Selection of Khamir *Saccharomyces spp.* Isolated from Colon of Native Chickens as a Probiotics Properties and has CMC-ase Activity

By

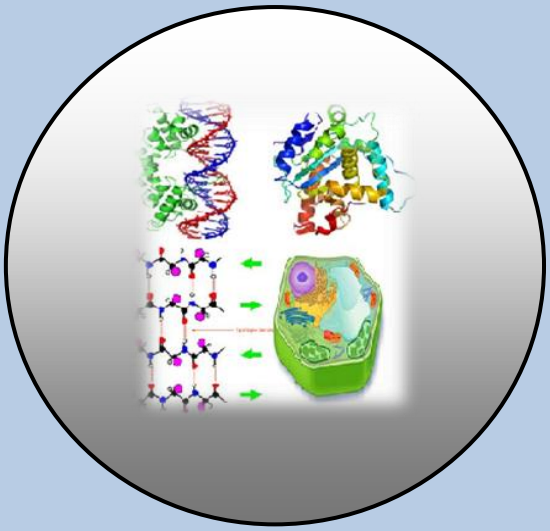
Bidura, I.G.N.G., D.P.M.A. Candrawati and

D. A. Warmadewi

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Global Impact factor of Journal: 0.756
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 2.93
Index Copernicus International Value
IC Value of Journal 6.01 Poland, Europe

J. Biol. Chem. Research
Volume 32 (2) 2015 Pages No. 683-699



Journal of Biological and Chemical Research

(An International Refereed/Peer Reviewed Journal of Life Sciences and Chemistry)

Indexed Abstracted and Cited in about 25 different Scientific Databases around the World

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 32, No. 2: 683-699, 2015
(An International Refereed/Peer Reviewed Journal of Life Sciences and Chemistry)
Ms 32/2/70/2015

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Bidura I.G.N.G
http:// www.sasjournals.com
http:// www.jbcr.in
jbiolchemres@gmail.com
info@jbcr.in

RESEARCH PAPER

Received: 29/07/2015 **Revised: 26/08/2015** **Accepted: 01/09/2015**

Selection of Khamir *Saccharomyces spp.* Isolated from Colon of Native Chickens as a Probiotics Properties and has CMC-ase Activity

Bidura, I.G.N.G., D.P.M.A. Candrawati and D. A. Warmadewi

Faculty of Animal Science, Udayana University, Jl. PB. Soedirman, Denpasar-Bali, Indonesia

ABSTRACT

The purpose of this research is to get isolates of Saccharomyces spp. as potentially as a probiotic agents and degrading of crude fiber (CMC-ase activity) were isolated from the colon of native chickens can be used in order to alleviate the negative effect of rice bran as poultry feed. The ability test of isolates has CMC-ase activity assay views of the clear zone surface area caused by isolates the user's media CMC (carboxymethyl celulose). The results of experiment showed that six isolates of Saccharomyces spp. (Gb5; Gb6; Gb7; Gb9, Gb10, dan Gb11) were isolated from colon of native chickens samples. The whole isolates of Saccharomyces sp. showed resistant grew on both in different temperature (10^0-45^0C), acid conditions (1.5-6.0), bile salts (0.20 to 0.60 NaDC) and has CMC-ase activity. All of isolates were potensial as probiotics sources and has a CMC-ase activity. But only two isolates of Saccharomyces sp. showed good potensial as probiotics sources and has CMC-ase activity (i.e. Saccharomyces sp.Gb7 and Gb9 isolates). The study showed that fermentation of rice bran used of Saccharomyces sp.Gb7 and Gb9 isolates culture could improve significant differences (P<0.05) on digestibility of its dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), and increased its metabolizable energy of rice bran. It was concluded that nutrient digestibility of rice bran might be improved by fermentation using both of Saccharomyces spp.Gb7 and Gb9 isolated from colon native chickens. The isolates (Saccharomyces spp.Gb7 and Gb9) can be used as a probiotic agency and crude fibre degradaded (has a CMC-ase activity) of feed.
Key words: Probiotics, CMC-ase, Fiber and Rice bran.

INTRODUCTION

Probiotics are food additives that contain living microbes that give beneficial effect for the host by improving the microbial balance in the digestive tract (Fuller, 1992). Alimyameen (2011) and Jin *et al.* (1997) defines probiotics as a single culture or a mixture of microbial life when consumed by humans or animals will be instrumental in maintaining the balance of microflora naturally present in the body of its host. Probiotics are often associated with gastrointestinal health, as it can help suppress the growth of harmful bacteria (Hegar, 2007). Probiotics are generally in the form of a group of microorganisms that nonpathogen positive effect on the digestive tract physiology and health of its host, if consumed regularly in sufficient quantities (Schrezenmeir and De Vrese, 2001). In the digestive tract, many probiotic were able to decipher the toxic compounds resulting from the metabolism of protein and fat, so that the concentration of toxic substances that can be reduced or even eliminated entirely. In other words, the health of the digestive tract will increase if the inside there are probiotics in sufficient quantities. Probiotics show functional effects, such as antidiarrheal effect, lowers blood cholesterol, improve intestinal motility and detoxification, inducing the immune system, resulting in a wide variety of metabolites (such as hydrogen peroxide, lactic acid and acetic acid) that is capable of maintaining pH balance and mikroekologi intestine, as well as helps the metabolism of vitamins, minerals and hormones. In addition, probiotics also act as an antitumor agent by preventing the formation of carcinogenic nitrosamines (Tjay and Kirana, 2007). Some of the requirements that must be met by a microorganism that can be developed into a probiotic agent was not pathogenic, tolerant to acid and bile salt tolerant (Hood and Zottola, 1998), because during its journey towards colonic probiotic must be able to pass through the stomach which has an acidic pH and deoksikolat acid which is a biological detergent for microorganisms. In addition, probiotic candidate should also have the ability to provide health effects, so it can be used for a prolonged period (Shortt, 1999). Research on probiotics have been carried out by selecting strains of microorganisms that have the potential to be developed into a local probiotics. Bacterial metabolism of the bile acids play an important role in the risk of colon cancer. It is assumed that the secondary bile acids (produced by microbial metabolism) can act as a promoter of the process of the formation of colon cancer. The process of dehydrogenation of steroids in generating core bond delta 1 and delta 4 that binds to the 3-keto group has an important role in relation to colon cancer. Certain strains of Clostridia in vitro are known to form this reaction (Wahyudi and Hendraningsih, 2007). *Saccharomyces cerevisiae* in the form of biomass has been widely used as a supplement in animal feed (Ahmad, 2005). According Kompiang (2002) and Wahyudi and Hendraningsih (2007), *Saccharomyces cerevisiae* supplementation in the diet significantly increased the growth rate, feed efficiency, and prevent the incidence of poisoning in birds caused by aflatoxin or aflatoxicosis. *Saccharomyces spp* is a genus of yeasts that has the ability to convert glucose into alcohol and CO_2. Saccharomyces yeast is a single-celled microorganisms lacking chlorophyll, including Eumycetes group and grow well at a temperature of 30^0c and a pH of 4.8. *Saccharomyces* is one form of single cell protein or referred to as single cell protein (Fuller, 1989). Rice bran is a by-product of rice milling industry, which is abundantly available (approximately 10% of paddy weight) during the harvesting season. Unfortunately this by-product contains anti-nutritive factors such as trypsin inhibitor, phytic acid as phytate, and high content of crude fiber.

