* is known as koji. Koji in Japan is usually used as a starter for making sake, in koji, there are microbes such as *Aspergillus oryzae* or *Monacu purpureus* (Hutkins, 2006). In West Sumatra Province, there are various types of *Ragi Tapai*. Different types of *Ragi Tapai* are caused by differences in processing and ingredients. Processing techniques for making *Ragi Tapai* based on community knowledge. Differences in the composition of additives in *Ragi Tapai* can provide different growth responses to the microbes present in *Ragi Tapai* (Allwood et al., 2021). Giving different *Ragi Tapai* can affect the texture, aroma, and taste of glutinous rice *Tapai*. Different doses of *Ragi Tapai* can affect the results of organoleptic tests in the form of aroma, color, texture, and taste (Tiara, 2021). The result of *Tapai* fermentation depends on the composition of the *Ragi Tapai* used. Besides the sweet taste, *Tapai* also contains alcohol. The use of different *Ragi Tapai* doses will have a significant effect (Berlian et al., 2016) on the *Tapai* alcohol content there is an effect of *Ragi Tapai* concentration on the acceptance of aroma and taste in *Tapai* cassava. However, it did not affect the color and texture of *Tapai* cassava.

There are various types of microbes in *Tapai* yeast including Genus Saccharomyces, Terulopsis, Rhodotorula, Mucor, Amylomyces, Aspergillus, Penicillium, Fusarium, Candida, Saccharomyces, and Hansenula (Steinkraus et al., 2008). Candida genus is a yeast in *Tapai* yeast that has amylase activity. Microbes on *Ragi Tapai* produce hydrolase enzymes such as amylase (Naiola, 2008). Mold on *Ragi Tapai* has strong amylolytic properties, so it can degrade carbohydrates into sugars, and then yeast degradation sugars into alcohol. *Aspergillus oryzae*, *Penicillium expansum*, and *Mucor racemosus* were isolates of amylolytic mold from several *Ragi Tapai* in West Sumatra (Putriani, 2006). On *Ragi Tapai* there are various types of amylolytic molds including Rhizopus and Amylomyces (Anasa et al., 2019). Although *Amylomyces rouxii* is found in *Ragi Tapai*, molecular identification based on ribosomes turns out not only *Amylomyces rouxii* but also *Mucor indicus* (Delva et al., 2022).

The conversion of starch into sugars is known as saccharification. Saccharification is an important step in the production of bioethanol. Bioethanol is one of the renewable alternative energy sources which is a solution to the problems of energy demand and consumption in Indonesia. Saccharification can use enzymes, microbes, and chemical compounds. However, saccharification using commercial microbes and enzymes will give less effective results. Saccharification that used enzymatic hydrolysis by *Aspergillus niger* resulted in higher reducing sugar levels, compared to acid hydrolysis using 1% $H2S0_4$ (Adini et al., 2015).

So it is necessary to find other alternatives that can optimize the saccharification process. The amylolytic potential possessed by the microbes in *Ragi Tapai* is used in the starch hydrolysis process without the use of amylolytic enzymes or other chemical compounds. In this regard, it is necessary to conduct research on “Selection and Potential In-Vitro Amylolytic Yeasts from Several *Ragi Tapai* in West Sumatra Province”.

METHOD

The study was conducted using a survey method with several stages including isolation, and characterization (morphology and in-vitro potency) of amylolytic yeast of several local *Ragi Tapai* of West Sumatra Province. All data obtained were analyzed descriptively. *Ragi Tapai* were obtined by purposive sampling from several local markets in West Sumatra Province (Padang, Batusangkar, Padang Panjang, Padang Pariaman, Payakumbuh, Solok, and Pesisir Selatan).

The yeast from several *Ragi Tapai* that were used as isolates were yeasts that had the largest clear zones of several types of colonies found on *Agar Pati Beras* medium (APB). Furthermore, yeast isolates that had the largest clear zones were propagated using Yeast Extract Agar medium (YEA). Amylolytic yeast isolates were characterized by observing the colonies macroscopically and microscopically of the cells. Macroscopic observations are shape, elevation, colony margins. Then microscopic observation by observing the cell shape and vegetative type of yeast cells.

