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By

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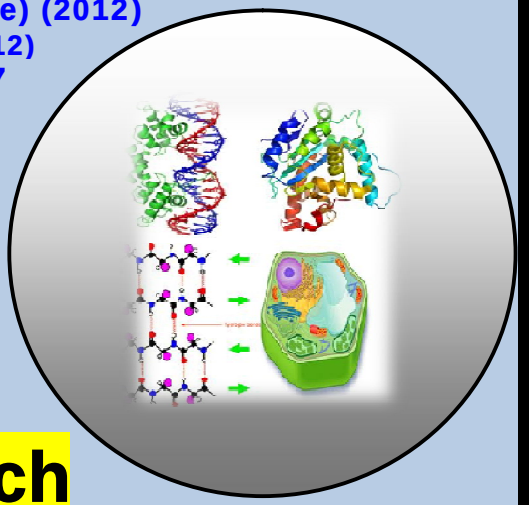

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RESEARCH PAPER
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Selection of *Saccharomyces Spp* Isolates (Isolation from Colon Beef of Bali Cattle) as Probiotics Agentand Colon Cancer Preventionandits Effect on Pollard Quality as Feed

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ABSTRACT

This experiment was carried out to study probiotics properties and colon cancer prevention of Saccharomyces spp isolation from colon beef of Bali cattle samples can be used to alleviate the negative effect of pollard as feed. Three isolates of Saccharomyces spp (Sc.SB-3; SB-6; and SB-7) were isolated from colon beef of Bali cattle samples in the first experiment. The whole isolate of Saccharomuces spp showed resistant growth in different temperature (10^0-45^0C) andacid conditions (2.5-4.5). However, only two isolates (Sc.SB-6 and SB-7) had potential as probiotics sources (resistant on both acid and bile salt, and couldprevent colon cancer). The study showed that fermentation of pollard using of Saccharomyces spp.SB-6 and SB-7 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 pollard. Fermentation caused increase of crude protein (CP) and crude fibre (CF) of pollard.It was concluded that two isolates (Sc.SB-6 and SB-7) were isolated from colon beef of Bali cattle samples.They were potential as probiotics sources and colon cancer prevention.Their utilization in pollardfermentation could increase nutrient composition and digestibilities.

Key words: Saccharomyces spp., Colon Cancer, Probiotics, Digestibility and Pollard.

INTRODUCTION

Probiotics are food supplements that contain living microbes and provide beneficial effects for the host by improving microbial balance in the digestive tract (Fuller 1992).

Probiotics as single culture or mixed of live microbes that when consumed by human or animals will be maintaining balance of microflora, naturally present in the body of its host (Havenaar and Huis, 1992). Probiotics are often associated with gastrointestinal health, as it can help suppress the growth of harmful bacteria (Hegar, 2007).

Generally, probioticsis group of non pathogen microorganisms that have positive effect on physiology and health of gastro intestinal tractofits hostif regularly consumed in sufficient quantities (Schrezenmeir and De Vrese, 2001). In the digestive tract, probioticgroupsable to decipher toxic compounds derived from protein and fat metabolism. In that case, concentration of toxic compound scan be reduced or even eliminated (Alimyameen, 2011).

Tests conducted to assess potential microbial probiotics could be developed such as: resistance to low pH, bile acids, and digestive tract enzymes (Ahmad, 2005). Besides, ability to transformcholic acid (CA) to deoxicholic acid (DCA) also required to betested in vitro to know whether candidate probiotics could stimulate the occurrence of colon cancer or not (Ariwati, 2012).

Bacterial metabolism of bile acids has an important role in colon cancer risk. It is assumed that the secondary bile acids (produced by microbialmetabolism) could be the promoter of colon cancer in formation process. The process of de hydro genation of steroid nucleus in generating bonddelta 1 and delta 4 bind to 3-keto grouphasan important role related to colon cancer. Certain strains of Clostridia *in vitro* are known to form this reaction (Wahyudi and Hendraningsih, 2007).

Probiotic group showed functional effects, such asantidiarrhealeffects, loweringbloodcholesterol, improvebowelmotilityanddetoxification, inducing immune system, resulting a wide variety of metabolites, such as: hydrogenperoxide, lactic acid and acetic acid. They wereable to maintain a balance pH and micro ecologyintestine and assist metabolize vitamins, minerals and hormones. In addition, probiotics alsoacts as an antitumor agent by preventing formation of carcinogenicnitrosamines (Tjay and Kirana, 2007).

