World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

PROBIOTIC CANDIDATES FROM NATURAL MICROFLORA OF BUFFALO MILK FROM THE DISTRICT OF LEMBAH GUMANTI

Syntia Resti, Nurmiati Nurmiati* and Periadnadi Periadnadi

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Indonesia.

***Corresponding Author: Nurmiati Nurmiati**
Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Indonesia.
Email ID: nurmiati@sci.unand.ac.id,

Article Received on 11/06/2019 **Article Revised on 01/07/2019** **Article Accepted on 21/07/2019**

ABSTRACT

Natural bacteria from three buffalo milk products from Lembah Gumanti District, Solok Regency, West Sumatra have been analyzed and compared. This study aims to analyze the composition of microflora and proteolytic bacteria in buffalo milk, to compare the character of potential acid fermentation isolates as probiotic candidates. The study was conducted with survey methods and data were analyzed descriptively. The results of this study include, the proportional presence of fermentative bacteria in fresh buffalo milk from the highest three different samples obtained in Sample B ($95.5x10^6$ *cfu/ml*), followed by Sample C ($88.5x10^6$ *cfu/ml*) and Sample A ($83.5x10^6$ *cfu/ml*), potential isolates of fresh buffalo milk including *Lactobacillus* (*Lactobacillus* sp. 1 (SSKA1, SSKB1, SSKC1), *Lactobacillus* sp2. (SSKA2, SSKB2) and *Lactobacillus* sp3, Hemolysis Tests of the six isolates (SSKA1, SSKA2, SSKB1, SSKB2, SSKC1, and SSKC2) were not pathogenic (negative pathogens) which were marked d with no formation of clear zone regions (zone of hemolysis) around isolates, and SSKA1, SSKA2, SSKB1, SSKB2, SSKC1, and SSKC2 including the class of lactic acid bacteria.

KEYWORD: buffalo milk, fermentatif, probiotic.

INTRODUCTION

In general, the composition of buffalo milk is the same as cow's milk or other ruminants only the proportion is different, which contains water, protein, fat, lactose, vitamins, and minerals. Buffalo milk is easily recognized because it is richer in fat, its fat molecule is smaller, and it forms emulsions in milk and its color has a whiter characteristic compared to cow's milk because of the absence of carotene (Hasinah and Haniwirawan 2007).

Another criterion that must be fulfilled to make certain microorganisms as probiotics is to ensure that these microorganisms are not pathogenic, so they do not endanger the host. Probiotic strains must also survive and be able to survive during the food processing and storage process, are easily applied to food products, and are resistant to psychochemical processes in food (Allameh et al, 2012).

Probiotics also have the ability to produce antimicrobial metabolism capable of suppressing pathogenic bacteria by becoming part of the microflora and contributing to the health of their hosts (Vine et al., 2004). and provide a positive influence on the physiology and health of the host (Yulinery et al., 2006). The requirement for probiotics to work effectively is being able to adapt to the environment (physical and chemical) conditions of host animals; can survive at low temperatures and high concentrations of organic acids in the digestive tract, also against pancreatic and bile fluids produced in the upper small intestine channel; does not produce toxic compounds that harm host animals; and able to live and metabolize in the host intestinal tract (Kesarcodi et al, 2008). The basic principle of the work of probiotics is the utilization of the ability of microorganisms to break down or break down the long chains of carbohydrates, proteins and fats that make up the feed given. This ability is obtained because of the special enzymes possessed by microbes to break these bonds. On the other hand, the microorganism of this breaking agent benefits from the energy obtained from the reshuffle of the complex molecule.

The application of probiotics is very important and at present studies on probiotics are being intensified.Based on the above statement, it can be seen that there is still little public understanding of the content contained in buffalo milk and very few reports that clearly describe the isolation, potential and characterization of microflora contained in fresh buffalo milk, as well as proportional microflora groups in it. Until now there have been no reports of the isolation, potential and characterization of the microflora groups as probiotic candidates in fresh buffalo milk. Therefore it is necessary to conduct research on the isolation, potential and characterization of probiotic candidates in fresh buffalo milk.

METHOD

Materials

While the ingredients used were 96% alcohol, spritus, aquadest, peptone, lactose, agar, glucose, CaCO3, milk buffalo milk from Lembah Gumanti District, Solok Regency, West Sumatra.