These anti-nutritive factors have been reported by Kahlique *et al.* (2003) causing reduction of feed intake and depress performance of poultry. These toxic factors are phytic acid as phytate and crude fiber (CF). These anti-nutritive factors have been reported to reduce feed intake and depress performance of poultry. Bach Knudsen (2001) reported that CF has been defined as the complex macromoleculer substances in food plants that are not degraded by mammalian digestive enzymes. Based on the above description of research conducted to assess isolates of *Saccharomyces spp*. were isolated from the colon of native chickens as potentially as a probiotic agents and degrading of crude fiber (CMC-ase activity) can be used in order to alleviate the negative effect of rice bran as poultry feed.

METERIAL AND METHODS

Animals and experimental design

Eighteen of Lohmann Brown laying hens was assigned to three treatments in a completely randomized design. Each treatment has six replications with one bird per replication (individual cage). All of the birds were fed experimental diets for two days. The treatments were (i) unfermented rice bran as control; (ii) fermented rice bran by 0.30% *Saccharomyces spp.Gb7* culture; and (iii) fermented rice bran by 0.30% *Saccharomyces spp.Gb9* culture; respectively. The objectives of this study is to determine the nutrient digestibility and the metabolizable energy value of rice bran using Lohmann Brown laying hens up to thirty two weeks of age.

Source Isolates and Rice bran

Source isolates in this study were grown chicken colonic digesta obtained from chicken in nearby research. Rice bran obtained from domestic industry in the manufacturing at Ubung Kaja, Denpasar-Bali.

Media

The medium used is a PDA medium (Oxoid), a solution of Bacteriological peptone 0.1%, oxy tetracycline 50 ppm, distilled water, 3% H_2O_2 solution, bile salts at each concentration of 0%; 0.50%; 1.0 %; 5%; and 10%; 2% sodium thioglycollate, 0.3% oxygall; 0.10% (0.10%/100 mg) cholesterol. Chemicals used in this study are: bacterio logical solution peptone 0.1%, OMEA (oxytetracycline extrax malt *agar)*, distilled water, nutrient broth, NaOH, H2SO4, glucose, glycerol, bile salts, cholesterol and alcohol.

Media Testing

Weigh OMEA (oxytetracycline extrax malt *agar*) of 50 g, and then diluted with distilled water until its volume to 150 cc. Furthermore OMEA solution is heated in the stove, and then put into a water-bath with a temperature of 60-70^0 C to keep OMEA solution does not compact.

Grow the yeast *Saccharomyces spp* Isolated from Colon of Native Chicken

Take as much as 1 cc pipette bacterio logical solution peptone 10^{-3}, then pour in a petri dish with code A-3, then pipette as much as 1 cc solution of peptone logical bacterio 10^{-2} and pour into a petri dish with code A-2. And so on. After the solution OMEA temperature of 40^0-50^{0}C, then pour into each petri dish, then shake by hand too evenly and allowed to stand until the solution solidifies (Candrawati *et al*., 2013). A few minutes after the media solidified, then the media inserted into a desiccator under anaerobic conditions. Anaerobic conditions can be determined by candlelight in a desiccator. When the flame dies, then the anaerobic conditions in the desiccator has been reached.

The position of the petri dish in a desiccator is in an inverted position, so that steam will arise not interfere with the growth of isolates. Isolates allowed standing in a desiccator for 2 x 24 hours.

Isolation of yeast *Saccharomyces spp* isolates from Colon of Native Chicken

Isolate colonies in petri dishes have started to grow after grown for 2 x 24 hours. Isolates spherical shape is small. Before being transferred, first prepared 10 pieces of petri dishes that have previously been sterilized. Prepare OMEA solid selective media, then take one ose isolates and scratched on a petri dish that already contains solid media OMEA. After two days of isolates in a petri dish begins to grow, the next will be bred back into the test tube.

Prepare as much as 3.4g OMEA media diluted with distilled water to 100 cc. Furthermore OMEA solution is heated in the stove, and then input into a water bath with a temperature of 60-70^0 C for approximately 15 minutes and pour into a test tube and sealed with cotton. Put to the test tube 10 into autoclaving for sterilization. After that, input in laminar flow (UV light) for approximately for 15 minutes. Tilt the test tube, let the media solidified. With the scratch method, isolates the petri dish was transferred to a test tube (Ahmad, 2005). Close the test tube already containing isolates with a cotton swab and let the 2 x 24 hours, incubated in an incubator in an inverted position at a temperature of 30^{0}C for 48 hours, and observed colonies that grow.

Colonies that have the characteristics of yeasts were isolated by following method reported Ahmad (2005). Purified, and cultured on solid media for the purposes of further analysis, and stored prior to characterization, test resistance to low pH, various levels of temperature, deoxicholic acid, and test transpormasi cholic acid into deoxicholic acid (Hyronimus *et al*., 2000; Prangdimurti, 2001)

Isolate yeast *Saccharomyces spp* morphology of Colon Native Chicken

Weigh nutrient brot 1.8 g, and then diluted with distilled water to 200 ml. Then put into to 20 a test tube, close the test tube with a cotton swab, then ditrerilisasi in autoclaving. Once cool, isolates available in the media slant transferred respectively by 1 ose into a test tube which already contains a nutrient brot, and grown for 18 hours. After 18 hours in a nutrient brot, isolates taken by ose needle and dab into a glass preparations. Observe in the microscope with magnification 10 times. If the results of the observations still found microbial contamination other than yeast, then the separation of the colonies as much as two times, to obtain a high purity level.

Growing Ability test yeast *Saccharomyces spp* at Various Temperatures

Test the growth of yeast *Saccharomyces spp* performed at 10^{0}C temperature variations, 37^{0}C, and 45^{0}C with the following procedure: weigh 1 g of nutrient brot dissolved in distilled water to 100 cc. Pour nutrient brot in a test tube that has been coded isolates were elected, namely *Saccharomyces spp.Gb7* and *Gb9*. All test tubes sterilized before hand in autoklav. Once cool, bred isolates into nutrient brot and save it for 24 hours. Weigh as much as 3 g of nutrient broth and dissolved in 300 cc of distilled water used. Sterilize in autoklav and input into 30 pieces of test tubes containing nutrient broth was then used to breed isolates that had been stored for 18 hours. Take 1 cc in each tube and culture. Save tenth isolates at room temperature (37^{0}C), 10 isolates in the temperature of 10^{0}C (in a refrigerator), and 10 isolates at a temperature of 45^{0}C (in an incubator). Storage is done for 1 x 24 hours. After 24 hours in view of turbidity levels arising. If there is turbidity, meaning no growth of yeast *Saccharomyces spp* temperature tested

Growing Ability *test Saccharomyces spp* at various pH

Test the ability of yeast *Saccharomyces spp* isolates grown at various pH using the method (Hyronimus *et al*., 2000). A total of 1 ose pure isolates of *Saccharomyces spp* cultured in a nutrient broth solution and stored for 24 hours. After 24 hours take 1 cc of pure isolates are then cultured in a nutrient broth solution that has been conditioned at pH 1.5; 3.0; 4.5; and 6.0. If the acidic conditions have not been reached, then add H_2SO_4 solution. Incubation was performed for 24 hours at a temperature of 37^0C. There are 10 tubes each isolates in different pH levels. Measurement of pH by using a pH meter.

