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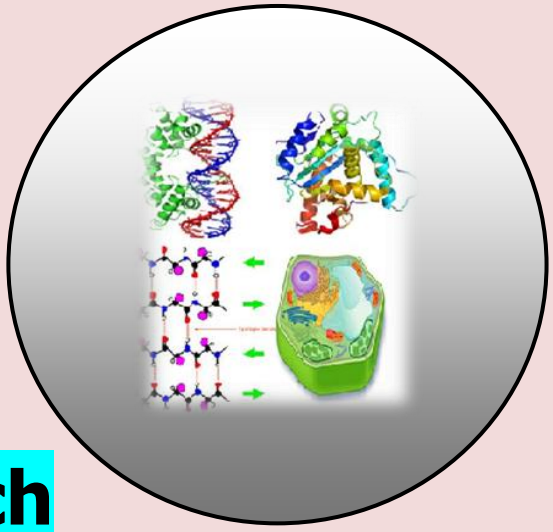

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RESEARCH PAPER

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Selection and Implementation of Probiotics *Saccharomyces Spp*. Kb-05 and *Saccharomyces Spp*. Kb-08 Isolated from Buffalo Ruments to Increase the Nutritional Value of Rice Bran

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ABSTRACT

The present study was conducted to find isolates Saccharomyces spp isolated from buffalo rumen which has cellulolytic activity (CMC-ase) and potential as a source of probiotics agents to increase the nutritional value of rice bran.

The study included the isolation of yeast Saccharomyces spp from buffalo rumen, then both test enzyme activity (CMC-ase) and test as probiotic agent (passing test of several temperature levels, pH, acid and bile salt, and assimilate cholesterol). The superior isolate further tested its ability to increase the nutritional value of rice bran.

Six isolates of Saccharomyces spp were isolated and selected from buffalo rumen as probiotics and had CMC-ase activity too. The six isolates, namely Saccharomyces spp.Kb-05 isolates; S.spp.Kb-08; S.spp.Kb-09; S.spp.Kb-10; S.spp.Kb-13; and S.spp.Kb-14, respectively. The use of both Saccharomyces spp.Kb-05 or Saccharomyces spp.Kb-08 isolate for rice bran fermentation were significantly (P<0.05) increased the crude protein and gross energy (GE) content of rice bran. Protein digestibility of rice bran that has been fermented by both isolates S.spp.Kb-05 and S. spp.Kb-08 were increased than control. Similarly, there was also an increase in the digestibility of crude fiber from fermented rice bran.

It can be concluded that the fermentation of rice bran by both Saccharomyces spp.Kb-05 and S.spp.Kb-08 isolated from buffalo rumen can increase the energy, protein, and metabolizable energy of rice bran.

Keywords: Saccharomyces Spp, Crude Fiber, Probiotics and Rice Bran.

INTRODUCTION

In general, almost 90% of poultry feed uses agro-industrial waste and 40-50% of feeding cell components are composed of cellulose that is very difficult/not digested by poultry digestive enzymes, which are caused by poultry generally not having cellulase enzymes (fiber degrading

enzymes). In order to be used, the cellulose fraction must first be described as low molecular weight compounds such as mono, di, and tri saccharides. The degradation involves the cellulase enzyme complex produced by yeasts, ie. endo-beta-glucanase and beta glucosidase (Wainwright, 2002). The yeast is present in buffalo rumen fluid which has the highest cellulolytic activity compared to the cellulolytic microbes in other animals (Prabowo *et al.,* 2007).

Cellulolytic yeasts play a very important role in the degradation process of such crude fiber polymers. The yeast is found in the gastrointestinal tract, especially in ruminants, such as goats, cattle, and buffalo (Purwadaria *et al*., 2004). Provision of yeast culture of buffalo rumen fluid isolate to poultry is expected to cause a synergistic effect between rumen buffalo species with poultry gastrointestinal microbe, so it can cause the ability of digesting poultry to fiber feed increased (Bidura *et al*., 2014).