Research methods

The study was conducted using the survey method and purposive sampling technique in buffalo milk sampling in the district. Lembah gumanti, Solok Regency. The first stage starts from the visual observation of the product on the texture, color and taste of buffalo milk, then the condition of the comparison of curd with serum and Ph value. Furthermore, the microbiological stages include the enumeration of the total microbial buffalo milk samples and proportional microbial fermentation and protein lysis using specific mediums. The second stage included isolation, characterization, biochemical tests and pathogenesis of fermentative, proteolytic, and amylolytic isolates as probiotic candidates and analyzed descriptively.

RESULTS AND DISCUSSION

The presence of fresh buffalo milk fermenting bacteria

Based on the results of research on isolation, potential and characterization of milk digestion natural microflora as probiotic candidates from 3 samples of fresh milk buffalo milk originating from Lembah Gumanti District, it was found that there were perfementative microflora in the fresh milk buffalo milk sample. The results of isolation, characterization and potential of natural milk digesting bacteria in fresh milk buffalo milk samples on a specific medium can be seen in the following tables.

Table 1: pH and Average Total Fermentative Natural Bacteria in GPA-CaCO3 Medium.

No	Sample	GPA $CaCO_3$ (…x10^6cfu/ml)	pH
1	SSKA	83.5	6.7
2	SSKB	95.5	6.8
3	SSKC	88.5	6.5

Description: (SSKA: Sample buffalo milk Aia Dingin A, SSKB: Sample buffalo milk Aia Dingin B, SSKC: Sample buffalo milk Aia Dingin C)

Characteristics of Fermentative Potential Isolates from Fresh Buffalo Milk

Based on macroscopic and microscopic observations on colony shape, colony edge, colony elevation, colony color, shape, cell shape, Gram staining, and motility as well as biochemical tests which included catalase and KOH tests of both potent isolates in buffalo milk, the results were obtained in Table 2.

Table 2: Morphological characters and biochemical tests of perfementative potent isolates in Fresh Buffalo Milk in Gumanti Valley, West Sumatra.

Character	Isolat					
	SSKA1	SSKA2	SSKB1	SSKB2	SSKC1	SSKC2
Makroskopis						
Form colony	Circular	Circular	Circular	Circular	Circular	Spindle
Edge colony	Undulate	Entire	Undulate	Entire	Undulate	Entire
Elevasi colony	Raised	Flat	Raised	Flat	Raised	Flat
Color colony	White	White	White	White	White	White
Mikroskopis						
Form cell	Basil	Basil	Basil	Basil	Basil	Basil
Gram	+	+	+	+	+	+
Acid Fast	-	-	-	-	-	-
Endospora	-	-	-	-	-	-
Motilitas	Motil	Motil	Motil	Motil	Motil	Motil
Biokim						
Katalase Test	-	-	-	-	-	-
Hemolysis Test	-	-	-	-	-	-
Asetat Test	-	-	-	-	-	-

Based on macroscopic, microscopic and biochemical tests and identification based on the Bergey Manual of Determinative Bacteria, it was assumed that from the six isolates, the genus *Lactobacillus* was obtained.

In Table 2, the results of macroscopic, microscopic and biochemical tests of bacterial isolates in fresh buffalo milk are seen. These six isolates have different characters and some of the same characters. SSKA1 isolates and SSKA2 isolates have colonies that are circular to the edges of Undulate and Entire colonies, and have colony elevations namely Raised and Flat, and have the same colony color that is white. SSKB1 and SSKB2 isolates have the form of a colony that is circular to the edges of Undulate and Entire colonies, and has a colony elevation that is Raised and Flat, and has the same colony color that is white. SSKC1 and SSKC2 isolates have a colony shape that is circular to the edges of Undulate and Entire

colonies, and has a colony elevation that is Raised and Flat, and has the same colony color that is white.

In microscopic characters, the six isolates had the same microscopic character, which had basil cell shape, Gram positive, motile and had no endospores. Similar to the biochemical test, the two isolates have the same character, the negative catalase test which is characterized by the absence of air bubbles in the test using 3% H2O2 and the negative KOH test which is indicated by the absence of mucus in the 3% KOH test.

The characters that appear visually are one of the macroscopic characteristics of bacteria that are likely to be influenced by environmental factors, namely the substrate used as a medium and incubation temperature. This is in accordance with Lay's statement (1994), that the morphological characteristics of bacterial colonies and pure cultures can be identified in the types of microorganisms, but to obtain the perfect identification results it must be continued with biochemical tests.

