Fermentation 3

by Ikan Budu 3

Submission date: 18-Oct-2020 10:20PM (UTC+0800)
Submission ID: 1418600130
File name: Turnitin3.doc (801.5K)
Word count: 3172
Character count: 16560

Potential Yeast Probiotic Isolated from Fermented Fish (ikan budu) an Indonesian Indigenous

Abstract-Budu is a fermented food brought about by the activities of microorganisms like lactic acid bacteria and yeast. Budu, therefore, serves as a source of probiotic which can have beneficial effects on livestock and humans. Nonetheless, their selection has to be done with caution. The current study purposed to find budu desirable probiotic properties in vitro, such as pH, bile acid tolerance, hydrophobicity and inhibition of pathogens such as *Stapphylacoccus aureus*, *Salmonella* Enteritidis and *Escherichia coli*. Experimental method was used to conduct this research and the experiment was repeated 4 times using three *Saccharomyces cerevisae* (coded as SC 11, SC 12 and SC 21) from budu. They were tested for their probiotic properties (low pH, bile salts, hydrophobicity and inhibition of pathogenic bacteria) in vitro. The results showed that the three *Saccharomyces cerevisae* survived in gastric juice and bile acid, exbibited good hydrophobicity and had the ability to inhibit pathogenic bacteria, both gram positive and negative pathogens, especially pathogens associated with poultry infections. They were able to survive at pH 2 for 3 h (40.70 to 55.1%), at pH 2 for 5 h (35.25 to 46.88%), in 0.3% bile acid incubated for 3 h (69.69 to 86.56%) and at 5 h (82.22 to 88.18%), and hydrophobicity ability of 97.0 to 98.1%. The inhibition against pathogenic bacteria, that is, *Escherichia coli* was 2.50 to 3.81 mm, *Staphylococcus aureus* was 1.66 to 3.71 mm, and *Salmonella* Enteritidis was 1.20 to 2.64 mm.

***Keywords*— coconut meat waste, thermophilic bacteria, thermostable mannanase,broiler, histomorphology**

I. INTRODUCTION

In recent times, the use of antibiotic growth promoters (AGP) in livestock business or broiler business in particular has been banned. This is due to consumer concerns about the presence of AGP residues in products such as meat, milk and eggs that can cause immunity to certain antibiotics. Farmers always try to find a substitute for AGP with organic compounds such as plant extracts, prebiotics in the form of MOS, FOS and probiotics such as giving live microbes to livestock.

Microrganisms of the lactobacillus genera are mostly use for the commerercial production of probiotics especially in fermented milk worldwide [1]. Probiotics promote the growth of healthy microflora in the gastrointestinal tract [1, 2].

Probiotics can be from bacteria, fungi and yeast. Bacteria that are widely used as probiotics are lactic acid bacteria, from fungi are *Rhizopus oligosporus*, while from yeast are *Saccharomyces cereviceae* and *Saccharomyces bulardi*. The source of microorganisms that will be used as probiotics is essential, which is usually obtained from the digestive tract of livestock because it is expected that the microorganism can already adapt in the intestine. There are not many reports showing that probiotics are isolated from fermented foods such as budu.

Traditional fermented fish also known as budu is produced mainly in West Sumatra, Indonesia. Budu is usually made from leatherskin (*Chorinemus sp*.) and Spanish mackerel (*Scomberomorus sp*.) known as ikan talang and ikan tenggiri, respectively in Indonisian language [3]. Budu processing starts with the hanging of fresh fish by the tail fin for four hours under room temperature. It is then eviscerated, washed and covered with a layer of salt in a tradional container. Afterwhich, it is stored for one day at room temperature and sun-dried for five days. Garlic and white pepper can be sprinkled on the fish during the drying process to improve the quality of the budu [4].

Anggraini et al. [5] isolated lactic acid bacteria from budu for the purpose of producting Gamma aminobutyric acid (GABA) as anti heat stress for broiler, and they also found that yeast undergoes symbiosis with lactic acid bacteria in budu. Stadie et al. [6] reported a symbiotic relationship between yeast and lactic acid bacteria of water kefir origin. Symbiosis (commensalism or mutualism) widely occur in fermented foods such as sourdough, milk kefir and yogurt [6].

