PROBIOTIC CANDIDATES YEAST ISOLATED FROM DANGKE—INDONESIAN TRADITIONAL FERMENTED BUFFALO MILK

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Abstract

The aim of the study was to isolate and identify the yeast isolated from Dangke and its potential as probiotics. The purified isolates obtained were identified based on observations of macroscopic characteristics of colonies and microscopic cells. The ability as a probiotic yeast is obtained by testing the resistance towards acid conditions, bile salt resistance test and aggregation ability test against pathogenic bacteria using Salmonella sp. The yeast isolates were identified using the RapID Yeast Plus System. The isolation result was obtained D.10.3.d isolate that identified as Candida guilliermondii which showed probiotic characteristic. The yeast colony is round, cream-colored, smooth surfaces, low convex elevations and entire edges, capable of growing on mediums with the pH of 4, containing 1 % and 5 % of bile salts and having the ability to aggregate Salmonella sp. at 15, 60, and 180 minutes.

Keywords: Dangke, yeast, identification, isolation, probiotics

INTRODUCTION

Dangke is a traditional fermented food that have similar tastes with cheese, but the appearance and texture are similar with white to yellowish tofu. As traditional fermented food, Dangke is an indigenous product for the Enrekang Regency that has been known by all the people of South Sulawesi and even Indonesia (Rahman, 2014).

Recently, the potential of Dangke has been developing into one of the functional food products. One of functional food product that is widely evolved is probiotic products that control about 60–70 % of functional food markets (Holzapfel, 2005).

Various microorganisms such as bacteria or even yeasts can be used as probiotics (Khotimah et al., 2015). Yeasts can be applied as a probiotic because it can survive in guts microflora and producing antibacterial metabolites (Vine et al., 2006, Indah et al., 2015).

The type of yeast used as a probiotic is very diverse. Several previous studies conducted in an in vitro and in vivo showed probiotic yeasts such Saccharomyces boulardii was able to prevent intestinal infections.
caused by *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Yersinia enterocolitica* and *Candida albicans* (Czerucka, et al., 2007).

The nature of yeast as probiotics in dairy products has not been explored. Based on the above description, traditional fermented products such as *Dangke* can have potential as probiotic food obtained from yeast activities. So that the exploration of yeast probiotics from *Dangke* felt necessary to do in-depth research.

**MATERIALS AND METHODS**

The raw materials used in this study are *Dangke* obtained from Makassar, medium PDA/Potato Dextrose Agar (Oxoid Ltd.), NB/Nutrient Broth (Oxoid Ltd.), Brain Heart Infusion / BHI broth (Oxoid Ltd.), Vegemite (Kraft), Amoxicillin, 0.1 N HCl, synthetic bile salts (Ox Bite). The research method used is an experimental method with descriptive analysis. The yeast isolation was done by isolating the potentially probiotic yeast candidate from *Dangke* product. The parameters were tested based on yeast ability on resistance towards low pH, bile salts, and the aggregation ability.

**Isolation and identification of yeast**

According to Roostita and Fleet (1999), yeast isolation can be obtained by taking 1 gram of *Dangke* and conducting a series of dilutions to be inoculated onto agar media. Media used for yeast growth is PDA which modified by adding 3 % vegemite as an extract of yeast and 10 ppm amoxicillin to prevent unwanted bacterial growth. Identification of yeast isolates done by using *Rapid Yeast Plus System* and continued with the analysis using ERIC (Electronic Code Compendium) through www.remel.com/eric (Utama et al., 2016).

**Resistance Test towards Acid Conditions**

The acid resistance test was performed using a modified PDA medium added with 0.1 N HCl to obtain pH 2, 3, 4 (corresponding to gastric pH). A total of 1 ml of each yeast isolate extracted from culture stock then inoculated on a modified-HCl PDA medium. Incubated for 48 hours at 37 °C. The yeast colony appears on PDA modified-HCl medium counted as acid resistance yeast probiotic candidates (Djide and Wahyuddin, 2008).