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The reference book used to characterize yeast is "yeast" by M. Th. SMITH and D. YARROW (Centraal Bureau voor Schimmel Cultures, Yeast Division Julianalaan 67, 2628 BC Delft, the Netherland) in introduction to food-Borneo Fungi by Robert A. Samson and Ellen S. Van Reenen-Hoekstra (1988) page 210. The in-vitro potential of amylolytic yeast isolates was observed by looking at the clear zones formed in each medium. APB medium to observe amylolytic in-vitro potential, CMCA medium to observe cellulolytic in-vitro potential and GPACaCO3 to observe fermentative in vitro potential.

RESULTS AND DISCUSSIONS

The results of observations on yeast isolates isolated from 7 *Tapai* yeast samples, it was found that 8 yeast isolates had macroscopic and microscopic differences. Macroscopic and microscopic characteristics of 8 yeast isolates obtained from several samples of West Sumatra Province *Ragi Tapai* can be seen in the Table 1 below.

Table 1. Macroscopic and Microscopic Characteristics of Yeast Isolates from Several Samples of West Sumatra Province *Ragi Tapai*.

No.	Isolates	Macroscopic				Microscopic		Amylolytic Potencial
		Shape Colony	Elevation Colony	Edge Colony	Colour Colony	Shape Cell	Type Reproduction	
1	*Trichosporon*	Circular	Convex	Entire	Cream	Silinder	Arthroconidia	+
2	*Schizosaccharomyces* 1	Circular	Convex	Entire	Cream	Silinder	Fission	+
3	*Schizosaccharomyces* 2	Circular	Convex	Entire	Cream	Silinder	Fission	+
4	*Debaryozyma*	Circular	Convex	Entire	Cream	Oval	Multi Lateral Budding	-
5	*Hanseniaspora* 1	Circular	Convex	Entire	Putih	Oval	Bipolar Budding	-
6	*Hanseniaspora* 2	Circular	Convex	Entire	Putih	Oval	Bipolar Budding	-
7	*Sporobolomyces* 1	Circular	Umbonate	Entire	Cream	Silinder	Ballistoconidium	-
8	*Sporobolomyces* 2	Circular	Umbonate	Entire	Putih	Silinder	Ballistoconidium	-

Information: (+) = The Result of a Positive Reaction; (-) = The Result of a Negative Reaction.

The results of the isolation of West Sumatra Province *Ragi Tapai* found the presence of 8 yeast isolates, 3 of which were yeast isolates that had amylolytic potential. The amylolytic yeast genera found in *Ragi Tapai* from West Sumatra Province include 2 Genus Schizosaccharomyces and Trichosporon. Based on Table 1 above, it can be seen that the genera *Schizosaccharomyces* and *Trichosporon* have different macroscopic and microscopic characteristics. For a clearer observation of the morphological characters and types of reproduction of amylolytic yeast isolates can be seen in Figures below.

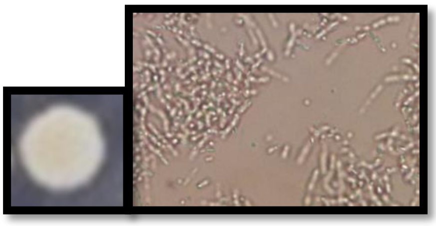
A  **B**

Figure 1. *Trichosporon.* (A) Macroscopic Colony; and (B) Microscopic Yeast Cells with 100 x 10 Magnification.

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Figure 2. ***Schizosaccharomyces*** **1. (A) Macroscopic Colony; and (B) Microscopic Yeast Cells with 100 x 10 Magnification.**

Figure 3. ***Schizosaccharomyces*** **2. (A) Macroscopic Colony; and (B) Microscopic Yeast Cells with 100 x 10 Magnification.**

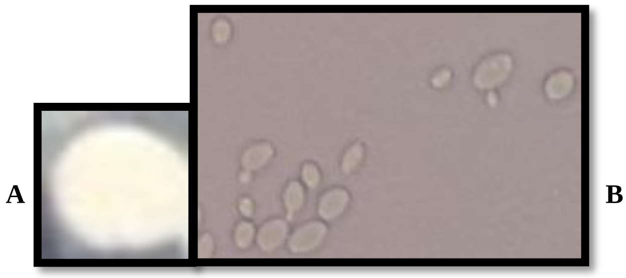

Figure 4. ***Debaryozyma.*** **(A) Macroscopic Colony; and (B) Microscopic Yeast Cells with 100 x 10 Magnification.**