As a probiotic, yeast must be able to withstand gastric pH, bile acids and pathogenic bacteria. Brandao et al. [7] found that, acidic pH was not affected by the fatty acid composition of *S. boulardii*. Yeast are capable of maintaining their their internal pH by consuminhg H+ through metabolic pathway and by using cell buffer systems. Ogunremi et al. [9] added that *Pichia kudriavzevii* ROM 11, that is, yeast from Ogi which is a cereal pudding fermented food from Nigeria usually made from corn, sorghum or millet, has a resistance of 86.36% against bile acids concentration of 0.3%. The yeast derived from fermented foods

from West Sumatra such as fermented fish (budu) has not been exploited, especially as a candidate for probiotics. Yeast as a probiotic can contribute a variety of metabolites to other microbes such as lactic acid bacteria that can produce amino acids and vitamin B to support the survival and growth of lactc acid bacteria in the gastrointestinal tract. The purpose of the research was to find yeast that can serve as a probiotic for potential application for poultry production.

II. The Material and Method

A.Isolation of yeast

Sample of budu was purchased from traditional producer at Pariaman Regency, West Sumatra, Indonesia. Budu was made from coral reef fish such as red Kakap (*L. campechanus*) and tenggeri fish (*Scomberomorini*) as shown in Figure 1. Isolation of yeast was conducted according to the method of Bajwa and Sharma [10]. The sample (1 g) was added to 9 ml of 0.9% NaCl (saline) solution and mixed thourougly for 60s. Serial dilution was then carried out in the saline solution and spread plated onto yeast universal agar. The yeast universal agar was composed of 3.0 g/L malt extract, 3.0 g/L yeast extract, 10.0 g/L glucose, 5.0 g/L peptone and 15.0 g/L agar. The yeast universal agar was plated with the aliqout and incubated for 72 h at 28 °C. Presumptive yeast showed white-to-yellow colonies under the miscroscope. Such isolates were randomly selected and puried on yeast universal agar. Yeasts, showing the typical appearance of *Saccharomyces* (white-to-yellow colonies) were selected. The selected yeast strains were further purified by successive streaking on yeast universal media. Three isolates were maintained at −80 °C in 20% (v/v) glycerol (Hi-Media).

Functional properties of yeast as probiotics

1. *pH resistivity test*: This was done using a modified nutrient broth in which 0.1 N HCl was added to achieve a pH of 2 which corrrsponds with gastric pH as described by Ogunremi et al. [9]. The yeast extract (1 ml) was inoculated in modified HCl nutrient broth and incubated at 37 °C for 3 and 5 hours. Afterwhich, the absorbance was read at a wavelength of 600 nm. This research was conducted with three replications. Isolates resistance was also expressed as a percentage according to Ogunremi et al. [9].
2. *Bile salt resistivity test:* The modified HCl nutrient broth was mixed with 0.3% oxygall (synthetic bile salts) and incubated for 3 and 5 h [9]. The culture was streaked onto a modified nutrient broth and incubated at 37 °C. 1 ml of yeast isolate taken from the culture stock was streaked onto the bile salt-modified nutrient broth medium. Incubation was done at 37 °C. [9]. The results were obtained qualitatively by comparison of the yeast absorbancy with the control (not streaked with yeast) with the streaked modified nutrient broth (0.3% bile salts).
3. *Hydrophobicity of on stainless steel plates*. Hydrophobicity test or attachment was carried out by the method of Vinderola et al. [11] using stainless steel plates. A clean and dried stainless stell plates were marked on one side. One hundred (100) ml of distilled water was used to dissolve 0.8 g of nutrient broth. Growth media and stainless steel were autoclave at 121 °C for 15 minutes. The stainless steel plate was placed in 25 ml nutrient broth inoculated with 1 ml of LAB in an erlenmeyer, and incubated for 24 hours at 29 °C. Furthermore, the surface of the stainless steel was evenly wiped with a swab. The swab was put in a tube containing 10 ml of phosphate buffer and homogenized. It was then measured by looking at the absorbance at a wavelength of 600 nm (A). To measure the growth in the liquid phase, 1 ml of liquid was taken from nutrient broth media and diluted in 9 ml of phosphate buffer solution. Afterwhich, it was measured by looking at the absorbance at a wavelength of 600 nm (Ao). The percentage hydrophobicity was calculated using the formula of Fadda et al. [12].
4. *Inhibition test against pathogenic bacteria*. The antimicrobial activity test of 3 yeast isolates against *Salmonella* Enteritidis, *Staphyloccocus aureus* and *Escherichia coli* was carried out based on a modification from Diosma et al. [13] with the following working procedures: Media nutrient agar (10 grams) in 500 ml of aquades, homogenized, heated in a water bath and autoclaved. The media was allowed to cool (± 45 °C) and 0.2% of test bacteria was added into ± 10 ml petri dishes and allowed to solidify. Meanwhile, a blank antibiotic disk was soaked in lactic acid bacterial solution for approximately 10 minutes and was placed on the surface of the nutrient agar medium containg the pathogenic bacterial isolates. It was then incubated aerobically at 37°C for 24 hours. After incubation, the diameter of the inhibition zones were measured using a caliper.