Fermentation with cellulolytic microbes can simplify the particles of feed ingredients, thereby increasing their nutritional value, and converting complex proteins into simple amino acids that are easily absorbed (Bidura *et al.,* 2008). The imperfect fermentation process seems to cause the development of other pathogenic bacteria that can cause health problems and death of livestock. Therefore, the selection of microbes as fermentation inoculants should be observed. Bacterial and fungal fermentations result in degradation of various anti-nutritional factors, an increase in amount of small-sized peptides and improved content of both essential and non-essential amino acids. However, the resulting fermented products vary in levels of nutritional components as the two species used for fermentation differ in their metabolic activities (Mukherjee *et al.,* 2016).

Zurmiati *et al*. (2017) reported that addition of 2000-3000 ppm of probiotics *B. amyloliquefaciens* to the drinking water of Pitalah ducks can improve the feed efficiency by >15% and provide economic benefits for farmers raising starter ducks. Supplementation two isolates of *Saccharomyces spp*.Gb-7 and Gb-9 isolated from the colon of chicken in the diet may increase the performance and reduce the content of fat and cholesterol in the body broiler (Bidura *et al*., 2016). According to Harmayani (2004), bacteria that are able to grow and assimilate cholesterol in the small intestine have the potential to control blood serum cholesterol levels host, because in the small intestine occurs cholesterol absorption process. The ability of cholesterol assimilation by probiotic bacteria varies between strains and requires anaerobic conditions and the presence of bile acids.

The present study was conducted to find isolates *Saccharomyces spp* isolated from buffalo rumen fluid which has cellulolytic activity (CMC-ase) and potential as a source of probiotics agents to increase the nutritional value of rice bran.

MATERIALS AND METHODS

Sources Isolate/Rumen Buffalo Liquid: Sources of isolate in this study is the contents of adult buffalo rumen obtained from slaughter of buffalo in Denpasar, Bali. The buffalo rumen fluid is immediately taken after the livestock is slaughtered. The sample is inserted in a thermos previously filled with warm water (temperatures around 39^0C) whose contents have been removed. The thermos is filled with samples, then sealed until free from air contamination and immediately used for research.

Isolation of *Saccharomyces spp* from Rumen Buffalo Liquid: Colonies of isolates in petri dishes have begun to grow after being grown for 2 x 24 hours. The isolate form is small round. Before transferred, firstly prepared 10 pieces of petri dish that had been sterilized. Prepare selective OMEA solid media, then take one ose isolate and scratch on the petri dish that already contains the OMEA solid media. After two days the isolates in the petri dish begin to grow, then it will be bred back into the test tube (Bidura *et al*., 2015).

Prepare OMEA media as much as 3.4 g dissolved with aquades to 100 cc. Furthermore, the OMEA solution is heated in the stove, then input into water bath with temperature 60-70^0C for about 15 minutes and pour into the test tube and sealed with cotton.

Put the 10 test tubes into autoclav for sterilization. After that, input in laminar flow (UV light) for approximately 15 minutes. Tilt the test tube, let the media solidify. With the scratch method, the isolates in the petri dish were transferred into the test tube (Ahmad, 2005). Close the test tube that already contains the isolates with cotton and leave 2 x 24 hours, incubated in the incubator in reverse position at 30^0C for 48 hours, and observed growing colonies (Bidura *et al*., 2015).

The colonies having the characteristics of yeasts were isolated by following the method reported by Ahmad (2005). Purified, and cultured on solid medium for further analysis, and stored prior to characterization, low pH resistance test, and various temperature levels, deoxycholic acid (Hyronimus *et al*., 2000; Prangdimurti, 2001).

Saccharomyces spp Capability Test in CMC-ase: Weigh as much as 11 g of OMEA and 3 g CMC-ase, subsequently dissolved into aquades. Heat in Waterbath and after that do strelilisasi on autoklav. Chill at a temperature of 45-50°C, then poured on a petri dish and let stand until solidified. Isolates that have been cultured in nutrient broth for 24 hours. Take a paper disk with tweezers and then dip in a nutrient broth solution and paste on a petri dish containing the OMEA and CMC-ase media. Leave on for 24 hours. After 24 hours measurements of the width of the clear zones were generated by using the sliding term (Van DevoordeandVerstraete, 1987; Bidura *et al*., 2014b).