**Resistance test towards bile salts**

Modified PDA medium is added with synthetic bile salts, with concentrations of 1 % and 5 %. As many as 1 loops of yeast isolate taken from the culture stock then streaked on the bile salt-modified PDA medium. Incubation was done for 48 hours at 37 °C (Djide and Wahyuddin, 2008; Khotimah et al., 2015). The results were obtained qualitatively from the comparison of the yeast colonies appears from control (not streaked PDA) with the streaked modified PDA (1 % and 5 % bile salts).

**Salmonella sp. Aggregation Test**

One ml of 10⁸ CFU/ml yeast isolates were grown in modified NB medium at 37 °C for 24 hours, with constant agitation (150 rpm). 0.5 ml of *Salmonella* sp. suspension contains 10⁹ CFU/ml was grown in BHI broth for 48 hours at 37 °C. Mix the two suspensions on six-well plates, the presence or absence of aggregation is observed microscopically after the 15th and 60th minutes, up to 180 minutes’ maximum (Perez-Sotelo et al., 2005; Khotimah et al., 2015).
RESULTS AND DISCUSSION

Yeast Isolation

Results of microorganism isolation from Dangke found 4 bacteria isolates and 1 yeast-like isolate. The yeast-like isolates were identified macroscopically and microscopically, which the results can be seen in Tab. I.

Yeast-like isolate has an appearance with features of rounded colony shape, slightly creamy white color, prominent elevation, glossy surface, flat edges, oval cell shape, and has a size of 3.8 μm. According to Bhatia (2016) and Utama, et al. (2015), yeast has a round shape, cream-colored, smooth surface, soft texture and has a varied size, usually has a diameter of 3-4 μm.

Yeast Identification using RapID Yeast Plus System

The results of the biochemical test used for yeast identification is using RapID Yeast Plus System (Tab. II). Based on the results of the biochemical test obtained, yeast-like isolate showed color changes in the media of glucose, sucrose, and raffinose, the color changed from red to yellow. According to Bhowmik, (2011), the color changes due to yeast activity ferment glucose, sucrose, and raffinose, so its produce acid and cause color changes in the media from red to yellow. If the carbohydrate particles are fermented, the final product produced is acidic, thus decreasing the pH so that the pH indicator will change (phenol red changes from red to yellow).

Positive test of ONPG will also produce a yellow color because according to Leboffe and Pierce (2011), β-galactosidase will break down o-Nitrophenyl-β-D-galactopyranose (ONPG) to yellow β-galactose and o-Nitrophenol (ONP). Positive results on enzyme tests showed that these isolates were able to degrade complex compounds to be simpler. Urease test results showed negative results, this was indicated because there was no color change in the urea test media. Urease test is useful to identify organisms that are able to hydrolyze urea which can produce ammonia and carbon dioxide, especially to find out whether these microorganisms have urease enzymes or not.

Urease is a constitutive enzyme that hydrolyzes urea to carbon dioxide and ammonia (NH2)2CO + H2O → CO2 + 2NH3 (Brink, 2013). Based on the biochemical test and after analyzed by ERIC, the isolates were identified similar to Candida guilliermondii species. Candida guilliermondii is a commensal yeast that can live as a saprophyte in the respiratory and