Fig.1. Fermented fish (budu) made from red Kakap (*L. campechanus*)

III. RESULT AND DISCUSSION

Resistance test to acid

The yeast resistance to gastric pH was tested at pH 2 because the pH in the proventriculus and gizzard is 2.0 - 3.5 long feed transit for 70 minutes [14]. The gastric pH was tested for 3 hours and 5 hours, the results for which can be seen in Table 1.The results of the study showed that all yeast isolates can survive at pH 2 with resistance > 30%. The three yeast isolates of budu origin grew at pH 2 with a viability of 55.1% for isolate SC 11, 43.70% for isolate SC 12 and 40.70% for isolate SC 21 that were contact for 3 h. When the time contact with acid increased to 5 h, the presentage viability decreased.

pH 2.0 - 3.5 is pH in the proventriculus, where digestive enzymes such as protease, pepsin, khimotrypsin or HCL are produced which can digest food substances. The probiotic yeast work in the GIT by providing nutrients which aid in digestion of food and inhibition of harmful bacteria. Probiotics are aalso mixed or added to feed to increase the rate of feed and nutrient utlization [15].

The results in Table 1 showed that yeast isolate that had the highest resistance was SC 11, with resistance > 50%. Nurnaafi et al. [16] explained that good probiotic candidate isolates are those that have a survival of more than 50% under low pH conditions and are resistant to bile salts. The resistance of isolate SC 11 with large resistance at 3 hours incubation time was 55.10% and decreased at 5 hours incubation time which was 46.88% with a difference of 8.22%. The results of the study are higher than those of Kumura et al. [17] who found that yeast (*Kluveromyces lactis* S25) isolated from commercial blue cheese and kefir, had a resistance of 54.7%. The results of this study were also higher than those of Diosma et al. [13]. They examined yeast isolates from kefir (tested at pH 2.5 with an incubation time of 3 hours), and reported that *Kluyveromyces marxianus* 8116 had 45.5% resistance, *Saccharomyces cerevisiea* 8115 had 40.5% resistance, and *Saccharomyces boulardii* had 45.5% resistance.

Tovar et al. [18] reported that when yeast isolates enter the digestive tract of poultry, it must be able to survive at low pH because proventriculus and gizzard have a pH of 2.0 - 4.5. Zubaidy and Khanda [19] added that *Saccharomyces cerevisiae* var boulardi (S.b32) was able to survive at low pH.