Fermented Rice Bran: Fermented rice bran by both *Saccharomyces spp*.Kb-05 isolate and *S.spp.*Kb-08 with the following procedure. Rice bran added 50% water (volume/weight), then stirred evenly, then steamed for for 45 minutes counted from boiling water. After steaming, the rice bran is cooled and then inoculated with *Saccharomyces spp.Kb-05* or *S.spp.*Kb-08 isolates with a dose of 0.50% of the weight of rice bran, then inserted into a black plastic which has been given small holes, then incubated in room temperature with a thickness of 2-4 cm for 2 days. After 2-3 days, the fermented of rice bran is dried for 24 hours at 50^0C (Wahyuni *et al*., 2008; Bidura *et al*., 2014b) and ready to be administered to the chicken**.**

Retention and excretion of nutrients by force feeding method: In order to determine the nutrient digestibility and metabolizable energy (ME) value of the rice bran. In this method, first prepared each 18 adult male Balinese ducks. The amount of rice bran used was 50 g. This amount as based on preliminary assays with male Balinese duck (aged 70 weeks) using rice bran. All the birds were deprived of feed for 24 h to ensure that their alimentary canals were empty from feed residues. They were then force-fed with the specific amount of rice bran (fermented and unfermented). Stainless steel funnel with 40 cm stem was used in *force feeding technique* (Mustafa *et al*., 2004; Bidura *et al*., 2012 and Bidura *et al*., 2014b). Water was available *ad libitum* during the experimental period. The total excreta were collected in plastic trays. The excreta samples were frozen, allowed coming to equilibrium with the atmospheric moisture, weighed, and groun through a 1 mm sieve. Samples of excreta and rice bran were subjected to appropriate analysis to determine DM, OM, CP, CF, and energy, respectively.

Laboratory analyses: Dry matter (DM), organic matter (OM), CP, and ash determinations were done according to the Assocciation of Official Analytical Chemists (l994). The CP content of the diets was determined using the Kjeldahl procedure (AOAC, 1994). Crude fibre in the feeds was determined using the procedure of Van Soest *et al*. (l991) on oven-dried samples. Gross energy (GE) was measured with an adiabatic oxygen bomb calorimeter (Parr, USA),

Calculations: The data were used to calculate AME value according to the following formulate (Mustafa *et al*., 2004): AME (apparent metabolizable energy) = IE – FE. Where IE = ingested energy; FE = fecal energy voided by the fed birds.

Statistical analysis

The data analysis of *Saccharomyces spp* yeast isolate including temperature, pH, cholesterol, bile salt, and CMC-ase test was done by descriptive method. To determine whether a culture can grow at various temperatures, the criterion is to see its turbidity. To determine whether the culture can grow at a certain pH with pH meters.

To determine the ability to conjugate cholesterol using a spectrophotometer at a wavelength of 660 nm (Sujaya *et al*., 2008). The data obtained in the analysis with the variance and if there is a marked difference (P<0.05) between treatments, then continued with Duncan's multiple-range test (Steel and Torrie, l989).

RESULTS

Isolation *Saccharomyces spp*

The results of the isolation of yeast *Saccharomyces spp* from the buffalo rumen fluid using the OMEA (Oxytetracycline Malt Extrax Agar) medium and successfully isolated as many as 15 Saccharomyces spp isolates. The results of morphological observations, it turns out yeast *Saccharomyces spp* has a white oval shape. In this research, the colony separation is done twice, so that high purity level is obtained.

Khamir *Saccharomyces spp* isolated from buffalo rumen, did not all have the ability as probiotic agents, so it is necessary to test several temperature levels, various acid concentrations, and various concentrations of bile salts. In addition, there is a CMC-ase test, which is an ability test of isolates in digesting crude fiber shown by the width of the clear zone generated.

Table 1. Number of colonies in isolates *Saccharomyces spp* (Log colony/ml) isolated from buffalo rumen fluid that can survive at various concentrations of pH.

