Isolation, Characterization, and Identification Lactic Acid Bacteria from Chicken Waste Faeces that Potential as Probiotics

Dr. Ir. ASTUTI, M.P

Department of Biology, Yogyakarta State University, INDONESIA

Abstract- The research is aimed at isolating, characterizing, and identifying lactic acid bacteria which have the potential as probiotic agent obtainedfrom the chyme of 34 days-old strain patriot broiler chicken intestine.

In order to reach the goal, pour plate and streak plate method on MRS (de Mann Rogosa Sharpe) are used to isolate the bacteria. It is done by adding $CaCO_3$ 0.5% as the indicator of the formation of clean zone around the colony. The incubation is conducted at 37ºC for 48 hours. The phenotypic characterization process of the obtained isolates are conducted by gram painting, morphology, catalyst test, temperature influence test, pH and salinity toward the growth, fermentation test, and acid formation test from the carbon source. The identification to find out the possibility of BAL genus and species is conducted through profile matching based on the phenotypic characteristic traced by using Bergey's Manual of Determinative Bacteriology. Meanwhile, the potential test on its possibility as probiotic agent is conducted by doing the acid resistance test. The production of lactic acid of each isolate of lactic acid bacteria is measured when the bacteria is already 24 hours-old.

The result of the research is 15 isolates of lactic acid bacteria. After the confirmation test is conducted, the characteristics show that there are 4 genuses of lactic acid bacteria, i.e. lactobacillus, pediococcus, streptococcus, and enterococcus. The result of the BAL test in producing the lactic acid shows that the AST 04 isolate has the highest amount of lactic acid, 57.69 %, followed by AST 05 which has 9.41 % lactic acid. The BAL isolate of those chicken faces waste still can grow until the concentration of bail salt reaches 0.5 %.

Index Terms- lactic acid bacteria, chyme, broiler chicken, probiotic, isolate

I. INTRODUCTION

The effect of probiotic bacteria toward the decreasing of cholesterol rate was estimated due to its ability to assimilate cholesterol and deconjugate bile salt (Gilliland and Speek, 1977; Gilliland *et al.,* 1985). Lactic acid bacteria which have specific ability will work effectively if it can stand in the condition of digestion system. Therefore, the strain of lactic acid bacteria must be able to stand the bile salt and the acid condition of the stomach (pH 1-2). The potential BAL strain that will be made commercial as probiotic products must have high viability and must be stable during the process. Some of the production processes that use freeze drying or spray drying may cause the decreasing of cell viability and affect the products.

Gilliland (1990) and Ray (1996) state some requirements in choosing lactic acid bacteria that will be used as probiotic, i.e: grow and can live in food or sample before it is consumed, can live after passing the digestion system, resistant toward stomach acid, antibiotic, lissome, give beneficial effect toward the intestines, produce acid in a large number, and are able to produce other antimicrobe components, besides the lactic acid, which are effective in blocking pathogen bacteria.

Gillian (1979) cited by Oh *et al.*, (2000) explains that the digestion system of human and animal is quite a hard environment for the development of bacteria. It is because it contains chyme, digestion enzymes, and bile acid. This condition affects the life of probiotic strain. Pereira and Gibson (2002) explains that in order to give a beneficial effect, the probiotic culture must be able to endure inside the digestion system and tolerant toward the concentration of bile solution inside the small intestine. The ability to stay in 3.0 pH for 2 hours and grow inside a medium which contains1.000 mgs/l bilesolutions is used as a standard in considering the microbe culture tolerance energy toward the acid and bile solution.

This research was aimed at finding out whether lactic acid bacteria (BAL) obtained from chicken feces can be used as probiotic agent which is able to assimilate and deconjugate bile salt and whether this bacteria can decrease the cholesterol of broiler chicken meat. It was done by keeping the chickens for 42 days and giving them lactic acid bacteria culture by using freeze drying method.