Test isolates the number of colonies that live after cultured at pH 1.5; 3.0; 4.5; and 6.0 is done with the work procedures as follows: Take as much as 1 cc isolates were grown at various pH them and put on a petri dish that has been previously sterilized. Add a solution OMEA and shake petri dish so well blended and coded. Bred for 3 days then calculated the number of colonies (Colony Form Unit).

Ability Test *Saccharomyces spp* in Bile Salts

A total of 1 ose pure isolates of *Saccharomyces spp* cultured in a nutrient broth solution and stored for 24 hours. After 24 hours, take as much as 1 cc of pure isolates, and then cultured in a bile salt solution for 24 hours. The concentrations of bile salts used were: 0.2 mM; 0.4 mM; and 0.6 mM. Each level of bile salts was made in 10 tubes. Isolates survival in bile salt levels were measured with a spectrophotometer with a wavelength of 660 nm (Hyronimus *et al*., 2000; Prangdimurti, 2001).

Ability Test *Saccharomyces spp* in CMC-ase.

OMEA weigh as much as 11 g and 3 g of CMC-ase, and dissolved in distilled water. Heat in a waterbath and then do strelilisasi on autoklav. Refrigerate at a temperature of 45-50^0C, then poured in a petri dish and left to solidify. Take a paper disk with tweezers and dipped in a solution containing nutrient broth which had been cultured isolates for 24 hours, and stick it on a petri dish containing OMEA media and CMC-ase. Let stand for 24 hours. After 24 hours the clear zone width measurements caused by using a caliper (Kanti, 2007).

Transformation test Cholic Acid (CA) to Deoxicholic Acid (DCA) By *Saccharomyces spp* Isolated from Colon of Native Chickens

This test is performed to determine whether Cholic Acid (CA) is transformed into Deoxicholic Acid (DCA) by isolates obtained in this study. Khamir pure cultures isolated from colon of native chicken stock made suspension of glycerol by way of suspending 50 mL culture into 5 ml of PDB and incubated at a temperature of 37^0C for 24 hours. Then 50 mL cultures were grown in PDB suspended in 5 ml of PDB plus Ca, and incubated at a temperature of 37^0C for 24 hours. Subsequently 1 ml of culture in Ca pipetted into Eppendorf then centrifuged for 5 minutes at a speed of 5000 rpm.

The number of 0.1ml of the supernatant was then pipetted, added with 500 mL of ethyl acetate and 20 mL of HCL, centrifuged for 5 minutes at a speed of 5000 rpm, the supernatant pipette and evaporated, the supernatant added with 500 mL ethyl acetate, centrifuged for 5 minutes at a speed of 5.000 rpm, the supernatant pipette and evaporated for 48 hours at room temperature, added 15 mL of methanol. Cyclohexane 10 mL, 15 mL and 4 mL Ethyl Acetate Acetic Acid is mixed in the Chamber, and allowed to stand for 30 minutes prior to TLC on silica gel alluminium. After 30 minutes, DCA, Ca, and each isolate was spotted in 1 mL on aluminum silica gel, dried with a hair drayer, which has been placed in the chamber containing the eluent solution,

allowed to stand until silica absorbs the solution, dried, sprayed with acid dyes phosporic molibdho, dried, and roasted until the spot of each isolate was visible on silica gel. If isolates transformation Cholic acid (CA) to dioxicholic acid (DCA), it will isolate the same spot by spot Dioxicholic acid (DCA), otherwise if it isolates did not transformation deoxicholic acid isolates the spot will be the same as the spot cholic acid (Ariwati, 2012).

Fermented of Rice Bran

Saccharomyces spp.Gb7 and *Gb9 isolate* in this study was isolated from colon of native chickens. Both isolate of *Saccharomyces spp.Gb7* and *Gb9* which has been approved from bile salt and poultry digestive tract *in vitro* test as potentially as a probiotic agents and degrading of crude fiber (CMC-ase activity). The study was carried out at the Bioscience Laboratory of Udayana University, Bali, Indonesia. Fermentation of commercial rice bran was prepared as follows. Comercial rice bran was used and approximately 0.30% (3.5×10^7 spores) *Saccharomyces spp.Gb7* and *Gb9 isolate* culture was added to 100 g of steamed rice bran. Then, water was added to bring the product to 50% content and left up to 2 days for fermentation. After that, fermented rice bran was dried at 45^0C for six hours and then it was ground for analysis. Unfermented rice bran was also ground for its chemical analysis.

Retention and excretion of nutrients

In order to determine the nutrient digestibility and metabolizable energy (ME) value of the rice bran. The amount of rice bran used was 100 g. This amount as based on preliminary assays with Lohman brown laying hens consumption of ration. 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). 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 ground 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.

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

Analysis of data from yeast *Saccharomyces spp* isolates which includes test temperatures, pH, bile salts, and CMC-ase done with descriptive method. To determine whether a culture can be grown on a wide range of temperature criterion is viewed turbidity. To determine whether culture can grow on a variety of specific pH with a pH meter (Sujaya *et al*., 2008). The data obtained in the analysis of variance and if there are significant differences ($P<0.05$) among treatments, then continued with Duncan's multiple range test (Steel and Torrie, l989).

RESULTS

Isolation of *Saccharomyces spp* from Colon Native Chicken

In this study, a total of 13 isolates *Saccharomyces spp.* successfully isolated using PDA medium. Isolation is aimed at having the possibility of yeast that can be developed into a new probiotic candidate to improve the productivity of poultry. The observation in the laboratorium, it turns out all isolates have an oval shape and are not motile (not able to move actively). All yeast isolates colon chicken has the ability to produce the enzyme catalase which is characterized by the formation of gas bubbles after the colony of yeast is spilled with a few drops of a solution of H_2O_2. In the glucose fermentation test, all isolates have the ability to ferment sugars to produce gas in the medium were added glucose. The characteristics shown by all the yeast *Saccharomyces spp* isolated from colon of native chicken in accordance with those reported by Pratidina *et al*. (2008).