Pollard is a by-product of wheat milling industry which is abundantly available during harvesting. This by-product unfortunately contains toxic factors as of: trypsin inhibitor, lectin, phytic acid as phytate, and high content of crude fiber. These anti-nutritive factors reported by Kahlique *et al.* (2003) caused reduction of feed intake and depress performance of poultry. The potency of pollard as poultry energy source depends on its cell wall content, degree of microbial fermentation in poultry large intestine, absorption and production of the volatile fatty acids (Wang *et al.,* 2003).

Polysaccharides in the cell wall such as: celluloses, pectins, and oligosaccharides are known as non starch polysaccharides (NSP). Non starch polysaccharides can not be degraded in digestive systems of birds due to minimum enzymes for NSPs degradation in their digestive systems (Choct, 2002). It coulddicrete microflora permanently, colonizing in gastrointestinal tract then mainly occurs in the hindgut of non ruminants by microbial fermentation (Wang *et al*., 2003). These enzymes are effective in degrading complex compounds such as: beta-glucans and arabino-xylans (Dubey, 2006). Most recent studies are focusing on the effect of bacterial and fungal enzymes used in cereal.

Hong *et al*. (2004) and Bidura *et al.* (2012) reported that fermentation of feed using khamir increased digestibility of its dry matter (DM), organic matter (OM), and crude fiber (CP). The inclusion of soluble dietary fiber (wheat bran) increased daily NSP excreted from feces (Wang *et al.,* 2004; Jaelani *et al*., 2008; Suprapti *et al*., 2008). Chen *et al.* (2005) reported that addition of 0.20% complex probiotic (*L. acidophilus* and *S. cerivisae*) in basal diets increased digestiblity of DM and CP.Related studies clearly conveyed that supplemented probiotics to diets in NSP results in significant reduction in the intestinal viscosity, enhances energy, and protein utilization (Bidura *et al*., 2012; Bidura *et al*., 2009).

It is suggested to study on probiotics properties and colon cancer prevention of *Saccharomyces spp* isolation from colon beef of Bali cattle samples in order to alleviate the negative effect of pollard as feed.

MATERIALAND METHODS

Source of isolates/Contents of Colon

Source ofisolatesin these study is excreta colon beefof Bali cattle obtained from abattoir (slaughter house) in Denpasar, Bali.

Media PDA (potato-dextrose-agar)

Oneliter of PDA weighed 39.4 ginstant, added 1000 ml distilled water, homogenized by heating and sterilize dat 121^0Cautocalve for 15 minutes temperature. The media was poured into petridishes and store dat 4°C temperatures inan ice cup board after expelled from auto calve.

Isolation of *Saccharomyces spp*

Khamir probiotic candidates were isolated using method followed by dilution and growth on Potato Dextrose Agar medium (PDA). In this research, colon beef of Bali cattle excreta samples were purchased from RPH Denpasar. 10 g of colon excreta beef added to 90 ml of 1% peptone which previously sterilized to obtain the level of dilution of 10^{-1}, shaked until homogeneous and taken as 1 ml for further diluted in 1% peptone solution seriesto obtain dilution rate of 10^{-2} - 10^{-6}. Moreover, 1 ml suspension of dilution 10^{-4},10^{-5}, and 10^{-6}inserted into sterile petri dish, added 20 ml of PDA medium between 40 - 45^0C , set andincubated in the incubator inverted position of 30^0C for 48 hours, and then observed growth of colony. Nikon (2004) and Ahmad (2005) reported that colonies which have khamir characteristics isolated within following the method: purified and cultured on PDA solid medium for further analysis; stored prior to characterization; testing its resistance to low pH, deoxicholic acid and cholic acid into transpormation deoksikolat acid (Hyronimus *et al*., 2000; Prangdimurti, 2001).

Probiotics properties

Acid tolerance: to determine the trancsit tolerance to low pH, the method of Corzo and Gilliland (1999) was used with slight modifications. Strains were grown in MRS broth at 37^0C for 24 hours. A 0.5 ml aliquot of the bacterial culture was inoculated in 10 ml of phosphate buffered saline adjusted to pH 2 with 4 N HCl. Phosphate buffer was prepared by dissolving NaCl (9 g/l), $Na_2HPO_42H_2O$ (9 g/l), and KH_2PO_4 (1.5 g/l) in destiled water.

The initial bacterial concentration was 10^{6-8} cfu/ml. Culture was incubated at 37^0C. After 0; 0.5; 1; 2; and 4 hours incubation, cell were serially diluted tenfold in 0.1 M sodium phosphate buffer (pH 7.2), and viable cells enumerated on MRS *agar* plate at 37^0C for 2 days.