The research needed to be done in order to find out the BAL of the chicken feces and the effect of its usage as probiotic toward the decreasing of the cholesterol rate of broiler chicken meat. Besides, by doing this research, it was hoped that there will be a much healthier broiler chicken poultry because the cholesterol rate is not too high. The aim of the first year research was to isolate, select, and test the characterization of lactic acid bacteria obtained from chicken feces; determine the fermentation type of the lactic acid bacteria; determine the catalyses type of the lactic acid bacteria; endurance test of the lactic acid bacteria toward bile salt; fermentation kinetic test of the lactic acid bacteria; lactic acid rate test of the lactic acid bacteria.

By doing some selection process, it was hoped that some potential lactic acid bacteria strain that have the characteristics as probiotic can be obtained. The chosen strain later would be applied into some fermentation products.So, later those foodswould be probiotic food that can give good effect toward

the body and can decrease the rate of LDL cholesterol and also the cholesterol rate of broiler chicken meat. Another aim of this research was that later there can be some healthier chicken broiler poultry because the cholesterol rate is low. This research would also beneficial for the development of science, particularly in poultry subject.

II. EXPERIMENTAL RESEARCH MATERIALS AND METHOD

The production of superior BAL isolate as the inoculums

Purification. It is done by removing every species of the separated colony in seaweed MRS media by using quadrant scratch method. The obtained colony later will be taken as the pure culture, and then the culture will be planted in liquid MRS.

Lactic Acid Bacteria Characterization Technique

The colony that grows and pure will be characterized based on the characteristics of its morphology, biochemical test, physiological test, and fermentation type.

a. Morphological Characteristics

- **Colony morphological form.** The colony forms clean and separated zone on seaweed plate (the colony which is estimated as lactic acid bacteria). The form, surface, edge, and pigmented color of the colony are observed.
- **Gram Staining, Cells Morphology (Benson, 2001**
- **Motility Test (Benson, 2001)**

b. Biochemical Characteristics

- **Catalyses**
- **Fermentation Type**

Lactic Acid Bacteria Identification Technique

Bacteria isolate that is already characterized and fulfill one of lactic acid bacteria characteristics, i.e. form clean zone (produce acid compound), is later identified based on *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*, 1994) by Profile Matching to estimate the possibility of the bacteria's genus and species based on the referenced lactic acid bacteria's genus and species.

Isolate Ability Test as Probiotic

In order to find out the ability of lactic acid bacteria isolate as probiotic agent, chosen BAL test is done on the fermentation kinetic and the endurance of bile salt.

- **Fermentation Kinetic**
- **Endurance test on bile salt**
- **Observed Variable**
- **Selected Isolate Characteristics**

The test toward the obtained isolate characteristics are the 24thhour pH, catalyses type, gram staining, morphological form, and the rate of the lactic acid.

- **Fermentation Kinetic**
  - **Growth Pattern**.
  - **Specific Growth Speed.**
  - **pH reduction**.
- **Endurance Test toward Bail Salt**
  - **Growth Pattern**.
  - **Specific Growth Speed**.
  - **pH reduction**.
- **Lactic acid production**.
- **Lactic Acid Rate Examination**
- **Lactic Acid Isolation Phase**.
- **Determining phase of the sample's lactic acid rate**.
- **Data Analysis**

III. RESULTS AND DISCUSSION

A. The result of the research

In this research, the isolation, characterization, and identification of lactic acid bacteria obtained from strain Lohman broiler chicken feces are administered.

1. Lactic acid bacteria isolation obtained from chicken feces

10 grams of chicken's internal digestion system is dissolved with 90 ml of sterile aquades inside Erlenmeyer. Administer series of dissolving 10-2, 10-4, and 10-6. The result of the dissolution is 20 ml is grown in dense medium by using MRS in the Petri dish.

The separated colony is taken by using *ose* and grown in dense medium by using scratching method and incubated in 39ºC in anaerob condition for 24 hours. After 24 hours incubation, take separated colony by using *ose* and grown in liquid medium and then incubated for 24 hours at the temperature of 39ºC. Lactic acid bacteria (BAL) isolation is administered by using pour method (pour plate) in MRS plate seaweed media + CaCO3 0.5 % which is the beginning step to differentiate lactic acid bacteria with non lactic acid bacteria. Lactic acid bacteria colony in MRS plate seaweed media + CaCO3 0.5 % is recognized by the appearance of clean zone around the colony of the bacteria. Each colony which has different appearance is isolated and purified. The purification is done by using scratching method (streak plate) in MRS plate seaweed + CaCO3 0.5 % until the pure isolate is obtained. The obtained lactic acids bacteria isolate are 15 isolates.

