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

Inelvi Yulia*1, Adewirli Putra2, Wiya Elsa Fitri3, and Gusliani Eka Putri4
1,2,4Department Medical Laboratory Technology, STIKes Syedza Saintika, Indonesia
3Department Public Health, STIKes Syedza Saintika, Indonesia
*E-Mail: yulianiely@gmail.com
DOI: https://doi.org/10.33394/bioscientist.v11i1.6924
Submit: 06-01-2023; Revised: 24-01-2023; Accepted: 28-01-2023; Published: 30-06-2023

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 GPACaCO3 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, and 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 a dessert. 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% H2SO4 (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.
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.

<table>
<thead>
<tr>
<th>No.</th>
<th>Isolates</th>
<th>Macroscopic Colony</th>
<th>Microscopic</th>
<th>Amylolytic Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trichosporon</td>
<td>Circular</td>
<td>Convex</td>
<td>Entire</td>
</tr>
<tr>
<td>2</td>
<td>Schizosaccharomyces 1</td>
<td>Circular</td>
<td>Convex</td>
<td>Entire</td>
</tr>
<tr>
<td>3</td>
<td>Schizosaccharomyces 2</td>
<td>Circular</td>
<td>Convex</td>
<td>Entire</td>
</tr>
<tr>
<td>4</td>
<td>Debaryomyces</td>
<td>Circular</td>
<td>Convex</td>
<td>Entire</td>
</tr>
<tr>
<td>5</td>
<td>Hanseniaspora 1</td>
<td>Circular</td>
<td>Convex</td>
<td>Entire</td>
</tr>
<tr>
<td>6</td>
<td>Hanseniaspora 2</td>
<td>Circular</td>
<td>Convex</td>
<td>Entire</td>
</tr>
<tr>
<td>7</td>
<td>Sporoholomyces 1</td>
<td>Circular</td>
<td>Umbonate</td>
<td>Entire</td>
</tr>
<tr>
<td>8</td>
<td>Sporoholomyces 2</td>
<td>Circular</td>
<td>Umbonate</td>
<td>Entire</td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Medium</th>
<th>Halo Zone (cm)</th>
<th>Diameter Colony (cm)</th>
<th>Index Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Schizosaccharomyces</em> 1</td>
<td>Amylolytic</td>
<td>0.7</td>
<td>0.2</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>Cellulolytic</td>
<td>0.7</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Fermentative</td>
<td>1</td>
<td>0.8</td>
<td>1.25</td>
</tr>
<tr>
<td><em>Schizosaccharomyces</em> 2</td>
<td>Amylolytic</td>
<td>0.7</td>
<td>0.3</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Cellulolytic</td>
<td>0.7</td>
<td>0.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Fermentative</td>
<td>1.3</td>
<td>0.7</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Trichosporon</em></td>
<td>Amylolytic</td>
<td>0.5</td>
<td>0.3</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Cellulolytic</td>
<td>0.6</td>
<td>0.5</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Fermentative</td>
<td>1.5</td>
<td>0.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

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.
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.

ACKNOWLEDGEMENTS

Thanks to my lecturer and tim research who support for completing this research.

REFERENCES


25(16), 2150-2157.