<table>
<thead>
<tr>
<th>Material</th>
<th>Results</th>
<th>%</th>
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<tbody>
<tr>
<td>GLU</td>
<td>+</td>
<td>91 %</td>
</tr>
<tr>
<td>MAL</td>
<td>–</td>
<td>00 %</td>
</tr>
<tr>
<td>SUC</td>
<td>+</td>
<td>92 %</td>
</tr>
<tr>
<td>TRE</td>
<td>–</td>
<td>04 %</td>
</tr>
<tr>
<td>RAF</td>
<td>+</td>
<td>38 %</td>
</tr>
<tr>
<td>LIP</td>
<td>–</td>
<td>03 %</td>
</tr>
<tr>
<td>NAGA</td>
<td>–</td>
<td>00 %</td>
</tr>
<tr>
<td>αGLU</td>
<td>+</td>
<td>63 %</td>
</tr>
<tr>
<td>βGLU</td>
<td>–</td>
<td>40 %</td>
</tr>
<tr>
<td>ONPG</td>
<td>–</td>
<td>00 %</td>
</tr>
<tr>
<td>αGAL</td>
<td>+</td>
<td>70 %</td>
</tr>
<tr>
<td>βFUC</td>
<td>–</td>
<td>01 %</td>
</tr>
<tr>
<td>PHS</td>
<td>+</td>
<td>91 %</td>
</tr>
<tr>
<td>PCHO</td>
<td>+</td>
<td>88 %</td>
</tr>
<tr>
<td>URE</td>
<td>–</td>
<td>07 %</td>
</tr>
<tr>
<td>PRO</td>
<td>+</td>
<td>99 %</td>
</tr>
<tr>
<td>HIST</td>
<td>+</td>
<td>70 %</td>
</tr>
<tr>
<td>LGY</td>
<td>+</td>
<td>29 %</td>
</tr>
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</table>

II: Biochemical Test Results using RapID Yeast Plus System
gastrointestinal tract. Changes from the form of saprophytes to pathogens, will not occur as long as the body's immunity is still stable, and there are no predisposing factors. This factor can increase yeast growth and facilitate yeast invasion into the system so that local colonization arises (Yang et al., 2010).

Candida’s presence in food products is a common thing. Candida has a beneficial composition so that it has a high potential for use in various industrial sectors. According to Nehal (2013), Candida has been found in many fermentation products in Asian countries especially in India and Nepal. Candida parapsilosis also found in soybean fermentation, C. famata, C. guilliermondii, C. kefyr, and C. inconspicua on milk fermentation from kefir (Simova et al., 2002; Sohliya et al., 2009; Pogacic et al., 2013).

**Probiotic yeast candidates**

**resistance towards acids**

According to Kanmani et al. (2010), resistance towards acid is one of the important conditions of an isolate to be categorized as probiotics. This is because if the isolate enters the digestive tract of the body, the isolate must be able to withstand the pH of the gastric acid which is around 3–5. The results of the resistance test of C. guilliermondii isolates for various pH values are presented in Tab. III.

Tab. III shows that the Candida guilliermondii isolated from Dangke were able to grow at pH 4 with the number of colonies around $9.9 \times 10^7$ CFU/g while at pH 2–3 showed no growth. This is in line with the opinion of Shah, (2007) and Hardiningsing et al., (2006), that the minimum number of probiotic strains present in probiotic foods is $10^6$ CFU/g. The number of probiotic strains that must be consumed every day around $10^8$ CFU/g and resistant to acidic conditions in the pH range 2–6, with the aim of compensating for the decreasing possibility to the number of probiotic microbes in the digestive tract.

Based on the testing results of low pH indicates that Candida guilliermondii isolates are able to survive acidic conditions at pH 4. Susanti et al. (2007) explained that very acidic conditions can cause membrane damage and release of intracellular components that can cause death. Acid resistant yeast has a greater resistance to membrane damage due to a decrease in extracellular pH. The high tolerance of yeasts to acids is usually due to the fact that the yeast is able to maintain a more alkaline pH of the cytoplasm than the extracellular pH. A more alkaline cytoplasmic pH defenses occur when cells have membranes which are barriers that limit the movement of compounds/protons. According to Usmiati, et al. (2011), microorganisms that are not resistant to acidic conditions are unable to maintain pH in their cells to become more alkalis than their extracellular pH, resulting in the cell membrane and the microbial intracellular component damage that causes death.

Composite components such as fatty acids and proteins among yeast species also affect the ability to resist acidic conditions (Halim and Zubaidah, 2013). The yeast cell wall is an elastic structure that provides physical protection and osmotic support and determines cell shape. This cell wall is a complex and dynamic structure containing glucans, chitin, and mannoproteins (Orlean, 2012).