Basically to find out the bacterial group not only with gram staining but also can be done with 3% KOH test, where if the negative test results are not slimy states that the bacteria are classified as gram positive bacteria because the cell wall of gram positive bacteria is more resistant to KOH so the cell wall is not broken.

Testing of the six bacterial movements of these isolates was non-motile in that, it was found that the bacterial growth did not spread to the semisolid NA medium. According to Volk (1988) the ability of a moving organism itself is called motility (motion power). The bacteria are said to be motile when the bacteria spread around the puncture, while the bacteria are said to be non-motile if the growth is only in puncture marks.

Catalase test in each isolate did not form air bubbles, this indicates that all of these bacterial isolates were catalase negative. Negative catalase is characterized by no formation of air bubbles when bacterial isolates are dripped with 3% H2O2 solution. Catalase is positive if air bubbles form when bacterial isolates are dripped with 3% H2O2 solution.

Based on macroscopic, microscopic and biochemical tests, and identification based on the Berge's Manual of Determinative Bacteriology, it was estimated that from the six isolates, the genus Lactobacillus was obtained. SSKA1, SSKA2, SSKB1, SSKB2, SSKC1 and SSKC2 isolates are Gram positive bacteria, bacilli, non-spore and negative catalase. This is in accordance with the Berge's Manual of Determinative Bacteriology that Lactobacillus bacteria belong to the class of Gram positive bacteria, rod-shaped, not sporoidal, not motile and negative catalase.

Hemolysis Test

Hemolysis Test in Blood Media To be carried out with the aim of knowing the toxic properties (pathogenicity) possessed by bacteria. Based on the hemolysis test performed, it was found that negative SSKA1, SSKA2, SSKB1, SSKB22, SSKC1, and SSKC2 isolates were tested for hemolysis in Medium Blood Agar, characterized by no formation of clear zone regions or hemolysis zones around the scratch of the six isolates. the hemolysis zone showed that isolates were unable to lyse erythrocytes.

This is in accordance with the opinion of McKane and Kandel (1998) which states that in solid media for blood, bacteria that produce hemolysin will show color changes in the bacterial growth zone. Bacteria that have the ability to damage erythrocytes show clear zones around the growth of colonies on blood agar media and are grouped as β-hemolysis bacteria and if around colonies growth shows a zone that is not clear is inserted into a group of α-hemolytic bacteria and bacteria that does not have the ability to damage erythocytes grouped into groups of non-hemolytic bacteria.

According to Yulinery et al., (2006) the toxic compounds produced in the metabolism of probiotic bacteria such as lactic acid, hydrogen peroxide, antimicrobial bacteriocin and antibiotics can reduce the growth of pathogenic bacteria. According to Kanmani et al. (2010), one of the characteristics of probiotic bacteria is that it has a high resistance to acid. Bacteria capable of lyhing erythrocytes are more virulent than bacteria that are not able to lyse erythrocytes. The ability of bacteria to lyse erythrocytes is determined by the substance of extracellular proteins called hemolysin (McKane and Kandel 1998).

Test of Lactic Acid Bacteria or Acetic Acid Groups

Tests of groups of lactic acid bacteria or acetic acid using Calcium Carbonate Medium Ethanol (Etanol + CaCO3) were carried out to determine the group of bacteria (groups of lactic acid bacteria or acetic acid) which were isolated from fresh buffalo milk. The isolates which were scratched on the Etanol + CaCO3 medium did not have any colonies that formed the clear zone region, this indicates that the six types of isolates were not classified as acetic acid bacteria but were a group of lactic acid bacteria. Acetic acid bacteria will form a clear zone in the Etanol + CaCO3 medium because only acetic acid bacteria can grow in this medium. According to Periadnadi and Nurmiati (2010), Etanol + CaCO3 medium is a selective medium to determine a group of bacteria (acetate or lactate), only acetic acid bacteria can use alcohol as a source of C from the media to acetic acid, which is characterized by the formation of clear zone around the colony.

Lactic acid bacteria are a group of bacteria that are able to convert carbohydrates in the form of glucose to lactic acid (Ray and Bhunia 2008). According to Axelsson (2004) lactic acid bacteria can be linked to habitats that are rich in nutrients such as milk, meat, vegetables, but some are microflora of the mouth, intestines, and vagina

of mammals. According to Reid et al. (2003) lactic acid bacteria are included in probiotic bacteria, which are live microorganisms which, if given in certain quantities, provide benefits to their host such as preventing diarrhea from maintaining the balance of intestinal flora, preventing cancer and lowering cholesterol.