Bile resistance: The resistance to bile was examined according to method of Hyronimus *et al*. (2000). Each strain was inoculated in MRS broth with 0.5 or 1% (w/v) bile salt (Difco). Culture was incubated at 37^0C for 24 hours, and numbers of viable cell were determined and compared to a control (without bile salt) on MRS *agar* plates.

Termsof khamirtobe developedas probioticwereresistant tobilesalts. Firstly, Kamirisolateswеregrownfromthe manufacturedivortexworkingculture.Secondly,put4 pieces oftubes containing5mLin each mediaof PDB.50mL of suspensionkamirwas added in each tube. Firsttubeas a controlwas notaddedwith sodiumDeoksikolat(NaDC). The secondtubewas addedwith 10mLof 100 mMNaDC(NaDC concentration of0.2mM). Thirdtubewas addedwith 20mLof 100mMNaDC(NaDC concentration of0.4mM). Fourthtubewas addedwith 30mLof 100mMNaDC(NaDC concentration of0.6mM).Then,the wholetubeswere incubatedat37°C for24hours. KhamirisolatesresilienceagainstNaDCmeasuredbyturbidity(OD 660nm) using aspectrophotometer. In order toobtainrepresentativedatathen 3 timesreplication was conducted(Hyronimus *etal*., 2000 andPrangdimurti, 2001).

Test of Cholic Acid Transformed into Dioxicholic Acid

This test was conducted to determine whether cholic acid transformed into deoxicholic acid by isolates obtained in this study. Pure cultures isolated khamir from colon beef of Bali cattle suspension derivedfrom glycerol stock by suspending 50 mL culture into 5 ml of PDB and incubated at 37^0C for 24 hours. Then, 50 mL of cultures grown in PDB suspended in 5 ml of GDP plus Ca and incubated at 37^0C for 24 hours. Subsequently, 1 ml of culture in Ca pipetted into Eppendorf centrifuged for 5 minutes at 5000 rpm.A total of 0.1 ml of the supernatant was then pipetted, added with 500 mL ethyl acetate and 20 mL HCL, centrifuged for 5 minutes at 5000 rpm, supernatant pipetted and evaporated, added with 500 mL ethyl acetate, centrifuged for 5 minutes at 5000 rpm, supernatant pipetted and evaporated for 48 hours in room temperature, add with15 mL methanol. 10 ml Cyclohexane, Ethyl Acetate 15 mL and 4 mL Acetic Acid mixed in the Chamber, and allowed to stand for 30 minutes prior to TLC on alluminium cilica gel. After 30 minutes, DCA, Ca, and each 1 mL isolatesplattered on aluminum silica gel, dried with hairdrayerplaced in the chamber containing eluent solution, allowed to stand until the silica absorbed solution, dried, sprayed with acid dyes Phosporic Molibddo and roasted until the spot of each isolate was visible on silica gel. If isolates transformed CA into DCA then isolated will be similar to spot with DCA spot, otherwise if DCA transformation isolated the spot will be similar to CA spot (Ariwati, 2012).

Fermentation of Pollard

*Saccharomyces spp.*SB-6 and SB-7 isolates in this study was isolated from colon beef of Bali cattle samples. The isolate of *Saccharomyces spp.*SB-6 and SB-7 which has been approved from bile salt and poultry digestive tract *in vitro* test could colon cancer prevention for probiotics agency.

The study was carried out at Bioscience Laboratory of Udayana University, Bali, Indonesia. Fermented commercial pollardwas prepared as follows:approximately 0.40% (2.56 x 10^6 spores) *Saccharomyces spp.*SB-6 and SB-7 isolates culture, respectively added to 100 g of steamed pollard. Then, water added to bring the product to 50% content and leave out for three days fermentation. After that, fermented pollardwas dried at 45^0C for six hours was analyzed. Unfermented pollard was also also conducted for its chemical analysis.

Retention and Excretion of Nutrients

In order to determine nutrient digestibility and metabolizable energy (ME) value of the pollard, twenty weeks old male native chickens were used in this study. All birds were deprived of feed for 24 hours to ensure that their alimentary canals were empty from feed residues. Then, they were force-fed with specific amount of pollard (fermented and unfermented). Stainless steel funnel with 40 cm stem was used in *force feedingtechnique* (Mustafa *et al*., 2004). The amount of pollard used was 40 g based on preliminary assays. The birds were kept over excreta collection trays and their housing time was recorded. Excreta voided from 0 to 24 hours and 24 to 48 hours (which represent metabolic plus endogenus excretion) after housing were collected quantitatively. Water was available *ad libitum* during the experimental period. Excreta were collected for two hours. Other substances (such as feathers, scurf, etc.) in the collected excreta were removed before drying at 60^0C for 48 hours and subsequent grinding. Feed and feces were analyzed by AOAC (l994) procedures for proximate components. The retention of nutrients was calculated by dividing the amount of retained nutrient (ingested nutrient minus excreted nutrient) by the amount of ingested. Gross Energy (GE) was measured with an adiabatic oxygen bomb calorimeter (Parr, USA), Crude Fibre (CF) was analysed according to Van Soest (1991).