<table>
<thead>
<tr>
<th>pH</th>
<th>Number of Colony</th>
<th>Picture</th>
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<tbody>
<tr>
<td>4</td>
<td>$9.9 \times 10^7$</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td><img src="image3.png" alt="Image" /></td>
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</table>

Tabl. III: The resistance of Candida guilliermondii towards acid conditions
Mannoproteins are involved in cell-to-cell recognition, determine the nature of cell surfaces and play an important role in interactions with the host (Vickova et al., 2004). The components of the yeast cell wall such as mannoprotein affect the ability of Candida to resist acidic conditions. Another component of the cell wall is glucomannan, the antioxidant properties of glucomannan can protect the system against harmful activities associated with free radicals, iron iron chelation, and intracellular modulation of signaling pathways (Miadokova et al., 2006).

Probiotic candidates yeast candidates resistance to bile salts

Salminen et al. (2004) explained that microorganism can be categorized as probiotic if it is able to survive and grow in the digestive tract, especially when it enters the upper intestinal tract, where bile salts are secreted in the intestine. The period of observing Dangke indigenous yeast resistance against bile salts was carried out for 48 hours. According to Surono (2004), the length of microorganisms living in the intestine is about 4–6 hours. However, yeast that has passed bile salts must be able to colonize the lower intestinal tract so that it can be defined as probiotic yeast, and so the observation time carried out in this study was extended to 48 hours. The ability of Candida guilliermondii to survive in bile salts for 48 hours with a concentration of 1% and 5% can be seen in Tab. IV.

The results showed that the isolates succeeded in living on a medium which added 1% and 5% bile salts, based on these results, Candida guilliermondii was resistant against bile salts. 1% concentration of bile salts is the lowest concentration and 5% is a critical concentration, a value that is high enough to select isolates resistant to bile salts (Pacheco et al., 2010).

Bile salts are a mixture of bile acids, cholesterol, fatty acids, phospholipids, bile pigments, and a number of detoxified xenobiotics. The combination is bactericidal for commensal microorganisms in humans so that a number of pathogenic microbes do not hold bile salts, except for some genera of intestinal inhabitants or out of it that have resistance to bile salts (Salen and Batta, 2004; Bijl et al., 2009).

Bile salt compounds will be toxic to living cells, so microorganisms in the digestive tract must have a defense mechanism to protect themselves from that toxic activity (Puspawati and Arihantana, 2016). A number of yeasts can survive against bile salts due to the presence of fatty acids which can reduce cell leakage caused by bile salts. The presence of fatty acids is to improve the stability of membrane lipids. Lipids are the main compounds found in gram-positive microbial cell membranes and play an important role in maintaining the membrane structure (Kimoto et al., 2002). The presence of other cell walls components such as polysaccharides can tolerate the presence of bile salts to prevent cell leakage (Puspawati and Arihantana, 2016). In addition, the ability to deconjugate bile salts is also thought to be a factor that allows an isolate to have resistance against bile salts. The deconjugation process can reduce the level of toxicity from conjugated bile salts to another microbe (De Smet et al., 1995).

Probiotic yeast candidates aggregation towards Salmonella sp.

One of many features of yeast that must be possessed as a candidate for probiotics is to have the ability to interact and stick among species, so as to prevent pathogenic bacteria from attaching to

<table>
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<th>Treatment</th>
<th>Result</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Bile Salt 1 %</td>
<td></td>
</tr>
<tr>
<td>Bile Salt 5 %</td>
<td></td>
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</tbody>
</table>

Probiotic Candidates Yeast Isolated from Dangke–Indonesian Traditional Fermented Buffalo Milk 183
the intestine. These conditions can improve health by improving the balance of intestinal micro-flora. Aggregation ability is an easy and reliable way to select various probiotic yeasts. Adhesion of pathogenic bacteria panel to the surface of various yeasts is determined as aggregation (Khotimah et al., 2015). The results of *Candida guilliermondii* aggregation on *Salmonella* sp. can be seen in Tab. 5.