The gas formed in the fermentation of glucose by the cell test of khamir is CO_2 (Anon., 2012), and this gas is a by product of the fermentation of sugar by yeast to form ethanol (C_2H_5OH). These CO_2 gas production capabilities to create a group of yeast, especially *Saccharomyces spp* agent widely used as a developer in the process of making bread dough. Most of the yeast used in bread-making process is the same species, with that used in the process of fermentation in alcoholic beverages (Anon., 2011).

Table 1. Test yeast *Saccharomyces spp* isolated from colon of native chicken against temperature and acid.

code isolates	Mo-ti-lation	Tests on various temperature levels			Test various levels of acid (pH)				The number of colony after a pH test (cfu)			
		10^0C	37^0C	45^0C	pH 1.5	pH 3.0	pH 4.5	pH 6.0	pH 1.5	pH 3.0	pH 4.5	pH 4.5
Gb1	+	+	+	-	+	+	+	+	92	-	13	-
Gb2	+	+	+	-	+	+	-	-	-	-	-	-
Gb3	+	+	+	+	+	+	-	+	204	-	16	5
Gb4	+	+	+	+	+	+	+	-	42	67	-	-
Gb5	+	+	+	+	+	+	+	+	98	206	67	28
Gb6	+	+	+	+	+	+	+	+	75	139	21	19
Gb7	+	+	+	+	+	+	+	+	87	175	42	12
Gb8	+	+	+	+	+	+	+	-	62	16	-	-
Gb9	+	+	+	+	+	+	+	+	152	170	75	35
Gb10	+	+	+	+	+	+	+	+	92	108	29	19
Gb11	+	+	+	+	+	+	+	+	88	97	34	7
Gb12	-	+	+	+	+	+	+	+	59	-	9	-
Gb13	-	+	+	+	-	+	+	-	-	7	-	-

Description: GB1 to Gb13 is the yeast Saccharomyces spp isolated from the colon of native chicken

Yeast *Saccharomyces spp* isolated from colon of native chicken by using the medium OMEA (*oxytetracycline extrax malt agar*) managed to get the 13 isolates. Results of morphological observation, it turns yeast isolates colon chicken (*Saccharomyces spp*) has a white oval shape.

In this research, the separation of the colonies twice, in order to obtain higher purity. Isolat yeast *Saccharomyces spp* derived from colon of native chicken do not all have the ability as a probiotic ingredient, so it is necessary to test for temperature, pH, and bile salts. In addition, there CMC-ase test is to test the ability of the isolates in digesting crude fiber.

Ability test isolates grown at various temperature levels

Test the ability of yeast *Saccharomyces spp* colon chicken isolates grown at various temperatures is presented in Table 1. In the temperature test, thirty-nine isolates of yeast *Saccharomyces spp* that has been cultured in a liquid nutrient broth each of the 13 isolates were stored at a temperature of 10^0C; 13 isolates were stored at a temperature of 37^0C, and 13 isolates were stored at a temperature of 45^0C for approximately 24 hours.

Colonies began to be seen on the third day after incubation. Test tube that looks turbid yeast *Saccharomyces spp* isolates showed survival at that temperature (positive). Conversely, if looks clear, showing yeast *Saccharomyces spp* isolates is not survival at that temperature.

Ability test isolates grown at various pH levels

Table 1 shows the test results for the thirteen isolates of yeast *Saccharomyces spp* isolated from colon of native chicken against a wide range of pH levels, namely pH 1.5; 3.0; 4.5; and 6.0. PH variation used is a variation of pH conditions which exist in the digestive tract of poultry, which ranges from pH 1.5 to 6.0. The test results showed that not all isolates of yeast *Saccharomyces spp* isolated from colon of native chicken can live to various pH conditions tested. In Table 1 looks isolate yeast *Saccharomyces spp.Gb2* not stand living at various pH conditions tested. Similarly, the yeast *Saccharomyces spp.Gb13* isolates not live in acidic pH conditions (pH 1.5). From Table 1, it appeared that isolates of yeast *Saccharomyces spp*. who qualified to the level of pH test is as much as six isolates, the yeast *Saccharomyces* spp.Gb5; Gb6; Gb7; Gb9, Gb10; and Gb11. There is a tendency the higher the pH, especially at pH 6.0, most of the isolates decreased the number of colony life. *Saccharomyces spp* colonies grow well at pH 1.5 to 3.0. Furthermore, all of the isolates were followed by a test of its ability to grow in bile salts. Ritonga (2008) reported that the very rapid growth of the yeast on environmental pH range between 4 - 4.5. Good growth in the medium pH 3 by 6 isolates indicated in Table 1 indicates that this yeast isolates have the potential to be developed into a candidate probiotic, because it was able to pass through the digestive tract pH poultry. The ability of yeast to survive on very low pH environments (pH 1.5) closely related to the microbe's ability to maintain internal pH so that is always higher than the pH of the surrounding environment (Hutkins and Nannen, 1993). Mechanisms of yeast isolates obtained in this study (Table 1) to maintain the internal pH is always higher than the external pH, is closely associated with the activity of the enzyme ATP-ase that functions to translocate protons from the cell out of the cell (Chou and Weimer, 1999). In this process, the enzyme ATP-ase will use the energy generated from the hydrolysis of ATP (cellular energy possessed cells), so that this enzyme will be able to move protons (H^+ ions) out of his cell. With this ability, allowing the yeast cells can live in an environment which is very low pH (Ariwati, 2012). Microbes are not able to perform the above mechanism will generally experience a disruption in the growth process which is generally caused by excessive acidification in the cell wall. Exposure of the cell wall in the acidic environment in excess will damage the plasma membranes that are just below the walls of the cell,

so that it will cause the release of important components of cells, such as Mg, K, and fat from cells. In severe circumstances, the cells will die (Bender and Marquis, 1987). At very low pH environment, acid resistant microbes (including yeast) will experience some damage to the cell membrane, so that the microbial growth rate will decline. In fact, when exposed in quite a long time, many of which can be experienced death in the low pH environment (Siegumfeldt *et al*., 2000).

Growing Ability test isolates *Saccharomyces spp* Isolated from Colon of Native Chicken on Bile Salts

Candidate probiotics (six isolates elected) who have passed the test temperature and various pH levels, then tested again viability at the various levels of bile salts. This is important, because one of the requirements that must be met by potential probiotic before it developed into a potential probiotic candidates are testing the ability of the probiotic isolate live in an environment containing acid/bile salts. On his way to the colon, probiotics must be able to pass some obstacle or an extreme environment along the digestive tract of poultry. Probiotics will be dealing with the environment in the small intestine, which included bile salts are removed by the liver through the gall bladder, after successfully passing the acidic conditions in the stomach. Therefore, in the process of development of new probiotic, a new probiotic candidate should be able to pass the test of resistance to bile or bile salts were performed in vitro. In this test, the candidate probiotic incubated in GYP medium supplemented with NaDC at various concentrations (0.2; 0.4; and 0.6 mM). The concentrations mentioned above in a row is a NaDC concentration in normal individuals, candidates for cancer patients, and cancer patients (Dawson, 1998).

Table 2. Ability of yeast Saccharomyces spp isolates were resistant living in bile salts.