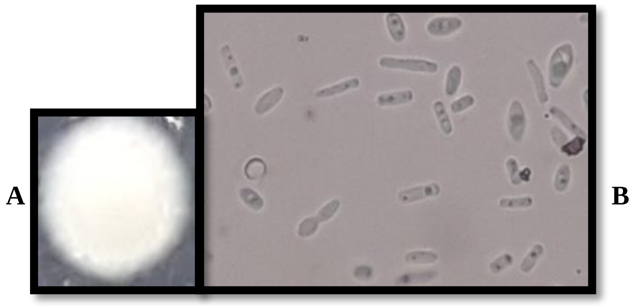

Figure 5. ***Hanseniaspora*** **1. (A) Macroscopic Colony; and (B) Microscopic Yeast Cells with 100 x 10 Magnification.**

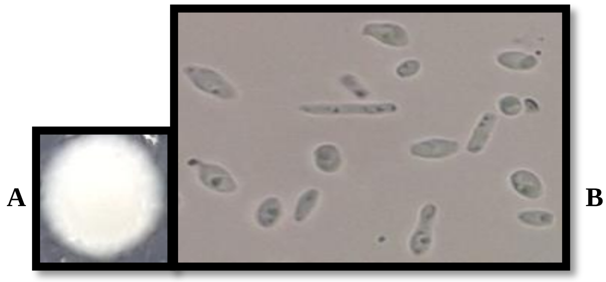

Figure 6. ***Hanseniaspora*** **2. (A) Macroscopic Colony; and (B) Microscopic Yeast Cells with 100 x 10 Magnification.**

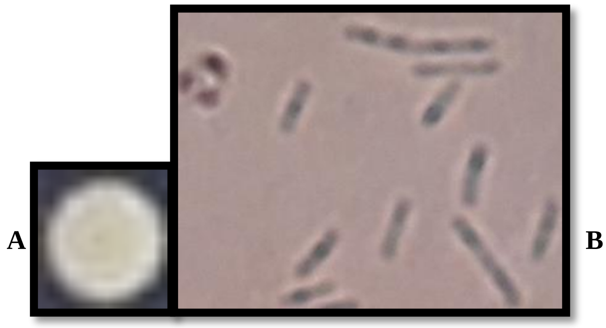

Figure 7. ***Sporobolomyces*** **1. (A) Macroscopic Colony; and (B) Microscopic Yeast Cells with 100 x 10 Magnification.**

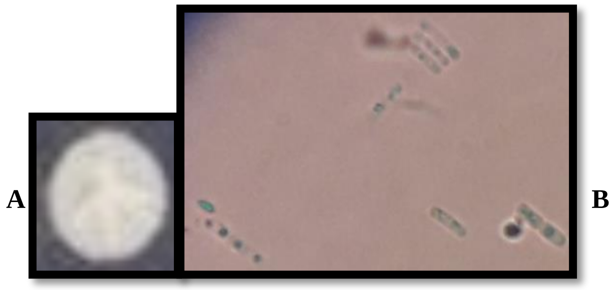

Figure 8. ***Sporobolomyces*** **2. (A) Macroscopic Colony; and (B) Microscopic Yeast Cells with 100 x 10 Magnification.**

Based on Figure 1, it is known that *Trichosporon* had morphological characteristics including circular colony shape, entire colony age, convex colony elevation, and cream colony color. While microscopically it has characteristics, namely the shape of cylindrical cells, and the type of arthroconidia vegetative reproduction. This is following the characteristics proposed by Samson and Van Reene-Hoekestra (1998). The yeast in Figure 3 is thought to be from the genus *Trichosporon*. The genus *Trichosporon* comprises 40 molecularly identified species (Middelhoven et al., 2004). One of the *Trichosporon* species is *T. mycotoxinivorans* which has potential biotechnology that can be used in the saccharification process. *T. mycotoxinivorans* has microscopic characteristics the cell is ovoidal, ellipsoidal and elongate (single or pair cells), and globose. A septa hyphae, giant cells, and arthroconidia (Khaled et al., 2013).