The total excreta were collected in plastic trays. The excreta samples were frozen, allowed to equilibrium with the atmospheric moisture, weighed, and ground through one mm sieve. Samples of excreta and pollard were subjected to appropriate analysis to determine DM, OM, CP, CF, and gross energy.

Measurements

Feed intake was determined by measuring feed residues on daily basis since the beginning of the experiment. Apparent metabolizable energy (AME) was calculated using the formula of Mustafa *et al*. (2004): AME = IE – FE, where IE = ingested energy and FE = fecal energy of the fed birds;

Dataanalysis

Analysis ofthe dataforthe endurance testisolateskamirtolow pHandthesodiumdeoxicholic(NaDC) andcholicacidtransformationtestperformedwith sourdeoxicholicdescriptivemethod. In order todeterminewhetheraculturecan begrownon amedium thathas alow pHorinmedium containinghighconcentrations ofNaDCwithin criteria of: (1) when absorbance(A) suspensionof khamirmeasuredwith a spectrophotometerat a wavelength of660nm<0.1,thenkhamirstrainsare not resistant tolow pHandNaDC; and(2) if thevalue of$A \geq 0.1$thenkhamirstrainsresistant tolow pHandNaDC(Sujaya *etal*., 2008).

RESULTS

Figure 5.1belowis themorphology oftwoisolates of*Saccharomycesspp*that can be isolatedfromcolonof beef Bali cattle. Thesecond cellweremicroscopicisolatesare the same, but the color ofthecolonytwodifferentstrains, i.e.isolates of*Saccharomycesspp*SB-6 translucent whitecolorand*Saccharomycesspp*isolatesSB-7 color is clear, so it is consideredtwoyeaststrainsaredifferent.

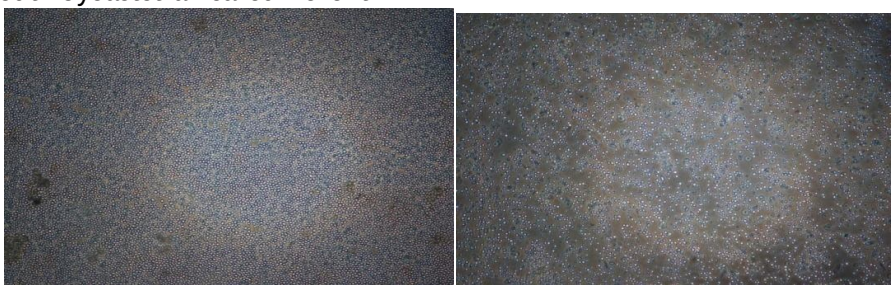

Figure1. Morphologyof *Saccharomyces spp* isolate with a magnification of400x

Resilienceisolate*Saccharomycesspp.*thelow pHis one ofthe characteristics requiredmetby acandidateor aprobioticthatcould be developed intoa potentialprobiotic. Onthistest, the mediumpHwas adjusted to1.5;3.0; 4.5; and 6.0by usingHCl(hydrochloric acid), becauseHClhascharacteristics similar tostomach acid. Resistant tohighlyacidicnatureneeds to be ownedby thecandidateprobiotic, becausetheapplicationlater, thisprobioticcandidatemustpassveryacidicstomachconditions, beforereachingthe colon.

Observationsas presentedin Table 1. In this study,as many asthreeisolates*Saccharomycesspp*. isolated fromcolon of beef Bali cattle sampleswas testedresistance tovariouspH, at pH1.5,3.0, 4.5, and 6. Sixisolates wereunable to growat pH1.5, whichisolatesthe SB-1; SB-2; SB-4; SB-5; SB-8; and SB-9.*Saccharomycesspp*.SB-1, SB-4; SB-5, and SB-8isolatesevensimply could notgrow atallpHtreatmentsgiven. Onlythreeisolateswereable togrow well, namely*Saccharomycesspp.*SB-3, SB-6, and SB-7 isolates. There is atendency ofthe higherpH, especiallyat pH6, most of theisolateshas decreasedthenumber ofcolonylife.

Table 1.The number of colony isolates *Saccharomyces spp* (Log colony/g) at various pH.