TABLE 1.
The resistance of yeast isolates towards acid and bile acid conditions

Isolates yeast	Time (3 h)(%)	Time (5 h) (%)
Acid conditions		
Saccharomyces cerevisiae (SC) 11	55.10 ± 2.19	46.88 ± 1.82
Saccharomyces cerevisiae (SC) 12	43.70 ± 1.35	39.36 ± 0.80
Saccharomyces cerevisiae (SC) 21	40.70 ± 0.87	35.26 ± 0.38
Bile acids 0.03%		
Saccharomyces cerevisiae (SC) 11	69.69 ± 0.14	82.02 ± 0.53
Saccharomyces cerevisiae (SC) 12	84.54 ± 1.37	87.43 ± 1.91
Saccharomyces cerevisiae (SC) 21	86.56 ± 1.71	88.18 ± 1.72

Glucomannan, chitin, mannoprotein, beta-glucan made up the cell component of *Candidda* sp. [20]. However, beta-glucan forms the largest (50-60%) component of the inner layer of the cell wall, while chitin forms 1-10%. Mannoproteins form mainly 30-40% of the outer layer of the cell wall. They play amajor role in interactions with the host, determine the nature of the cell surface and cell-to-cell recognition [21].

Resistance test to bile salts

The results of the study in Table 1 shows that isolate SC 11 had an ability with a resistance of 69.69% at the incubation time of 3 hours and increased to 82.02% at the incubation time of 5 hours with a difference of 12.33% increase. The results of this study were higher than those of Chen et al. [22], who examined yeast isolated from fresh milk on Beijing and Heilongjiang farms against 0.3% bile salts and reported that *Pichia fermentans* HJ15 isolate had 79% resistance, *Pichia kudriavzevii* BY10 isolate had a resistance of 25.9%, and *Yarrowia lipolytica* HJ6 isolate had a resistance of 62.9%.

Yeast is able to survive in bile salt solutions because of its extreme environmental resistance, in accordance with report by Chen et al. [22] who explained that there are resistant properties in stressful environments (salt, acids and sugars) and in competition with other microbial yeasts, they are able to live a normal life. The difference in the results of this study can be caused by differences in the type of yeast tested against 0.3% bile salts. Yeast cell walls are mostly composed of beta-glucan [23]. Ooi and Liu [24] reported that beta-glucan is a linear polysacharride that contains monomers of glucose that are linked by gycosidic bonds. Beta-glucan is water soluble, and a small concentration will produce high viscosity [38] and will form a gel in the digestive tract so as to increase the excretion of bile acids. By this, the fat is not emulsified and absorbed in the stomach.

Inhibition test against pathogenic bacteria

The ability of yeast to act antagonistically is due to medium pH changes, competition for nutrients, secretion of antimicrobial agents and production of ethanol in hgh concentrations. The results of the study (Table 2) shows that the inhibitory zone of isolate SC 11 against *Eschericia coli* was 3.81 mm greater than the inhibition against *Staphylococcus aureus* of 3.71 mm. This is influenced by bacterial cell walls, since *Stapphyloccocus aureus* is gram-positive while *Escherichia coli* is gram-negative. Saidi et al. [25] reported that gram negative bacteria have a thinner layer of peptidoglycan (5-10 cm), while gram-positive bacteria have a thicker layer of peptidoglycan (20-80 cm). Therefore, it was more difficult for yeast isolates to penetrate the cell wall of *Staphylococcus aureus* bacteria than the cell wall of *Eschericia coli* bacteria.

Table 2 showed that, the yeast inhibition was lower against *Salmonella* Enteritidis which can be influenced by antigens present in *Salmonella*. *Salmonella* has three main antigen types namely: somatic antigens or O antigens, flagellate antigens or H antigens, and capsule antigens or Vi antigens [26] which produce enterotoxins and cytotoxins, making it difficult for yeast to inhibit their growth.

TABLE 2.
The resistance of yeast isolates towards pathogenic bacteria associated with poultry

Yeast isolates	Diameter inhibition zone (mm)		
	Escherichia coli	*Staphylococcus aureus*	*Salmonella* Enteritidis
Saccharomyces cerevisiae (SC) 11	3.81	3.71	2.64
Saccharomyces cerevisiae (SC) 12	2.50	2.56	2.52
Saccharomyces cerevisiae (SC) 21	1.63	1.66	1.20