CONCLUSION

The proportional presence of fermentative bacteria in fresh buffalo milk from the highest three different samples obtained in Sample B (95.5x10^6*cfu/ml*), followed by Sample C (88.5x10^6*cfu/ml*) and Sample A (83.5x10^6*cfu/ml*), potential isolates of fresh buffalo milk including Lactobacillus (Lactobacillus sp. 1 (SSKA1, SSKB1, SSKC1), Lactobacillus sp2. (SSKA2, SSKB2) and Lactobacillus sp3, Hemolysis Tests of the six isolates (SSKA1, SSKA2, SSKB1, SSKB2, SSKC1, and SSKC2) were not pathogenic (negative pathogens) which were marked d with no formation of clear zone regions (zone of hemolysis) around isolates, and SSKA1, SSKA2, SSKB1, SSKB2, SSKC1, and SSKC2 including the class of lactic acid bacteria.

REFERENCES

1. Allameh Dr, M. Sayyed, Harooni, Alireza, Chaleshtari, S. Mohsen, Asadi dan Ali., Investigate the Relationship between Variables and Role Clarity Effects on the Perceived Service Quality of Front Line Employees (Studied on the Clerks of the Keshavarzi Bank in the Province of Chaharmahal-EBakhtiary). *International Journal of Academic Research in Business and Social Sciences*, 2012; 3(5).
2. Axelsson, L.T. Lactic Acid Bacteria: Clasification and Physiology. Di dalam: Lactic Acid Bacteria: Microbiological and Functional Aspect Third Edition. Salminen S, von Wright A dan Ouwehand A(Eds). Marcel Dekker Inc. New York, 2004.
3. Bergey's Manual of Determinative Bacteriology., 7th ed. Edited by R. S. Breed, E. G. D. Murray and N. R. Smith. Baltimore: The Williams and Wilkins Co., 1957.
4. Hasinah H, Haniwirawan E., *Pemanfaatan penciri gen κ-kasein untuk seleksi pada sapi dan kerbau. Prosiding Seminar dan Lokakarya Nasional Usaha Ternak Kerbau.* 2007 Jun 22-23; Jambi, Indonesia. Jambi (ID): Pusat Penelitian dan Pengembangan Peternakan, 2007.
5. Kanmani P, Kumar RS, Yuvaraj N, Paari, Pattukumar V, Arul V., Comaprison Ofantimicrobial Activity of Probiotic Bacterium Streptococcus phocae P 180, Enterococcusfaecium MC 13 and Carnobacterium Divergens Against Fish Pathogen. *World J Dairy & Food Sci*., 2010; 5: 145-151.
6. Kesarcodi W.A, Kaspar H, Lategan M.J, dan Gibson L., Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes.*Aquaculture*, 2008; 274: 1–14.
7. Khotimah K, Kusnadi J, Aktivitas Antibakteri Minuman Probiotik Sari Kurma (Phoenix dactilyferaL.) Menggunakan Lactobacillus plantarum dan Lactobacillus casei. *Jurnal Pangan dan Agroindustri*, 2013; 2(3): 110-120.
8. Periadnadi dan Nurmiati, *Bakteri Indigenous pada Buah-Buahan Tropis*, 2010.
9. Jurusan Biologi FMIPA UNAND. Padang. (Unpublished).
10. McKane, L and J. Kandel., *Microbiology, essential and Aplication.* 2nd. Ed., 1998.
11. McGraw-Hill, Inc. Philadelphia.
12. Reid G, Jass J, Sebulsky T, McCormick JK, Potential uses of probiotics in clinical practice. Clin Microbiol, 2003; 16: 658-72.
13. Vine, N.G., W.D. Leukes, H. Kaiser, S. Daya, J. Baxter & T. Hecht., Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. *J. Fish Dis*., 2004; 27: 319–326.
14. Volk., *Mikrobiologi Dasar*. Erlangga. Jakarta, 1988.
15. Yulinery, T. Eko, Y. dan Novik, N., *Uji Fisiologis Probiotik Lactobacillus* sp. *Yang Telah Dienkapsulasi dengan Menggunakan Spray Dryer untuk Menurunkan Kolesterol*. Biodiversitas, 2006; 7(2): 118-122.