Code Isolate	pH			
	1.5	3.0	4.5	6.0
Saccharomyces spp.SB_1	-	-	-	-
Saccharomyces spp.SB_2	-	+	+	+
Saccharomyces spp.SB_3	3.45	4.53	4.11	3.13
Saccharomyces spp.SB_4	-	-	-	-
Saccharomyces spp.SB_5	-	-	-	-
Saccharomyces spp.SB_6	4.06	4.71	4.58	4.29
Saccharomyces spp.SB_7	4.13	4.65	4.51	4.27
Saccharomyces spp.SB_8	-	-	-	-
Saccharomyces spp.SB_9	-	-	**+**	**+**

Descriptions: SB-1 s/dSB-9is a*Saccharomycesspp.*isolateswereisolatedfromcolon of beef Bali cattle samples.

In the testof resistance tobilesaltsas showedin Table2shows, threeisolates wereable to surviveandgrowon amedium containingbilesaltsboth atconcentrations of0.2mMand0.4mM, whereasat a concentration of0.6mMnoisolatescansurvive. Microbialisolatesresistancetobilesaltsis usedtoassess theability tosurvivedigestive tractisolatescontainedbilesaltsonthesurface of theintestine.

Table2. Ability ofyeast*Saccharomycesspp*isolateswereresistantlivinginbilesalts.

Isolate	Bilesaltconcentration(absorbance)			
	0.0 mM	0.2 mM	0.4 mM	0.6 mM
Saccharomyces spp.SB_3	++(0.698)	+(0.317)	+(0.245)	+(0.217)
Saccharomyces spp.SB_6	+++(1.661)	+++ (1.629)	+++ (1.140)	+++(1.096)
Saccharomyces spp.SB_7	+++(1.325)	+++ (1.198)	+++ (1.219)	+++(1.296)

Description:

- : A<0.1 (not bear bilesalt)
+ : A0.1-0.5(abilesalt-resistant)
++ : A0.5-1.0(bile salt-resistant)
+++ : A>1.0(highly resistantof bilesalts)

SB-3s/dSB-7is a*Saccharomycesspp*isolateswereisolatedfromcolon of beef Bali cattle

Probioticswill bedealingwiththe environmentin thesmall intestine, in which there werebileorbilesaltsareremovedby the liverthroughthe gall bladder, aftersuccessfullypassingthe acidic conditionsinthe stomach. Therefore, in the process ofdevelopingnew probiotic, or anewprobioticcandidatemust beable to pass thetest ofresistance tobileorbilesaltswereperformed *in vitro*. Based on thenature of theresistanceshown bysomeisolates, strain SB-6 and SB-7 indicates thatthestrainhas the potentialtobe developed into apotentialprobiotics.

Chemical composition and nutrient digestibility of pollard (fermented compared to the unfermented) were shown in Table 3 as below:

Table 3. Chemical composition and nutrient digestibility of unfermented and fermented pollardby *Saccharomyces Spp.*SB-6 and SB-7 isolates culture (in % Dry Matter).

Parameters	Pollard			
	Unfer-mented (UFP)	Fermented by SB-6 Isolate (FP)	Fermented by SB-7 Isolate	SEM
Chemical composition:				
Dry Matter (%)	90,75a	89.03a	88,97a	0.592
Organic Matter (%)	91,04a	91.51a	91,79a	0.497
Crude Protein (%)	14,85b	16,25a	16,08a	0.278
Crude Fibre (%)	14,07b	15.63a	15,59a	0.405
Gross Energy (Kcal/kg)	3174.09a	3206,72a	3240,91a	58.904
Nutrient digestibility (%):				
Dry matter digestibility (%)	51.93a	55.92b	55.47b	0.735
Organic Matter digestibility (%)	52.64a	56.79b	56.41b	0.694
Crude Protein digestibility (%)	40.36a	45.94b	45.17b	1.093
Crude fibre digestibility (%)	18.08a	20.75b	20.39b	0.318
Metabolizable energy (Kcal/kg)	1882.75b	2084.52a	2105.07a	25.704

Note:

- *UFP = Unfermented pollard; FP = Fermented pollard by 0.20% yeast culture*
- *SEM = standart error of the treatment means*
- The different superscript at the same row is significantly different (P<0.05)

Table 3 showed that nutrient in the content of crude protein, crude fibre (CF), and gross energy were slightly increased by fermentation. In contrast, fermentation caused decreasing dry matter (DM) and organic Matter (OM) of the pollard. The result indicated that all of nutrient digestibility of pollard fermented by *Saccharomyces spp*isolates culture were increased significantly (P<0,05) different rather than unfermented pollard.