Code of Isolate *Saccharomyces spp*	pH concentration			
	1.5	3.0	4.5	6.0
Kb-01 (Log colony/ml)	3.17	3.84	3.89	3.72
Kb-02 (Log colony/ml)	1.58	2.06	3.73	3.61
Kb-03 (Log colony/ml)	-	-	-	-
Kb-04 (Log colony/ml)	3.18	3.25	4.04	3.16
Kb-05 (Log colony/ml)	4.84	4.72	4.51	4.03
Kb-06 (Log colony/ml)	2.79	3.52	3.07	3.53
Kb-07 (Log colony/ml)	3.05	3.47	4.05	3.25
Kb-08 (Log colony/ml)	4.63	4.15	4.79	3.16
Kb-09 (Log colony/ml)	1.87	2.52	2.16	2.62
Kb-10 (Log colony/ml)	2.84	3.17	3.46	2.81
Kb-11 (Log colony/ml)	1.68	2.95	3.46	2.06
Kb-12 (Log colony/ml)	-	2.79	1.26	1.05
Kb-13 (Log colony/ml)	4.73	4.92	4.67	2.12
Kb-14 (Log colony/ml)	3.95	4.16	4.02	3.78
Kb-15 (Log colony/ml)	-	-	1.83	1.75

Note: Kb-01 s / d Kb-15 is isolate *Saccharomyces spp* isolated from buffalo rumen fluid

Test Various Levels of Acid

The results showed that not all isolates of *Saccharomyces spp* isolated from buffalo rumen survived various pH conditions tested. The pH variations tested were variations of pH conditions present in the gastrointestinal tract of poultry, ranging from pH 1.5 to 6.0. Table 1, shows that *Saccharomyces spp*.Kb-03 isolate did not survive under various pH conditions. Similarly, both isolate *Saccharomyces spp*.Kb-12 and Kb-15 does not survive in acidic pH conditions (pH 1.5). More detailed number of colonies of each isolate *Saccharomyces spp*.Kb-01 to *Saccharomyces spp*.Kb-15 presented in Table 1.

Hydrochloric acid (HCl) was used in testing the resistance of isolates to various pH concentrations. In this test used HCl, because HCL has characteristics similar to stomach acid.

Table 2. The ability of Isolate Yeast *Saccharomyces spp* to survive in bile salts.

Code of Isolate *Saccharomyces spp*	Bile Salts Concentration (Absorbance)		
	0.2 mM	0.4 mM	0.6 mM
Kb-04	+(0.128)	+(0.149)	-(0.048)
Kb-05	+(0.307)	+(0.275)	-(0.084)
Kb-07	+(0.185)	+(0.142)	-(0.038)
Kb-08	+(0.281)	+(0.237)	-(0.063)
Kb-09	+(0.163)	+(0.142)	-(0.037)
Kb-10	+(0.205)	+(0.183)	-(0.029)
Kb-13	+(0.347)	+(0.276)	-(0.052)
Kb-14	+(0.297)	+(0.251)	-(0.049)

Note: *S.spp*.Kb-01-Kb-15 is Code of isolate *Saccharomyces spp* isolated from buffalo rumen

- : A <0.1 (not resistant to bile salts)
+ : A 0.1 - 0.5 (Slightly resistant to bile salts)
++ : A 0.5 - 1.0 (resistant to bile salts)
+++ : A> 1.0 (highly resistant to bile salts)

Table 3. Activity Test of CMC-ase isolate yeast *Saccharomyces spp*. isolated from buffalo rumen fluid based on clear zone diameter.

Isolate Code	Diameter of the Clear Zone (Cm)
*Saccharomyces spp.*Kb-04	-
*Saccharomyces spp.*Kb-05	4.06
*Saccharomyces spp.*Kb-07	-
*Saccharomyces spp.*Kb-08	3.92
*Saccharomyces spp.*Kb-09	0.74
*Saccharomyces spp.*Kb-10	0.39
*Saccharomyces spp.*Kb-13	3.70
*Saccharomyces spp.*Kb-14	1.83

Note: *S.spp*.Kb-04-Kb-14 is Code of isolate *Saccharomyces spp* isolated from buffalo rumen fluid

The acid conditions in the medium used are set at the range of: pH1,5; pH 3.0; pH 4.5; and pH 6.0, respectively. The resistant nature of the isolates tested against various acidic conditions is necessary for probiotic candidates, because in its application later, this probiotic candidate must pass through a highly acidic stomach, before reaching the colon area of the poultry.

There is an increasing trend of pH, especially at pH 6, most of the isolates experiencing a decrease in the number of living colonies. The colonies of *Saccharomyces spp* grow well at pH 3 to pH 4.5. Isolate khamir *Saccharomyces spp*.Kb-05; Kb-08; Kb-13 and Kb-14 growth is quite good from ph 1.5 to pH 6.0. *Saccharomyces spp*.Kb-05 and Kb-08 for example, the number of colony at pH 1.5 reaches 4.84 and 4.63 log colony per milli liter of rumen fluid.