Based on the appearance results in the microscope, it can be seen that *Candida guilliermondii* has an aggregation ability towards *Salmonella* sp. The aggregation ability of *Candida guilliermondii* was shown at intervals of 15, 60 and 180 minutes. The attachment of *Salmonella* sp. aggregation is influenced by the presence of structural virulence factors possessed by bacteria such as capsule, surface protein and hemagglutinin (Wahyuni et al., 2006). Bacteria that have hemagglutinin can be more easily attached to the mucosal surface. Hemagglutinin will initiate colonization by mediating bacteria to receptors binding (usually oligosaccharides) in human cells because this bacterium requires hemin for growth, so its bond with the cell body's erythrocytes also provides nutritional functions (Irianto, 2008).

The results show *Candida guilliermondii* is potential to be used as a probiotic. According to Annuk et al., (2001) and Leboffe and Pierce, (2011), some microorganism can maintain their ability and autoagglutination properties because they have an S layer that binds tightly to the cell surface. Some researchers have suggested the role of carbohydrates such as teichoic acids, lipoteichoic acids and polysaccharides to the aggressiveness of *Salmonella* from yeast (Annuk et al., 2001; Corcoran et al., 2005). Some researchers also state that adherence in the mucosal epithelium is mediated by lectins (Jin et al., 2001; Rickard et al., 2000). Lectin is a non-immunologic protein or glycoprotein that can be involved in adhesion phenomena with specific molecules (carbohydrates, glycoproteins or glycolipids). The multimeric lectin structure causes the lectin to have the ability for agglutinate cells or form precipitates with glycoconjugates in similar ways as antigen-antibody reactions. The aggregation ability of isolates in a single form (aggregation) is influenced by the production of EPS (Extra Cellular Polysaccharides) in the outer membrane of the cell, in addition to gravity and motility or bacteria movement when EPS production is low (Surono, 2004).

Beside microscopically, aggregation ability of *Candida guilliermondii* towards *Salmonella* sp. can qualitatively be supported by observing the presence of sediments in the broth which are incubated for 48 hours. The aggregation ability is asserted positively when the incubated culture

<table>
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<tr>
<th>Time (minutes)</th>
<th>The appearance of aggregation ability on a microscope</th>
</tr>
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<tbody>
<tr>
<td>15</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>60</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>180</td>
<td><img src="image3.png" alt="Image" /></td>
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</table>

1: Results of a qualitative aggregation test of *C. guilliermondii* and *Salmonella* sp.
Description: A = Suspension of *C. guilliermondii* and *Salmonella* sp. before Incubation
B = Suspension of *C. guilliermondii* and *Salmonella* sp. After Incubation
looks clear with the aggregate of cells precipitate at the base of the tube. Aggregation is considered negative (non-aggregating) if the incubated culture exhibits significant turbidity. The result of sedimentation at *Candida guilliermondii* during the incubation period can be seen in Fig. 1. Based on the precipitate, the results showed that the aggregate formed at the base of the liquid culture was an indication that the isolate had aggregation ability as one of the preconditions of a yeast isolate before being determined as probiotics. The aggregation ability itself will cause the probiotic to be able to colonize the mucosal epithelium by forming bacterial films that play a role in removing pathogenic bacteria from the intestinal mucosa (Khotimah et al., 2015).

**CONCLUSIONS**

Yeast isolated type from Dangke is *Candida guilliermondii* species with the morphology of colonies showed characteristics have a round shape, cream color, smooth surface, elevation of low convex and entire edge. Based on the results of acidic, bile salts and aggregation ability against *Salmonella* sp., *Candida guilliermondii* were able to resist pH 4, against bile salts with concentrations of 1% and 5% and had the ability to aggregate *Salmonella* sp. which increased until minutes 15, 60 and 180.

**Acknowledgement**

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**REFERENCES**


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