Table 3 shows the nutrient digestibility and metabolizable energy of unfermented pollard (UFP) and fermented pollard (FP) ingredient. The digestibility of crude protein, organic matter, and crude fiber, were slightly increased significantly different (P<0.05) by the fermentation, both by *Saccharomyces spp.*SB-6 and SB-7, respectively. The metabolizable energy of FP was increased significantly different (P<005) than metabolizable energy of UFP. Fermented of feeding ingredient were caused increased of crude protein, organic matter, and crude fibre digestibilities, resvectively than UFP ingredients.

DISCUSSION

Actually, this probiotic yeast already present in the human digestive tract or other animals, such as beef Bali cattle colon to help suppress microbial populations gastrointestinal pathogens (Alimyameen 2011). Microbes can be developed into a candidate probiotic microbes if they meet the following requirements: (i) these microbes are pathogenic to animals and humans; (ii) is a microbial flora of the gastrointestinal tract and have the ability to colonize the intestinal wall; (iii) the microbes resistant to stomach acids, bile acids and salts, digestive enzymes, immune system, animal or human; and (iv) does not transform deoxy cholic acid into cholic acid, so that no potential to induce colon cancer (Fuller, 1989).

The degree ofacidity(pH) of the ventriculusand proventriculusranged from1-2, so theonlyacid-resistant microbesthatcanliveonlyinthispartofthe digestivetractof poultry. Microfloraandthe interactionbetweenthe types ofdifferent microorganismsinthe digestive tractof poultryis verycomplex(Sabini *etal*., 2000). The yeast*Saccharomycesspp*SB-6 andSB-7 turned out to beresistant topHextrimsso thatpotential asprobioticagents.

Probioticswill be dealingwith theenvironmentof the small intestinein which there isbileorbilesaltsareremovedby the livervia thegall bladder, aftersuccessfullypassingthe acidic conditionsinthe stomach. Therefore, in the process ofdevelopingnew probiotic, newprobioticcandidatemust beable to pass thetest ofresistance tobilesalts. Inthistest, the candidateprobioticGYPincubatedin mediumsupplemented withNaDCat various concentrations(0.2; 04, and 0.6mM). Theconcentrationin a rowisNaDCconcentrationin normal individuals, candidate'scancer patients, andcancer patients, respectively (Dawson, 1998).

The results showed that only three isolates of *Saccharomyces spp* potential as probiotic agents.

To three isolates showed resistance to acid and bile salts that is characteristic of probiotic bacteria. *Saccharomyces* is a unicellular microorganisms lacking chlorophyll, grew well at 30^{o}C and pH 4.8. pH value in the gastrointestinal tract of poultry in each section, are: crop (4.5), proventriculus (4.4), gizzard (2.6), duodenum (5.7-6.0), jejunum (5.8), ileum (6.3), colon (6.3), and ceca (5.7) (Hidayat, 2010). According to Ahmad (2005), temperature optimum environment for yeast growth is 25-30^{0}C and a maximum temperature of 35-47^{0}C. Some advantages *Saccharomyces* microorganisms in the fermentation process that is rapidly proliferating, resistant to high alcohol content, resistant to high temperatures, has held steady and rapid nature of the adaptation.

Cholesterol is a precursor of primary bile salt formed in the liver and stored in the form of conjugated bile salts in primary gallbladder henceforth gradually secreted into the digestive tract. In the digestive tract (especially in the small intestine), conjugated bile salts will be instrumental in helping the absorption of fat, kolesteol, hydrophobic vitamins, and other components that are soluble in fat. The conjugated bile salt are absorbed from the small intestine and is passed by circulation system (hepatic portal system) to the liver (the liver to be returned). Only a small fraction of bile salts (250-400 mg) that is not absorbed will be wasted in the feces in the form of free bile salts (Corzo and Gilliland, 1999). In the form of free bile salts, the products of the liver is less soluble in water and less able to be absorbed by the intestinal lumen (absorbality is lower than in conjugated form), so that bile acids can push conjugated decrease serum cholesterol by increasing the formation of new bile acids to replace lost during enterohepatic circulation or by decreasing cholesterol absorption in the intestinal lumen.