Bile salt test

The test of the isolate ability of yeast *Saccharomyces spp* on the various concentrations of bile salt is presented in Table 2. The test results showed that at 0.6 mM bile salt concentration, almost all isolates were not able to grow. The eight isolates of *Saccharomyces spp* isolated from the buffalo rumen fluid are able to survive and grow on a medium containing bile salts at a concentration range between 0.2 mM and 0.4 mM.

CMC-ase Activity Test

In Table 3, there were observed results of CMC-ase activity test against eight *Saccharomyces spp* isolates isolated from buffalo rumen fluid which have passed the test of various acid levels (1.5; 3.0; 4.5; and 6.0), various temperature levels (10^0C; 37^0C; and 45^0C); and various levels of bile salt concentration (0.2 mM; 0.4 mM; and 0.6 mM). The CMC-ase activity test is a test of the ability of *Saccharomyces spp* isolates having celulolytic activity, which is the activity of the isolates to degrade cellulose (crude fiber fraction). The cellulolytic activity of the isolates can be measured from the diameter of the clear zone formed.

From the results of this study only six isolates of yeast *Saccharomyces spp.* isolated from buffalo rumen fluid having CMC-ase activity. The six isolates are *Saccharomyces spp*. isolates. Kb-05; *Saccharomyces spp*. Kb-08; *Saccharomyces spp*. Kb-09; *Saccharomyces spp*. Kb-10; *Saccharomyces spp*. Kb-13; and *Saccharomyces spp*.Kb-4, respectively. Isolate yeast *Saccharomyces spp*. Kb-05 has the widest clear zone, while the *Saccharomyces spp*. Kb-08 has the lowest clear zone, but the two isolates of yeast *Saccharomyces spp*. Kb-04 and isolates *Saccharomyces spp*. Kb-07 does not show clear zone or does not have CMC-ase activity.

The result of research of rice bran digestion by force feeding method by using duck to test the ability of both isolate *Saccharomyces spp*.Kb-05 and *Saccharomyces spp*.Kb-08 as rice bran fermentation inoculant to increase the value of rice bran nutrition presented in Table 4. Isolate yeast *Saccharomyces spp*.Kb-05 and Saccharomyces spp.Kb-08 are isolates that have passed the acid test, bile salts, and are able to deconjugate cholesterol, and have the highest activity of CMC-ase compared to other isolates (Table 4).

The use of culture isolates *Saccharomyces spp.*Kb-05 and *Saccharomyces spp.*Kb-08 as fermented rice bran inoculant was not significant (P>0,05) to dry matter, organic matter, and crude fiber of rice bran. However, significantly (P<0.05) affected the crude protein and gross energy (GE) content of rice bran (Table 4). The crude protein from rice bran after fermentation increased significantly (P<0.05), respectively: 9.65% and 6.74% compared with control (rice bran without fermentation). Similarly, the gross energy content of rice bran that has been fermented by *Saccharomyces spp*.Kb-05 isolates or *Saccharomyces spp*.Kb-08 has significantly increased (P<0.05) higher: 6.84% and 6.67%, respectively compared to the gross energy content (GE) of rice bran control (unfermented).

Table 4. Chemical composition of rice bran with and without fermentation by yeast *Saccharomyces spp* isolated from buffalo rumen fluid (% Dry matter).

Variable	Rice Bran			SEM[1)]
	unfermented (kontrol)	Fermented by *Saccharomyces spp.*		
		Kb-05	Kb-08	
Chemical composition*:*				
Dry matter (%)	88.52a	88.39a	87.95a	1.053
Organic matter (%)	89.74a	89.25a	88.82a	1.037
Crude protein (%)	13.06b[2)]	14.32a	13.94a	0.205
Crude fibre (%)	14.83a	15.06a	15.27a	0.832
Gross energy/GE (Kcal/kg)	3261.91b	3485.18a	3479.25a	50.035

Note:

1. SEM:Standard Error of Treatment Means
2. Means with different superscripts within raw values are significantly different (P<0.05)

Table 5. Digestibility of rice bran with and without fermentation by *Saccharomyces spp* isolated from buffalo rumen fluid (% Dry matter).