Code Isolate	Growth indication of *Saccharomyces spp* (Absorbance)			
	Control	NaDC 0.2 mM	NaDC 0.4 mM	NaDC 0.6 mM
Gb5	++ (0.992)	++ (0.892)	+++ (1.093)	++(0.525)
Gb6	++ (0.735)	++ (0.704)	++ (0.782)	+ (0.403)
Gb7	++ (0.906)	++ (0.908)	++ (0.904)	+(0.478)
Gb9	++ (0.972)	++ (0.891)	+++ (1.175)	++(0.521)
Gb10	++ (0.895)	++ (0.793)	++ (0.961)	+(0.496)
Gb11	++ (0.703)	++ (0.807)	++ (0.877)	++(0.517)

Description **-** *: A<0.1 (can not resistant to NaDC)*
+ *: A 0.1-0.5 (slightly resistant to NaDC)*
++ *: A 0.5-1.0 (resistant to NaDC)*
+++ *: A>1.0 (highly resistant to NaDC)*

Table 2 presents the growth response of each test isolates were characterized by culture absorbance value at a wavelength (λ) to NaDC. Six isolates of yeast *Saccharomyces spp*. isolated from colon of native chicken were grown for 24 hours at GYP media (*Glucose Yeast Pepton*) is added that the concentration NaDC arranged so that its concentration reaches 0.2; 0.4; and 0.6 mM. The test results showed that all six test yeasts showed good growth (with a value of absorbance between 0.5-1.0) in medium containing NaDC to a concentration of 0.4 mM.

At a concentration of 0.6 mM NaDC, visible growth of yeast *Saccharomyces spp* isolated from colon of native chicken slightly inhibited (Table 2) which is characterized by a decline in suspension culture uptake value below 0.5 OD units. Tolerance to six good yeast isolates to bile salts allegedly closely associated with the role of enzymes capable of degrading bile salts. The role of bile salts degrading enzymes, such as bile salt hydrolase on lactic acid bacteria have been reported by Smet *et al.* (1995). This enzyme is able to modify the physical and chemical capabilities possessed by bile salts, so it is not toxic to the lactic acid bacteria. Based on the nature of resistance shown by all of the isolates test indicated that these strains have the potential to be developed as a candidate probiotic. In the event of the death of isolates in environments containing NaDC (bile salt) in certain concentrations, generally caused by the failure of these isolates in maintaining their membrane permeability after exposure in a long time by bile salts. Reported by Farida (2006), that the death of the cells in an environment exposed to NaDC caused by increased activity of the enzyme β-galactosidase to bile salts, because of the high activity of this enzyme would restrict the cell to control metabolism.

CMC-ase Activity Test

CMC-ase test is to test the ability of *Saccharomyces spp* isolated from colon of native chicken to degrade raw fiber. This can be measured by the diameter of the clear zone produced (Table 3). The results show turned out to six isolates were tested have the ability to degrade raw fiber. This is shown by the clear zone around the isolates. More specifically, the difference in diameter of clear zones produced by these isolates presented in Figure 1.

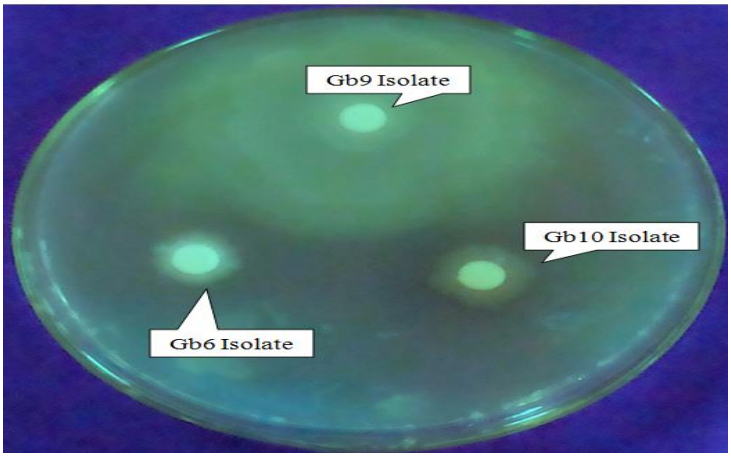


Figure 1. Isolates of the yeast *Saccharomyces spp.Gb6* and *Gb10* has clear zone is smaller than the *Saccharomyces spp.Gb9* isolates.

Of the six isolates were tested indicate isolates of *Saccharomyces spp.Gb.9* has the most wide clear zone, while the yeast *Saccharomyces spp.Gb.6* have a clear zone at least. This means that *Saccharomyces* spp.Gb.9 isolates have the ability to digest crude fiber high compared to most isolates of *Saccharomyces spp*. other.

Test Capability Isolates *Saccharomyces spp* isolated from Colon of native chickens to Improve digestibility of Rice Bran

The isolates of *Saccharomyces spp* isolated from colon of native chicken were elected, namely isolate *Saccharomyces spp.Gb7* and *Gb9* has proven to have the potential as probiotics and CMC-ase activity is highest, then used as an inoculant fermentation of rice bran.

The test results of yeast *Saccharomyces spp* isolates elected (*Saccharomyces spp.Gb7* and *Gb9*) on digestibility and metabolizable energy of rice bran presented in Table 4.

Dry matter digestibility from rice bran is fermented by inoculant *Saccharomyces spp* Gb7 and Gb9 isolated from colon of native chicken were increased significantly (P<0.05) different higher respectively: 8.99% and 9.02% than rice bran without fermented. Organic matter digestibility of fermented rice bran were increased significantly (P<0.05) different higher respectively: 7.94% and 9.07% than rice bran without fermented.

Table 3. CMC-ase activity test isolates of *Saccharomyces spp* isolated from colon of native chickens based on the diameter of clear zone posed.

Code Isolate	Clear zone diameter (cm)
Gb5	1.97
Gb6	0.75
Gb7	3.19
Gb9	4.69
Gb10	0.82
Gb11	2.37

Description: Gb5 to Gb11 is *Saccharomyces spp* isolated from colon of native chickens

Digestibility of crude protein fermented rice bran by using inoculant *Saccharomyces spp*Gb7 and Gb9 isolated from colon of native chicken showed significant differences (P<0.05) respectively: 24.88% and 23.70% higher than the not fermented (Table 4). Fermentation of rice bran were significantly different (P<0.05) increase the digestibility of crude fiber respectively: 21.60% and 20.54% higher than the rice bran without fermented.

Table 4. Nutrient digestibility of unfermented and fermented rice bran by *Saccharomyces Spp.Gb7* and *Gb9 isolated from colon of native chickens* (in % Dry Matter).