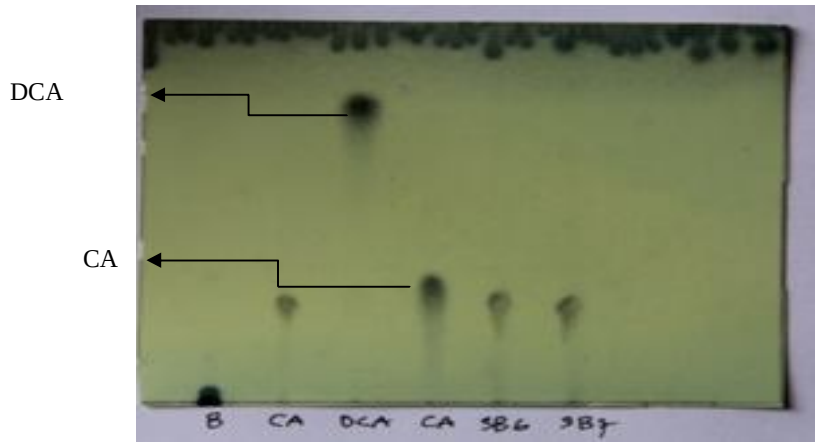

Figure2. Chromatogramtest resultsbiotransformationof cholicacidinto aciddeoxicholicin*Saccharomycesspp*isolates. SB-6 andSB-7 isolated fromcolonbeef of Bali cattle.

Figure 2, indicating that bothisolateskhamir(*Saccharomyces spp*. SB-6 andSB-7) wereisolatedfrom thecolonof beef Bali cattlelikelytobe developed intoprobiotics, because ofall therequirementsthatwere testedin this studycan be metbyallisolatestested.

Oneimportanttestthatshould beperformedbeforeaspecies ofmicrobes(bacteria orkhamir) canbe developed intoprobioticsis thebiotransformation ofcholic acidinto atestdeoxicholicacid. Candidateprobioticsshould not bedoingthe biotransformationof cholicacidintodeoxicholicacidsinthe digestive tractof its host, becausecholicacid derivativeissuspectedas one of thecompoundsthatmaystimulatethe onset ofcoloncancer(Brady *etal*., 2000;Pato, 2003;Liong, 2008). Therefore, althoughaspecies ofprobioticsshowother potentialproperties, thisspecieswillnotbe usedas aprobioticcandidateifthe speciesdobiotransformation ofcholicacidinto aciddeoksikolat.
In this study,twoisolatesof *Saccharomycesspp.*probioticsthathave passed the test(test temperature, pH, andbilesalts) as well asisolatesthatshowedgrowthresponse(resistance) inenvironments containingNaDCtested for their abilitytotransformation cholicacid(CA) to Deoxicholicacid(DCA). The result of this test (chromatograms) is shown inFigure2. Chromatogramin figure2, indicating thatthetwoisolatestesteddidbiotranspormationCA toDCA, whichis notindicatedbythe formation ofspots(black spots) on theretention time(Rt) isthe same as thestandarddeoxicholic(DCA).

In thedigestivetractof poultry, biotransformation ofcholicacidintodeoxicholicacidis generallycarried outbythe groupand the*Clostridiumbacterium.*Clostridiumscindenshiranois(Wells *etal.,*2003). Thebacteriumhasa set ofgenesthatproduceenzymescapable7-alfa-dehydroxilase. This enzymeis thought to workto break the tieshydroxycompoundsof cholicacid(Sujaya *etal*., 2008).
Table 3 shows the nutrient the content of crude protein, crude fibre (CF), and gross energy were slightly increased by fermentation. On the other hand fermentation caused decreasing dry matter (DM) and organic Matter (OM) of the pollard. These results indicated that carbohydrate was used for microbial growth and the reduction of dry matter. The result indicated that all of nutrient digestibility of fermented rice bran by *Saccharomyces spp* culture were increased significantly (P<0,05) different rather than unfermented rice bran.
Table 3 shows the chemical composition of unfermented pollard (UFP) and fermented pollard (FP) ingredient. Fermentation feed purpose is to improve the usability of feed and elimination of anti- nutritional substances, as well as utilize biomass formed. Pollard fermented with yeast will be able to increase the microbial biomass, so that the crude protein content of pollard increased (Utama *et al.,* 2006). Pollardnutrient content, such ascrude protein, organic matter, crude fibre, and metabolizable energy were slightly increased by *Saccharomyces spp.*SB-6 and SB-7 isolatesfermentation, resvectively. The increase inMEcontent ofpalm kernel cake(palm kernelcake/meal) asa result offermentationby thefungus*T.reesei*of1824.13kcal/kg to1930.44kcal/kgsuspected because of thedegradationof mannanpolysaccharidesexist inpalm kernelbyfungus*T.reesei*intosimpler forms(monosaccharides) that producesenough energyvaluebetter thanin the form ofpolysaccharidesmannan (Jaelani *et al.,* 2008). Fermentedpalm kernel cakecan markedly increasethe crude protein contentcompared topalm kernel cakewithoutfermentation.