The colony morphology of 15 obtained lactic acid bacteria is observed on plate seaweed related to its color, form, elevation, edge, and structure in the colony. Most of the lactic acid bacteria colony is white and circular. The shape of most of the colony edge is entire and the internal structure is opaque. For the details of the features or the characteristics of lactic acid bacteria colony see table 1.

Table 1. Colony morphological observation on 24 hours lactic acid bacteria in MRS spread plate and poure plate medium.

No.	Code	Colony Color	Colony Edge	Internal Structure	Colony Shape	Pigmen Color
1	AST 001	Milky white	entire	opaque	circular	clear
2	AST 002	Milky white	entire	Opaque	circular	clear
3	AST 003	Milky white	entire	Opaque	circular	clear
4	AST 004	Milky white	entire	Opaque	circular	clear
5	AST 005	Milky white	entire	Opaque	circular	clear
6	AST 006	Milky white	entire	Opaque	circular	clear
7	AST 007	Milky white	entire	Opaque	circular	clear
8	AST 008	Milky white	entire	Opaque	circular	clear
9	AST 009	Milky white	entire	Opaque	circular	clear
10	AST 010	Milky white	entire	Opaque	circular	clear
11	AST 011	Milky white	entire	Opaque	circular	clear
12	AST 012	Milky white	entire	Opaque	circular	clear
13	AST 013	Milky white	entire	Opaque	circular	clear
14	AST 014	Milky white	entire	Opaque	circular	clear
15	AST 015	Milky white	entire	Opaque	circular	clear

Isolation is a process to get a particular kind of bacteria from many sources. The isolation process in this research is focused on lactic acid bacteria. Lactic acid bacteria are defined as bacteria that have high ability in producing lactic acid from any fermented carbohydrate sources.

Lactic acid bacteria isolation is taken from the sample of patriot broiler strain chicken's small intestine. It uses seaweed MRS medium CaCO3 0.5 % by pour plate method. In this phase, the dilution series is administered. The series starts from 10-10 and then 10-5, 10-6, 10-7 and 10-8, 1 ml of the dilution is taken and poured into Petri dish. Pour MRS media and CaCO3 0.5 % and spread it. The aim of this dilution is to obtain bacteria which the growth is not too dense. So, there are some differences between the colonies.

The addition of CaCO3 5 % in the media is aimed for selecting lactic acid bacteria. The lactic acid bacteria that grow in the media is recognized by the appearance of clean zone around the colony. According to Harimurti (1999: 376), the clean zone around the lactic acid bacteria is formed as the result of acid neutralization which is produced by the bacteria by the CaCO3 in the media. The clean zone has initial characteristics in selecting lactic acid bacteria. Each of the colonies that have different appearance is isolated and purified by using scratch method (Streak plate) in seaweed MRS media until the pure isolate is obtained. Every isolate bacteria are then culturally purified into oblique seaweed medium and liquid medium.

2. Lactic acid bacteria isolate characteristics

Characterization is a process to observe and measure each part of bacteria as a special characteristic of the bacteria. The characterization of each lactic acid bacteria isolate involves its morphology (macroscopic, microscopic), biochemical and physiology. Macroscopic characterization is done by inoculating lactic acid bacteria in MRS medium seaweed plate to find out the morphological characteristics of the colony that related to its color, shape, elevation, edge, and internal structure. Colony morphological characterization is also done in oblique seaweed MRS related to its shape and the growth of lactic acid bacteria.
Those 15 lactic acid bacteria which are successfully isolated is characterized related to its cell, biochemical characteristics, and physiological characteristics. The characteristics group of the cells morphology includes some characters, i.e cells form, cells arrangement, and gram reaction/gram staining and motility.

The biochemical characteristic group includes character unit, i.e. catalyses test, fermentation test. Almost 90 % of lactic acid bacteria have coccus or circular cells. The others are bacil or bar and the sequence of the cell is tetrad and solitaire. Lactic acid bacteria isolate shows positive reaction toward gram staining test. However it shows negative and positive reaction toward motility test (non-motil).