Variables	Rice Bran			SEM[1)]
	Unfermented (control)	Fermented by *Saccharomyces spp.*		
		Kb-05	Kb-08	
Digestibility (%):				
Dry matter digestibility (%)	39.37b	42.08a	41.84a	0.509
Organic matter digestibility (%)	40.28b	43.35a	42.62a	0.513
Crude protein digestibility (%)	45.61b	49.16a	48.87a	0.837
crude fibre digestibility (%)	19.07b	22.16a	21.75a	0.305
Metabolizable energy (kcal/kg)	1805.71b	2001.92a	1995.83a	50.081

Note:

1. SEM: Standard Error of Treatment Means
2. Means with different superscripts within raw values are significantly different (P<0.05)

Table 5, shows the nutrient digestibility of rice bran that has been fermented by *Saccharomyces spp*.Kb-05 or *Saccharomyces spp*.Kb-08 isolates. Dry matter digestibility of rice bran that has been fermented by *Saccharomyces spp*.Kb-05 or *Saccharomyces spp*.Kb-08 had a significant increase (P<0.05) of 6.88% and 6.27%, respectively higher than control (unfermented rice bran). Similarly, a significant increase (P<0.05) on the digestibility of organic matter from rice bran has been fermented, namely: 7.62% and 5.81%, each higher than the control.

The digestibility of crude protein and crude fiber from rice bran fermented by *Saccharomyces spp*.Kb-05 or *Saccharomyces spp*.Kb-08 isolates, showed significant differences (P<0.05) compared with unfermented rice bran (Table 5). The results showed that the crude protein digestibility of rice bran fermented by *Saccharomyces spp*.Kb-05 or *Saccharomyces spp*.Kb-08 isolates increased: 7.78% and 7.15%, respectively higher than control. Similarly, there was also a significant increase (P<0.05) on the digestibility of crude fiber of fermented rice bran: 16.10% and 14.05%, each higher than control (Table 5).

Fermentation of rice bran by *Saccharomyces spp*.Kb-05 isolate or *Saccharomyces spp*.Kb-08 significantly (P<0.05) increased the metabolizable energy of rice bran ie: 10.87% and 10.53%, respectively rather than the metabolizable energy of rice bran without fermentation.

DISCUSSION

The resistance of *Saccharomyces spp* isolates to low pH is one of the characteristics required or must be possessed by probiotic candidates in order to be developed into potential probiotics. In general, probiotic candidates should be able to live within the digestive tract of host animals and mutualism with their host bodies in the pH range between 2-4. Microbial probiotics do not cause negative things on the host body, not pathogens, generally do not form spores, saccharolytic, anaerobic, do not disturb the gastrointestinal ecosystem, as well as live and grow in the intestine (Fuller, 1989). In addition, the optimal living temperature that must be possessed by the probiotic candidate is resistance to the host body temperature (poultry). According to Ahmad (2005), the optimum environmental temperature for yeast growth is between 25-30°C and maximum temperature between 35-47°C.

The results showed that only six isolates of *Saccharomyces spp* were potential as probiotic agents and had CMC-ase activity. The six isolates showed resistance to acids and bile salts which are characteristic of probiotic microbes. Saccharomyces is a one celled microorganism not chlorophyll, growing well at 30°C and pH 4.8.

According to Hidayat (2010), pH values in the digestive tract of poultry in every gastrointestinal organs are: crop (pH 4.5), proventrikulus (pH 4.4), ventriculus (pH 2.6), duodenum (pH 5.7-6.0), jejunum (pH 5.8) (pH 6.3), ceca (pH 5.7), and bile (H 5.9). Some of the advantages of Saccharomyces in the fermentation process, such as microorganisms are rapidly multiply, resistant to high alcohol levels, resistant to high temperatures, have stable properties and quickly hold adaptations in the digestive tract of the host.

The growing ability of *Saccharomyces spp* isolates against bile salts is used to assess the ability of isolates to survive in the gastrointestinal tract of hosts in which there is bile salts on the upper surface of the intestine. Microbial probiotics will deal with the environment in the small intestine in which there is bile or bile salts released by the liver through the gallbladder, after successfully passing acid conditions in the stomach. Therefore, in the process of developing new probiotics, or potential new probiotics should be able to pass the resistance test against bile or bile salts conducted in vitro. Based on the resistant properties shown by some isolates, it is indicated that the strain or isolate has the potential to be developed into a probiotic candidate.