Variable	Rice bran			SEM[1)]
	Unfermented/ Control	Fermented by *Sc.Gb7isolate*	Fermented by *Sc.Gb9 isolate*	
Dry matter digestibility (%)	30.81b	33.58a	33.59a	0.801
Organic Matter digestibility (%)	31.74b	34.26a	34.62a	0.703
Crude Protein digestibility (%)	40.72b	50.85a	50.37a	2.096
Crude fibre digestibility (%)	20.79b	25.28a	25.06a	1.179
Metabolizable energy (kcal/kg)	1703.61b	1973.92a	1965.82a	57.905

Note:

1. *Standart Error of the treatments means*
2. Means with different superscripts within raws are significantly different (P<0.05)

Rice bran fermentation using inoculant of *Saccharomyces* spp.Gb7 and Gb9 significantly different (P<0.05) was increases metabolizable energy content of rice bran respectively: 15.87% and 15.39% higher than metabolizable energy content of rice bran unfermentation (Table 4).

DISCUSSION

Results of laboratory studies indicate that of some samples isolated from the colon chickens have been isolated 13 species of *Saccharomyces sp* isolates. On the third day of incubation at a temperature of 39^0C in liquid media roll tube, the colony began to become evident. Clear zone around the colony as a characteristic of the yeast *Saccharomyces spp*. does not seem real even though indistinguishable from other types of colonies. Colonies began to appear clearly on the seventh day of incubation. Colonies are round with a diameter of between one to two millimeter, brown beige or white, and opaque. Isolates of *Saccharomyces spp*. is that form colonies with clear zones, rod-shaped cells, catalase test negative, Gram-positive and gram staining. Morphological observations showed, it turns yeast *Saccharomyces spp* isolated from colon of native chicken has a white oval shape. These results are the same as by Bidura *et al*. (2012) were isolated *Saccharomyces spp* from "tape" and Candrawati *et al*. (2014) were isolated from the feces of Bali cattle.

The test results for the thirteen isolates of yeast *Saccharomyces spp* isolated from colon of native chicken at various temperatures, showed mixed results. The yeast of *Saccharomyces spp*.Gb1 and Gb2 isolates survival at a temperature of 10^0C and 37^0C, but not survival at a temperature of 45^0C. But, isolates of *Saccharomyces spp*.Gb12 and Gb13 not stand life at a temperature of 10^0C. Some of the advantages of *Saccharomyces* in the fermentation process, the microorganism is rapidly proliferating, resistant to high temperatures, has held steady and rapid nature of adaptation. According to Ahmad (2005), optimum environment for the growth of yeast is 25-30^0C and the maximum temperature is 35-47^0C.

In general, probiotic microbes can live in the digestive tract of poultry and mutualism with its host body at a pH of between 2 to 4. The probiotic microbes do not result in a negative thing in the body of the host, not the pathogen, and generally do not form spores, *saccharolytic*, anaerobic, not disturbing the ecosystem of the body, as well as live and grow in the digestive tract of poultry (Fuller, 1989).

The results showed that only six isolates of *Saccharomyces spp* isolated from colon of native chickens potential as a probiotic agent and has a CMC-ase activity. The six isolates, is isolates of *Saccharomyces spp.*Gb5; Gb6; Gb7; Gb9; Gb10; and Gb11, showed resistance to acid and bile salts that are characteristic of probiotic bacteria. *Saccharomyces* is a single-celled microorganism and do not have chlorophyll, grows well at 30°C and pH 4.8. According to Rezanezhad *et al*. (2003), the value of pH in the gastrointestinal tract of poultry at any digestive organs, respectively, are: crop (pH 4.5), proventriculus (pH 4.4), gizzard (pH 2.6), duodenum (pH 5.7-6.0), jejunum (pH 5.8), illeum (pH 6.3), colon (pH 6.3), cecum (pH 5.7), and biliary (H 5.9).

Resilience of microbial isolates to bile salts used to assess the ability to survive in the digestive tract isolates contained bile salts on the top surface of the intestine. Probiotics will be dealing with the environment in the small intestine, in which there are bile or bile salts are removed by the liver through the gall bladder, after successfully passing the acidic conditions in the stomach (Fuller, 1989). Therefore, in the process of development of new probiotic, or a new probiotic candidate should be able to pass the test of resistance to bile or bile salts were performed in vitro. Based on the nature of the resistance shown by some isolates, indicates that these strains have the potential to be developed into a candidate probiotics.

Test CMC-ase enzyme activity (endo-1, 4-β-glukonase) is characterized by the presence or absence of clear zone around the colony isolates were tested. The wider the clear zone shown by the isolates tested, meaning the yeast isolates have strong extracellular cellulase enzyme activity. The size of the clear zone and the apparent absence of a clear zone, an indicator of the ability of these microbes to break down cellulose, as well as fast and slow arise the clear zone. Cellulolytic yeasts capable of producing the enzyme endo-1, 4 β glukonase, exo-glukonase 1. 4 β, and beta-glucosidase that can degrade components of raw fiber into soluble carbohydrates (Hatakka, 2000)

The fermentation process is expected to alter the chemical structure of the cell wall tissue, breaking bonds of lignocellulose, and decreased levels of lignin. Lignolitik yeasts those are able to degrade lignin through the establishment of a set of mycelia and then multiply asexually through spores (Erika, 1998). Fermentation of rice bran by *Saccharomyces spp.Gb7* and *Gb9* may increase microbial biomass, so the crude protein content of rice bran increased (Bidura *et al*., 2012). It was also reported that the success of the fermentation process is influenced by the type and number of microbes are used, the type of substrate, pH, and temperature during fermentation. Biomass is a form of mass of the results of the biological processes of microorganisms. Microorganisms capable of converting the material into microbial protein better known as single cell protein. The fermentation process has the objective to produce a product (material feed) that have nutritional content, texture, and better biological value, as well as lowering antinutrisi substances.

Jaelani et al. (2008) reported that fermentation of feedstuffs with *Trichoderma reesei* can increase the energy content, total soluble sugars, and crude protein content. Increase the energy content of fermented rice bran was due to the formation of sugars derived from the breakdown of crude fiber. Chen et al. (2005) reported that the addition of complex 0.20% probiotic (*L. acidophilus* and *S. cerivisae*) in the diet can increase the digestibility of dry matter feed. Rice bran fermented by yeasts will be able to soften and break down the cell walls of rice bran and yeast capable of releasing microfibrils, so that the structure of the cell wall of rice bran become brittle and more open. The yeast work gradually in breaking the cell wall components. Through fibrils and hyphae yeast secrete extracellular peroxidase enzymes. The extracellular peroxidase enzymes actively working on lignolisis activity, thus breaking the bond lignocellulose and lignin fraction breaks down into CO_2. Fermentation using microbes can improve the digestibility of feed nutrients (Arsyad *et al*., 2001; Bidura and Suastina, 2002). Hong et al. (2004) reported that feeding fermented using *Aspergillus oryzae* significantly increased dry matter digestibility and crude protein. According to Jaelani et *al.* (2008), an increase in metabolizable energy content of palm kernel cake as a result of fermentation by the fungus *T.reesei*. This is due to the degradation of mannan polysaccharides exist in palm kernel cake by the fungus *T. reesei* into a simpler form (monosaccharides) which generates enough energy value better than in the form of polysaccharides mannan. The same thing was reported also by Sabini *et al.* (2000), that the fungus *T.reesei* capable of degrading polysaccharides mannan to mannotriosa, mannobiosa, and monnosa. According to Jaelani *et al.* (2008), fermented palm kernel cake can markedly increase the crude protein content of palm kernel cake compared to without fermentation. Utama (2011), reported that the administration of *S. cerevisiae* in the diet can increase the digestibility of protein and crude fiber components, such as cellulose and hemicellulose, as has been overhauled in the form of simple monosaccharide.