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Based on Figures 2 and 3, it is known that the yeast has morphology characteristics, including circular colony shape, entire colony edge, convex colony elevation, and cream colony color. While microscopically it has characteristics, namely the shape of cylindrical cells, and the type of fission vegetative reproduction. This is following the characteristics proposed by Samson & van Reenen-Hoekestra (1988). The characteristics of the yeast above indicate that the yeast isolated from *Ragi Tapai* belongs to the genus *Schizosaccharomyces*. Classification *Schizosaccharomyces* consists of one genus with three species, namely *S. japonicus*, *S. octosporus*, and *S. pombe*. *Schyzosaccharomyces* can be isolated from substrates rich in dissolved carbon sources, such as trees, fruit, and honey. Individual cells are spherical to cylindrical and haploid (Walker & White, 2005). One of the species of the genus is *Schizosaccharomyces* pombe isolated from wine. Features of *Schizosaccharomyces* pombe include the characteristic of the cell is rod shape and sizes between 3-5 x 5-24 µm (Loira et al., 2018). After cell division, new cells have a more rounded shape (Atilgan et al., 2015).

Table 2. In-Vitro Potential Index Value of Amylolytic Yeast Isolates.

Isolates	Medium	Halo Zone (cm)	Diameter Colony (cm)	Index Value
Schizosaccharomyces 1	Amylolytic	0.7	0.2	3.5
	Cellulolytic	0.7	0.5	1.4
	Fermentative	1	0.8	1.25
Schizosaccharomyces 2	Amylolytic	0.7	0.3	2.3
	Cellulolytic	0.7	0.5	1.4
	Fermentative	1.3	0.7	1.8
Trichosporon	Amylolytic	0.5	0.3	1.6
	Cellulolytic	0.6	0.5	1.2
	Fermentative	1.5	0.8	1.8

Table 2 shows the results that *Schizosaccharomyces* yeast has the largest amylolytic index. *Schizosaccharomyces* have index values of 3,5 and 2,3. This proves that this yeast is the most potential yeast in hydrolyzing starch into sugar. Isolates with clear areas with a diameter twice the diameter of the colony are potential enzyme producers. The amylolytic potential of *Schizosaccharomyces* and *Trichosporon* shows that these yeasts have amylase activity that can hydrolyze starches such as amylose and amylopectin. The existence of amylolytic ability in yeast isolates is caused by the content of starch as a substrate in the raw material for making *Tapai* yeast. In addition to the amylolytic potential, *Schizosaccharomyces* has cellulolytic and fermentation potential. *Schizosaccharomyces* is an isolate that has potential enzymes to break down the starch in the medium into simple sugars because isolates that produce a clear zone diameter twice the diameter of the colony are potential enzymes producers (Ochoa-Solano & Olmos-Soto, 2006).

Schizosaccharomyces 1 has the greatest amylolytic potential than *Schizosacharomyces* 2 and *Trichosporon*. In addition to amylolytic potential, this yeast isolate also has cellulitic and fermentation potential. This potential can be seen in Figure 9 below.

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Figure 9. In-Vitro Potential of *Schizosaccharomyces* on Several Mediums. (A) Amylolytic; (B) Cellulolytic; and (C) Fermentative.

CONCLUSION

Based on the research that has been done, it can be concluded that of the seven *samples* of West Sumatra Province *Tapai* yeast, 2 genera of yeast that have amylolytic potential in vitro were found, namely *Schizosaccharomyces* (1 and 2) and *Trichosporon*. The genera *Schizosaccharomyces* and *Trichosporon* also have cellulolytic and fermentative potential. The highest amylolytic potential is *Schizosaccharomyces*. So that this *Schizosaccharomyces* isolate is a yeast isolate that has the most potential in the saccharification process compared to the other 2 isolates.

RECOMMENDATIONS

This study only focuses on the identification of amylolytic yeast. Measurements were made by measuring the further research will be able to measure the activity of the amyloltric yeast amylase enzyme.