The activity of the enzyme CMC-ase (endo-1,4-b-gluconase) is indicated by the presence of clear zones around the colony indicating that the isolate has strong extracellular cellulase enzyme activity. The small width of the clear zone diameter and the clearness of the clear zone, is an indicator of the ability of the yeast isolate to break down the cellulose, as well as the rapid and slow occurrence of the clear zone (Van Devoorde and Verstraete, 1987). According to Howard *et al*. (2003), cellulolytic yeasts are capable of producing 1.4 b-gluconase endo-enzymes, 1.4 b-glukonase eko, and beta-glucosidase which can degrade crude fiber components into soluble carbohydrates.

According to Fuller (1989), one of the conditions that must be possessed by microbial probiotics in each gram is as much as 106 CFU/gram. Bidura *et al*. (2012) reported that one of the conditions that must be possessed by microbial probiotics is easy to grow and the amount does not decrease if the culture is stored in a certain period of time before use. The results showed that the number of yeast colonies of yeast *Saccharomyces spp*.Kb-05 and *Saccharomyces spp*.Kb-08 in the second week (14 days) after planting high enough.

Fermentation of rice bran by both isolates *Saccharomyces spp*.Kb-05 and *Saccharomyces spp*.Kb-08 caused a decrease (P>0.05) of dry matter and organic matter in rice bran. This is because when fermented by *Saccharomyces spp*.Kb-05 or *Saccharomyces spp.*Kb-08 isolates, there will be a loss of carbohydrates to heat and other food substances for the formation of microbial proteins (Bidura *et al*., 2012). The content of dry matter and organic matter in rice bran has undergone fermentation decreased, due to the presence of carbohydrates and crude fiber fractions used by yeast *Saccharomyces spp*.Kb-05 or *Saccharomyces spp.*Kb-08 for the growth of yeast itself by utilizing nitrogen free extract. According to Sumarsih *et al.* (2007), the longer the feeding time when fermented, the more loss of dry matter and organic matter feed. Cellulase enzymes, namely selobiohidrolase may attack the crystal part of cellulose, and the enzyme endogluconase may attack the amorphous portion of the cellulose structure, whereas the b-glucosidase enzyme will decompose selobiose into glucose.

Rice bran was fermented with both the isolates of *Saccharomyces spp*.Kb-05 and *Saccharomyces spp*.Kb-08 found to increase microbial biomass, so that the crude protein content of rice bran increased (Bidura *et al*., 2012; Citrawati *et al*., 2017). The success of the fermentation process is influenced by the type of microbe and the amount of microbe used, the type of substrate, pH, and temperature during the fermentation process (Bidura *et al*., 2014). Biomass is a mass form of biological process results from microorganisms. Microorganisms are able to convert materials into proteins. The fermentation process has a purpose to produce a product (feed material) that has better nutritional, texture, and biological value of feed, and decrease antinutrition in fermented feed.

Increase in the crude protein content of rice bran after fermentation, due to the NPN conversion process by yeasts into microbial proteins, so that feed proteins become increasing (Mangisah *et al.,* 2008; Citrawati *et al.,* 2017). Similarly, the content of ash, Ca, and P in fermented feed products is higher than nonfermented feed (Suparjo *et al*., 2003). Mangisah *et al.* (2008) reported that feed fermentation process can significantly increase feed protein content (increased 65,41%). According to Suparjo *et al.* (2003), rice bran fermented with 0.20% *Aspergillus niger* culture for three days can significantly increase the protein and phosphorus content of rice bran. Increased protein and energy content in fermented feed is due to the ability of yeast to utilize nutrients in rice bran to form the body protein (microbial protein).

Fermentation of rice bran by both isolates of *Saccharomyces spp*.Kb-05 and *Saccharomyces spp*.Kb-08 can significantly increase the gross energy and energy content of rice bran. As reported by Jaelani *et al.* (2008) that the fermentation of feed ingredients with *Trichoderma reesei* may increase the energy content and crude protein content. The increased energy content of the fermented rice bran is due to the formation of sugars derived from crude fiber component breakdown. *Trichoderma reesei* was able to degrade mannan by increasing the value of true metabolizable energy (TME), total dissolved sugar, but a decrease in nitrogen retention and mannan content. Sabini *et al.* (2000) states that *T. reesei* is capable of degrading polysaccharides, such as mannan to mannotriose, mannobiose, and monnosa.