Reported by Bidura *et al.* (2014), that the pollard fermentation by *Saccharomyces spp* isolated from colon of Bali cattle can markedly increase the digestibility of dry matter, organic matter, protein and crude fiber of pollard, and can significantly increase the metabolizable energy of pollard. The same thing was reported by Candrawati *et al.* (2014), that the use of *Saccharomyces spp* isolated from feces of Bali cattle in the process of fermentation of rice bran, can markedly increase the digestibility of dry matter, organic matter, and crude fiber of rice bran, and can significantly increase the metabolizable energy content of rice bran.

CONCLUSION

It can be concluded that successfully discovered six isolates of *Saccharomyces spp.* (ie: Gb5; Gb6; Gb7; Gb9; Gb10; and Gb11) isolated from colon of native chickens are potential as probiotics, but only two isolates that have the highest activity of CMC-ase, is *Saccharomyces spp.Gb7* and Gb9 isolates. The use of *Saccharomyces* isolates *spp.Gb7* and Gb9 as inoculant fermentation of rice bran, can significantly improve the digestibility of dry matter, organic matter, crude protein, crude fiber and metabolizable energy content of rice bran.

ACKNOWLEDGEMENTS

The authors wish to thank you very much to the Directorate of Research and Community Services, Directorate General of Higher Education, Ministry of Education and Culture, In accordance with the Agreement on Implementation of Research Number: 52/UN14.2/PNL.01.03.00 / 2015, dated March 3, 2015, on funds provided so that research can be carried out.

REFERENCES

Ahmad, R. Z 2005. Pemanfaatan Khamir *Saccharomyces cerevisiae* untuk Ternak. *Wartazoa,* Vol. 15 (1): 49-55

Alimyameen 2011. Penggunaan Probiotik dan Prebiotik Pada Ternak. Available from: http://alimyameen. Blogspot. Com/2011/04/penggunaan-probiotik-danprebiotik-pada-13.html. cited: 25/02/2012.

Anonymous 2011. Apa itu khamir (cited 28 Mei 2012) Available from: www.sehatcommunitycom/2011/05/apa-itukhamir.html#4xzz1x5vxhRMB.

Anonymous 2012. *Saccharomyces* Bersifat Fermentatif Melakukan Fermentasi, yaitu Memecah Glukosa menjadi Karbondioksida dan Alkohol kuat. (cited 20 mei 2012) Available from: wikipedia.org/wiki/*Saccharomyces-cerevisiae.*

Ariwati, N. L.P. 2012. Isolation and Identification of Yeast *Saccharomyces spp* as Agencia Probiotics and Prevention of Colon Cancer. Thesis, Department of Animal Husbandry, Graduate Program, University of Udayana, Denpasar

Arsyad, M., H. Syam, dan R. Islamiyati. 2001. Kandungan Kalsium dan Fosfor Buah Kakao yang Difermentasi dengan EM-4 pada Berbagai Lama Penyimpanan. Buletin Nutrisi dan Makanan Ternak, *Fapet Unhas* 2 (1): 1-10.

Association of Official Analytical Chemists. 1994. Official Methods of Analysis. 15thEdition. Associoation of Analytical Chemists, Arlington, Virginia pp. 1230

Bach Knudsen, K. E. 2001. The nutritional significance of "dietary fiber" analysis. *Anim. Feed Sci. Tech*. 90: 3-20

Bender, G.R., dan Marquis, RE. 1987. Membran ATPase and Acid Tolerance of Actinomyces Viscosus and *Lactobacillus casei*. Appl and Environ Microbiol. 53(9): 2124-2128.

Bidura, I.G.N.G. 2012. Isolasi, identifikasi dan uji kemampuan khamir Saccharomyces cerevisiae yang diisolasi dari ragi tape sebagai agensia probiotik dan peningkatan produktivitas itik Bali. Disertasi, Program Studi Doktor Ilmu Ternak, Program Pascasarjana, Universitas Udayana, Denpasar.

Bidura, I.G.N.G. dan I.G.P.B. Suastina. 2002. Pengaruh Suplementasi Ragi Tape Dalam Ransum terhadap Efisiensi Penggunaan Ransum. *Majalah Ilmiah Peternakan* 5 (1): 06-11.

Bidura, I.G.N.G., D.P.M.A. Candrawati and I.B.G. Partama. 2014. Selection of *Saccharomyces spp* isolates (isolation from colon beef of Bali cattle) as probiotics agent and colon cancer prevention and its effect on pollard quality as feed. *Journal of Biological and Chemical Research*. July to December Vol. 31 (2): 1043-1047.

Bidura, I.G.N.G., I.P. Suyadnya, I.G. Mahardika, I.B. Gaga Partama, I.G.L. and Oka dan IG.A.I. Aryani. 2012. The implementation of *Saccharomyces spp.n-2* isolate culture (isolation from traditional yeast culture) for improving feed quality and performance of male Bali duckling. *Agricultural Science Research Journal*. ISSN-L: 2026-6073. September: Vol. 2 (9): 486-492.

Candrawati, D.P.M.A., D.A. Warmadewi and I.G.N.G. Bidura, 2013. Isolasi dan Uji Kemampuan Khamir *Saccharomyces Cerevisiae* Dari Feses Sapi Sebagai Agensia Probiotik Dan Inokulan Pendegradasi Pakan Serat. Laporan Penelitian Hibah Bersaing, Lembaga Penelitian dan Pengabdian Kepada Masyarakat, Universitas Udayana, Denpasar.

Candrawati, D.P.M.A., D.A. Warmadewi and I.GN.G. Bidura, 2014. Isolation of *Saccharomyces spp* from Manure of Beef Bali cattle as a probiotics properties and has CMC-ase activity to improve nutrient quality of rice bran. *Journal of Biological and Chemical Research*. January-June, Vol. 31 (1): 39-52

Chen, Y. H., H. K. Hsu, and J. C. Hsu. 2002. Studies on the fine structure of caeca in domestic geese. AJAS 15 (7): 1018-1021

Chou, L.S., and B. Weimer, 1999. Isolation and Characterization of Acid and Bile Tolerant Isolates from Strains of *Lactobacillus acidophilus*. *Journal Dairy Sci*. 62: 23-31.

Dawson, P.A. 1998. Bile Secretion and the Enterohepatic Circulation of Bile Acids. P., 1052-1063.