The inhibitory strength possessed by yeast varies, so the strength of different yeast species will result in inhibition and activity of different metabolite components during the fermentation process. Freimoser et al. [27] studied the antagonistic activity of *Kloechara* and *Kluyveromyces* species against bacteria and found that they produce intracecullar and extracellelar antimicrobial compounds that inhibit the growth of gram negative and gram positive bacteria. Research by Suzuki et al. [28] and Marquina et al. [29] found yeast antagonistic activity, namely the production of killer toxins or mycocins, because this toxin is an extracellular protein or glycoprotein that can damage the function and cell membrane. A group of researchers reported on the antimicrobial activity of yeast through the secretion of organic acid or antimicrobial peptide [30, 31, 32]. *S. bourlardii* secretes mainly capric acid a medium chain fatty acid, which showed bioactivity againt *Candida albicans* and formation of biofilms [33, 34]. *S. cerevisiae* secretes antimicrobial peptides (saccharomycin) which inhibits the growth of pathogenic bacteria [35]. Antimicrobia peptides inhibit bacteria growth by absorbing the cell membrane recepters of the pathogens, membrane permeability and alteration intracellular pH [35, 36].

Hydrophobicity using stainless steel plates

Table 3 shows the hydrophobicity of the yeast isolates. From Table 3, all the yeast isolates had a hydrophobicity percentage > 90%. The results of this study were higher than those of Sourabh et al. [37] who showed that, *Saccharomyces cerevisiae* isolated from traditional West Himalayan fermentation food, Sc01 had a hydrophobicity percentage of 59.65%. Fadda et al. [12] *Saccharomyces boulardii* isolated from codex has a hydrophobicity ability of 55.9%, *Kluyveromyces lactis* isolated from kefir had a hydrophobicity ability of 74.1- 79.4% and *Kluyveromyces marxianus* had a hydrophobicity ability of 75.9%.

TABLE 3.
The hydrophobicity yeast on *stainless steel plates*

Yeast Isolates	Hdrophobicity (%)
Saccharomyces cerevisiae (SC) 11	97.00 ± 0.24
Saccharomyces cerevisiae (SC) 12	97.96 ± 0.72
Saccharomyces cerevisiae (SC) 21	98.71 ± 0.19

The ability of microorganisms to attach to the digestive tract becomes one of the selection criteria for probiotics, the formation of colonies in the digestive tract is influenced by the ability of microorganisms to attach to the digestive tract that are specific to the host [38]. Tovar et al. [18] reported that some yeast species can synthesize and secrete polyamine molecules which can stimulate the development of the digestive tract and produce digestive enzymes.

IV. Conclusions

The results indicated that, all the isolates identified by macroscopic and microscopic were *Saccharomyces* sp, (SC 11; SC 12 and SC 21) and showed notable potential probiotic properties. They exbibited a better survival in gastric juice and bile acid, showed high hydrophobicity and the ability to inhibit pathogenic bacteria, both gram-positive and negative pathogen, especially pathogenic bacteria associated with poultry. They were able to live at pH 2 for 3 h (40.70 to 55.1%), pH 2 at 5 h (35.25 to 46.88%), in bile acid 0.3% incubated for 3 h (69.69 to 86.56%) and at 5 h (82.22 to 88.18%), and hydrophobicity ability of 97.0 to 98.1%. The inhibition against pathogenic bacteria *Escherichia coli* was 2.50 to 3.81 mm, *Staphylococcus aureus* was 1.66 to 3.71 mm, and *Salmonella* Enteritidis was 1.20 to 2.64 mm.

Acknowledgment

The authors are grateful to the Ministry of Research, Technology and Higher Education of Indonesia for funding the BOPTN Andalas University Grants through Research Cluster Grant No: 57/ UN.16.17/HGB/LPPM/2019.

Fermentation 3

ORIGINALITY REPORT

3%	1%	3%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Gemilang Lara Utama, Siska Meliana, Mohamad Djali, Tri Yuliana, Roostita L. Balia. "Probiotic Candidates Yeast Isolated from Dangke – Indonesian Traditional Fermented Buffalo Milk", Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2019 Publication	2%
2	Submitted to University of Newcastle upon Tyne Student Paper	1%
3	Submitted to essex Student Paper	1%
4	archive.org Internet Source	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		