Fermentation of rice bran by both *Saccharomyces spp*.Kb-05 isolates and *Saccharomyces spp*.Kb-08 significantly improved the digestibility of dry matter, organic matter, crude protein, crude fiber, and metabolized energy of rice bran compared to non fermentation. The increase is due to the fermentation yeast having the catabolic ability of transforming complex organic components into simple components. The process arises because of the activity of some enzymes produced by yeasts. Cellulolytic yeasts are capable of producing 1.4 b-glukonase endo-enzymes, 1.4 b-glukonase eko, and beta-glucosidase which can degrade crude fiber components into carbohydrates in an easily digestible form (Howard *et al*., 2003).

The results of Utama (2011) study, reported that giving of *S. cerevisiae* in feed can improve protein digestibility and crude fiber component, such as cellulose and hemicellulose. Reported by Bidura *et al*. (2014), that the use of isolate yeast *Saccharomyces spp* isolated from colon in Bali cattle for pollard fermentation can improve the digestibility of dry matter, organic matter, protein, and crude fiber, and can increase the metabolizable energy content of pollard. The same thing was reported by Candrawati *et al*. (2014), that the use of isolates Saccharomyces spp isolated from faeces in Bali cattle for rice bran fermentation, can significantly improve the digestibility of dry matter, organic matter, and crude fiber from rice bran, and can significantly increase the energy content of rice bran (Bidura *et al*., 2012; Citrawati *et al*., 2017).

Chen *et al*. (2005) reported that the addition of 0.20% complex probiotic (*L. acidophilus* and *S. cerivisae*) in the diet can improve the digestibility of the dry matter feed. Fermentation of rice bran with yeast will soften the cell wall of rice bran and break the cell wall of rice bran by yeast by releasing the bands of its microfibril fibers, so that the structure of the rice bran cell selt wall becomes brittle and more open. The yeast works gradually in breaking down the cell wall components. Khamir will excrete extracellular peroxidase enzymes through its fibrils. The extracellular peroxidase enzyme acts actively on the activity of lignolysis, so that the lignocellulosity bond is broken, and the lignin fraction breaks down into CO_2. The results of this study are similar to those reported by Arsyad *et al*., 2001; Bidura *et al*., 2012; Bidura *et al*., 2014; Citrawati *et al*., 2017; Hong *et al*., 2004.

According to Jaelani *et al*. (2008), an increase in ME content of palm kernel cake (meal palm kernel cake/meal) as a result of fermentation by *Tricodherma reesei*, which increased from 1,824.13 kcal/kg to 1930.44 kcal/kg caused by degradation of mannan polysaccharide on palm cake by

T. reesei into a simpler form (monosaccharide) which can produce a good enough energy value than in the form of a mannan polysaccharide. The same thing is also reported by Sabini *et al*. (2000) reported that *Tricodherma reesei* has the ability to degrade polysaccharide mannan into mannotriose, mannobiose, and monnosa compounds. Utama (2011), reported that addition of *S. cerevisiae* yeast in feeds can improve protein digestibility and crude fiber components, such as cellulose and hemicellulose, which have been overhauled in the form of simple monosaccharides. Bedford and Classen (1992), adding that fungi are highly effective in degrading complex compounds, such as β-glucans and arabinoxylans.

CONCLUSION

We conclude that six isolates *Saccharomyces spp* have been successfully isolated and selected from buffalo rumen fluid that can potentially as probiotics and have CMC-ase activity,namely: *Saccharomyces spp*.Kb-05 isolates; *Saccharomyces spp*.Kb-08, *Saccharomyces spp*.Kb-09, *Saccharomyces spp*.Kb-10, *Saccharomyces spp*.Kb-13, and *Saccharomyces spp.*Kb-14, respectively. The use of the superior isolates, both *Saccharomyces spp*.Kb-05 and *Saccharomyces spp*.Kb-08 for rice bran fermentation can increase the nutritional value of rice bran.

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