Erika, B. L. 1998. "Peningkatan Mutu Pod Kakao Melalui Amoniasi dengan Urea dan Biofermentasi dengan Kapang (*Phanerochaete chrysosporus*) serta Penjabarannya ke dalam Formulasi Ransum Ruminansia". (*Disertasi*). Bogor: Program Pascasarjana, Institut Pertanian Bogor.

Farida, E. 2006. Seleksi Pengujian Bakteri Asam Laktat Kandidat Probiotik Hasil Isolat Lokal serta Kemampuannya dalam menghambat Sekresi Interleukin-8 Dari Alur Sel HCT 116. (Tesis) Pascasarjana. Institut Pertanian Bogor.

Fuller, R. 1992. *Probiotics*; the scientific basis, First edition, Chapman & Hall, London, p; 209–221.

Fuller, R. 1989. History and Development of Probiotics, in: Probiotics the Scientific Basis. Ed.Fuller, R. First Ed. Fuller, R. First Ed London: Chapman and Hall.

Hatakka, A. 2000. Biodegration of Lignin. University of Helsinki, Viikki Biocenter, Department of Applied Chemistry dan Microbiology. Helsinki.

Hegar, B. 2007. Digestive microflora on Children's Health. Journal of Health and Pharmacy. Jakarta: Dexa Media.

Hong, K. J., C. H. Lee, and S. W. Kim. 2004. *Aspergillus Oryzae* GB-107 Fermentation Improves Nutritional Quality of Food Soybeans and Feed Soybean Meal. *J. Med. Food*. 7: 430.

Hood, S.K. and Zottola. 1998. Effect of low on the ability of *Lactobacillus acidophilus* to survive and adhere tohuman intedtinal intestinal cell. *Journal of Food Science* 53: 1514 – 1516.

Hutkins, R.W., and Nannen, N.L. 1993. pH Homeostatis in Lactic Acid Bacteria. *Journal Dairy Sci*. 76 : 2354-2365.

Hyronimus, B., C. Le Mareec, A.H. Sassi, and A. Deschamps, 2000. *Acid and Bile Tolerance of spore-forming Lactic Acid Bacteria. Journal Food Microbiology Volume 61: 193-197*.

Jaelani, A., W.G. Piliang, Suryahadi, dan and I. Rahayu. 2008. Hidrolisis bungkil inti sawit (*Elaeis guineensis* Jacq) oleh kapang *Trichoderma reesei* pendegradasi polisakarida mannan. *Animal Production*, Vol. 10 (1): 42-49.

Jin, L. Z., Y.W. Ho, N. Abdullah and S. Jalaludin. 1997. Probiotics in Poultry: Modes of Action. *Worlds Poultry Sci. J.* 53 (4): 351-368.

Kanti, A. 2007. Screening yeast *Cryptococcus sp cellulolitic*. Isolated from garden soil biology Wamena, Jaya Wijaya, Papua Province. *Journal of Biology* XI (1): 17-20.

Kahlique, A., K. P. Lone, T. N. Pasha, and A. D. Khan. 2003. Chemical composition and nutritional evaluation of variously treated defatted rice polishing for broiler. *Asian-Aust. J. Anim. Sci.* Vol 16 (6): 873-879

Kompiang, I. P. 2002. Effect of *Saccaromyces cerevisiae* and sea yeast as a feed additive probiotic on the performance of poultry. *JITV*. 7 (1): 18-21.

Mustafa, M. F., A. R. Alimon, M. W. Zahari, I. Idris, and M. H. Bejo. 2004. Nutrient digestibility of Palm kernel Cake for Muscovy ducks. *Asian-Aust. J. Anim. Sci. Vol* 17 (4): 514-517.

Prangdimurti, 2001. Probiotik dan Efek Perlindungan Terhadap Kanker Kolon (Cited 2010 Des, 17) Available from: http://www.rudyct.com.

Pratidina, A., Agustin, K.W. and Erni Sofia Murtini, 2008. Isolasi dan Identifikasi Mikrob Dari Tempe Sorgum Coklat (*Sorgum bicolur*) Serta Potensinya Dalam Mendegradasi Pati dan protein. *Journal Teknologi Pertanian*, Vol. 9 no 2 (Agustus 2008) 95-105.

Rezanezhad, D., B. S. Hejazi and A. Zakeri, 2003. Effect of dietary supplementation of prebiotics, probiotics, sybiotics, and acidifier on growth performance and organs weights of broiler chicken. *Euro. J. Exp. Bio. Vol*. 2 (6): 2125-2129.

Ritonga, H. 2008. Mikroflora saluran Cerna. 2008. Diakses tanggal 10 Juli 2013. Available from: technicalservice.wordpress.com.

Sabini, E., K.S. Wilson, M. Siika-aho, C. Boisset and H. Chanzy. 2000. Digestion of single crystals of mannan I by an endo-mannanase from Trichoderma reesei. *Europe Journal Biochemestry*, 267:2340-2344

Schrezenmeir, J. and M. de Vrese, 2001. Probiotics, Prebiotics and Symbiotics-Approaching and Definition. *American Journal of Clinical Nutrition*, 73 : 361S – 364S.

Shortt, C. 1999. The Probiotic Centure: Historial And Current Perspective**,** Review on Trend, *Food Science and Technology,* 10 : 411-417.

Siegumfeldt, H., Rechninger B.K. and Jacobsen, M. 2000. Dynamic Changes of Intracellular pH in Individual Lactic Acid Bacterium Cells in Response to a Rapid Drop in Extracellular pH. *Appl and Environ Microbiol*. 66 : 2330-2335.

Smet, I.D., L. Van Hoorde., M.V. Woestyne., H. Christiaens., and W. Verstraete. 1995. Significance of Bile Salt Hydrolytic Avtivities of Lactobacilli. *Journal Appl Bacteriol*. 79 : 292 -301.

Steel, R.G.D. and J.H. Torrie. l989. Principles and Procedures of Statistics. 2nd Ed. McGraw-Hill International Book Co., London.

Sujaya, N., Y. Ramona., N.P. Widarini, N.P. Suriani., N.M.U Dwipayanti., K.A. Nocianitri. and dan N.W. Nursini, 2008. Isolation and characteristics of lactic acid bacteria Sumbawa horse milk. *Veterinary Journal,* Volume 9 March 2008 2: 52-59.

Tjay, T.H. and dan Kirana R. 2007. Essential Drugs. Jakarta: Elex Media Komputindo.

Utama, C. S. N. 2011. Potensi Probiotik Bekatul. *Poultry Indonesia*. Vol VI, September: 78-80

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J.Dairy Sci*. 74:3583-3597.

Wahyudi, A. dan L. Hendraningsih. 2007. Probiotics. Concept, Implementation, and Expectations. Textbook. Faculty of Animal Husbandry and Fisheries, University of Muhammadiyah Malan.

Corresponding author: Dr. Bidura, I.G.N.G., Faculty of Animal Science, Udayana University, Jl. PB. Soedirman, Denpasar-Bali, Indonesia.
Email: bidura_unud@yahoo.com