Thefungus of *T.reesei*is able todegradeapolysaccharidemannanmannotriosa, mannobiosa, andmonnosa (Sabini *et al*., 2000). AccordingSumarsih*etal.*(2007), the longer thestorage timeof feed ingredientswhenfermented by*Trichodermaviride*, the more theloss ofdry matterandorganic mattermaterial, insteadincreasingthe crude protein contentandmaterialsdecreasedcrude fiber contentmaterial. Alsoreportedthatthe longer thefermentationprocessormaterialsstorage time, theincreaseddry matter digestibilityandorganic mattermaterials. Suparjo *et al.* (2003) reported that fermentation of rice bran with 0.20% *Aspergillus niger* cultured for 72 hours can markedly increase protein and phosphorus content of rice bran, on the contrary lower crude fiber content and acid phytat rice bran.

The digestibility of crude protein, crude fiber, and ME were slightly increased by the fermentation. These results indicated that carbohydrates other than fibres were used for microbial growth (*Saccharomyces spp*) and the reduction of nitrogen free extract resulted in increased concentration of the other components. Yi *et al.* (l996) reported that supplementation of microbial in diets improved Nitrogen retention in broiler chickens and *in vitro* digestibility of vegetable protein. Also, Chen *et al.* (2005) reported that addition of 0.20% complex probiotic (*L.acidophilus* and *S.cerivisae*) in basal diets were inceased digestiblities of dry matter.

Fermented of pollard by *Saccharomuces spp.*SB-6andSB-7isolates culture had better digestibility, because *Saccharomuces spp* culture in the gastro intestinal tract can to be part of probiotic sources. Supplementation of probiotic could improve protein and energy retention in the gastro intestinal tract of the birds (Piao *et al.,* l999). Chen *et al.* (2005) reported that dietary supplementation of complex probiotic slightly improved digestibility of nutrients. Also, Piao *et al.* (l999), suggested that probiotics in the gastro intestinal tract can improve protein and energy retention on the body of birds. These fungal are effective in degrading of the complex compounds such as beta-glucans and arabinoxylans (Bedford and Classen, 1992). Cho *et al. (*2007) reported that supplementation of microbe in diet could improve the bioavailability of dietary.Theyeast*Saccharomycesspp*is yeastcapable of producing theenzymesamylaseandselulolase (Utama, 2011), so as toincreasethe digestibility ofproteinandcrude fibersuch ascelluloseandhemicellulose, ashas beenoverhauledin the form ofa simplemonosaccharide.Cellulolyticyeastscapable ofproducingthe enzyme 1,4 beta-endo-glukonase, 1,4beta-exo glukonase, andbeta-glucosidase thatcandegradecomponents ofcrude fiberintosolublecarbohydrates(Howard *etal*., 2003)

Wang *et al.* (2004) reported that the inclusion of fiber sources such as wheat bran or potato starch reduced the maintenance of energy requirement. These fungi are effective in degrading complex compounds such as beta-glucans and arabinoxylans (Dubey, 2006). The high level of non-starch polysaccharides (NSP) in pollard is limiting its unrestricted use in poultry feeding. The NSP is known increasing the gut viscosity, reduce nutrient absorption in the intestine and affect indirectly the growth and performance of bird (Rhames *et al*., 2006; Cho *et al*., 2007).

The studies have clearly demonstrated that the addition of probiotics culture or enzymes to diets rich in NSP resulted in a significant reduction of the intestinal viscosity enhances energy and protein utilization. Wang *et al.* (2004) reported that degree of microbial fermentation in the large intestine improves the bioavailability of dietary. Chen *et al.* (2005) reported that dietary supplementation of complex probiotic slightly improved digestibility of nutrients. Inconsistent reports about the effect of probiotics may be due to several aspects such as strains of bacteria, dose level, diet composition, feeding strategy, feed form, and interaction with other dietary feed additives (Chesson, 1994).

Supplementation of microbial in diets improved N retention in broiler chickens and *in vitro* digestibility of vegetable protein. Besides, Chen *et al.* (2005) reported that addition of 0.20% complex probiotic (*L. acidophilus* and *S. cerivisae*) in basal diets increased digestiblity of DM and CP (Chen *et al.*, 2005; Yi *et al.*, 1996; and Hong *et al*., 2004). The inclusion of soluble dietary fiber (wheat bran) increased daily NSP excreted from feces (Wang *et al.,* 2004; Suprapti *et al*., 2008), increased in both metabolizable energy and crude protein contents of palm kornel meal (Jaelani *et al*., 2008).

CONCLUSION

It was concluded that two isolates (*Saccharomyces spp*.SB-6 and SB-7) were isolated from colon of beef Bali cattle samples, both were the potensial as a probiotics sources and has prevention colon cancer activity and its utilization in the pollard fermentation could increase nutrient composition and digestibilities.

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