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REFERENCES

Adini, S., Kusdiyantini, E., dan Budiharjo, A. (2014). Produksi Bioetanol dari Rumput Laut dan Limbah Agar *Gracilaria* sp. dengan Metode Sakarifikasi yang Berbeda. *Bioma : Berkala Ilmiah Biologi*, *16*(2), 65-75.

Allwood, J.G., Wakeling, L.T., and Bean, D.C. (2021). Fermentation and the Microbial Community of Japanese Koji and Miso: A Review. *Journal of Food Science*, *86*(6), 2194-2207.

Anasa, R.A., Nurlaila, W., Dharmastuti, W., Santoso, I., Maryanto, A.E., Sitaresmi, S., and Yasman, Y. (2018). Isolation and Screening of Amylase Activity of Primary Moulds in *Ragi Tapai* of Indonesia. In *Proceedings of the 4th International Symposium on Current Progress in Mathematics and Sciences, ISCPMS 2018* (pp. 1-10). Depok, Indonesia: American Institute of Physics Inc.

Atilgan, E., Magidson, V., Khodjakov, A., and Chang, F. (2015). Morphogenesis of the Fission Yeast Cell through Cell Wall Expansion. *Current Biology*,

Bioscientist : Jurnal Ilmiah Biologi
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Vol. 11, No. 1, June 2023; Page, 110-117
https://e-journal.undikma.ac.id/index.php/bioscientist

25(16), 2150-2157.

Berlian, Z., Aini, F., dan Ulandari, R. (2016). Uji Kadar Alkohol pada Tapai Ketan Putih dan Singkong melalui Fermentasi dengan Dosis Ragi yang Berbeda. *Jurnal Biota*, *2*(1), 106-111.

Delva, E., Arisuryanti, T., dan Ilmi, M. (2022). Genetic Diversity of *Amylomyces rouxii* from *Ragi Tapai* in Java Island Based on Ribosomal Regions ITS1/ITS2 and D1/D2. *Mycobiology*, *50*(2), 132-141.

Hutkins, R.W. (2006). *Microbiology and Technology of Fermented Foods*. Iowa, USA: Blackwell Publishing.

Khaled, J.M., Khalel, A.S., Kandeal, S.A., and Mothana, R.A. (2013). Cultural and Microscopic Characteristics of *Trichosporon mycotoxinivorans* and Its Effect on Some Reproductive Aspects in Mice. *Journal of Pure and Applied Microbiology*, *7*(3), 2141-2147.

Loira, I., Morata, A., Palomero, F., González, C., and Suárez-Lepe, J.A. (2018). *Schizosaccharomyces pombe*: A Promising Biotechnology for Modulating Wine Composition. *Fermentation*, *4*(3), 61-70.

Middelhoven, W.J., Scorzetti, G., and Fell, J.W. (2004). Systematics of the Anamorphic Basidiomycetous Yeast Genus Trichosporon Behrend with the Description of Five Novel Species: *Trichosporon vadense*, *T. smithiae*, *T. dehoogii*, *T. scrabaeorum* and *T. gamsii*. *International Journal of Systematic and Evolutionary Microbiology*, *54*(3), 975-986.

Naiola, E. (2008). Isolasi dan Seleksi Mikroba Amilolitik dari Makanan Fermentasi/Ragi Tapai Gambut di Kalimantan Selatan. *Berkala Penelitian Hayati*, *13*(2), 109-114.

Ochoa-Solano, L.J., and Olmos-Soto, J. (2006). The Functional Property of Bacillus for Shrimp Feeds. *Food Microbiology*, *23*(6), 519-525.

Steinkraus, K.A., Kaeberlein, M., and Kennedy, B.K. (2008). Replicative Aging in Yeast: The Means to the End. *Annual Review of Cell and Developmental Biology*, *24*(1), 29-54.

Tiara, C. (2021). Pengaruh Pemberian Dosis Ragi Tapai terhadap Sifat Organoleptik dan pH Tapai Pisang Gedah. In *Prosiding Biology Achieving the Sustainable Development Goals with Biodiversity in Confronting Climate Change* (pp. 113-118). Gowa, Indonesia: Universitas Islam Negeri Alauddin Makassar.

Walker, G.M., and White, N.A. (2017). *Introduction to Fungal Physiology*. New Jersey: John Wiley & Sons, Inc.