The result of the research related to the biochemical characteristics of lactic acid bacteria isolate shows that the 15 lactic acid bacteria are negative catalyses and there are 9 isolate of homofermentative fermentation type, i.e. AST 01, AST 04, AST 06, AST 07, AST 09, AST 13, and AST 14. The lactic acid bacteria are able to form acid from any sources of carbon. However, it does not followed by the formation of any gases.

Table 2. Characteristic test on cells form and also biochemical characteristic by administering catalyses test and gram staining

No	Isolate Code	Characteristics Type		
		Cells Shape	Gram Nature	Catalyses
1	AST 001	Coccus	(+)	(-)
		Coccus	(+)	(-)
2	AST 002	Basil	(-)	(-)
		Basil	(-)	(-)
3	AST 003	Basil	(+)	(-)
		Basil	(+)	(-)
4	AST 004	Basil	(+)	(-)
		Basil	(+)	(-)
5	AST 005	Coccus	(-)	(-)
		Coccus	(-)	(-)
6	AST 006	Coccus	(-)	(-)
		Coccus	(-)	(-)
7	AST 007	Coccus	(+)	(-)
		Coccus	(+)	(-)
8	AST 008	Coccus	(+)	(-)
		Coccus	(+)	(-)
9	AST 009	Coccus	(+)	(-)
		Coccus	(+)	(-)
10	AST 010	Coccus	(+)	(-)
		Coccus	(+)	(-)
11	AST 011	Coccus	(-)	(-)
		Coccus	(-)	(-)
12	AST 012	Basil	(+)	(-)
		Basil	(+)	(-)
13	AST 013	Coccus	(-)	(-)
		Coccus	(-)	(-)
14	AST 014	Basil	(-)	(-)
		Basil	(-)	(-)
15	AST 015	Coccus	(+)	(-)
		Coccus	(+)	(-)

Microscopic characterization is done to find out the cells' morphological characteristic. The cells morphology is observed until the result of lactic acid bacteria colony morphological observation shows many similarities on the characteristic of the isolate. The similarity can be seen from its color, shape, and internal structure. By looking at the observation result table (Table 1), it can bee seen that there are 2 colony color, white and milky white. Almost 70 % of the lactic acid bacteria isolate colony is milky white. Most of the colony shape is circular or circle while the others are curled or curly. The edge of lactic acid bacteria isolate colony is entire type or flat while the others are undulate or wavy. 99 % of the lactic acid bacteria colony has internal structure which is opaque while the others have finely granular structure.

Table 3. Biochemical character test through fermentation test

Isolate Code	Fermentation Testst	Notes
AST 001	(+)	Redish yellow
AST 002	(+)	Clouded yellow
AST 003	(+)	Clouded yellow
AST 004	(+)	Clouded yellow
AST 005	(+)	Clouded yellow
AST 006	(+)	Redish yellow
AST 007	(+)	Clouded yellow
AST 008	(+)	Clouded yellow
AST 009	(+)	Redish yellow
AST 010	(+)	Clouded yellow

AST 011	(+)	Redish yellow
AST 012	(+)	Clouded yellow
AST 013	(+)	Redish yellow
AST 014	(+)	Redish yellow
AST 015	(+)	Clouded yellow

Through glucose fermentation test, it is found out that all isolates can produce acid. However, all bacteria isolate cannot produce gas, so they can be grouped into homofermentative acid lactic bacteria. It means that the main fermentation product is only lactic acid (Axelsson, 2004: 20). Biochemical characterization phase is administered by completing some series of test, i.e. catalyses test, fermentation type, acid and gas formation test taken from any carbon sources.

Table 4, Biochemical test and physiological observation of 24 hours old lactic acid bacteria

No.	Code	Motility	Gas production	Fermentation type
1	AST 001	Non mtl	-	Homofer
2	AST 002	Non mtl	-	Homofer
3	AST 003	Non mtl	+	Heterofer
4	AST 004	Non mtl	-	Homofer
5	AST 005	Non mtl	+	Heterofer
6	AST 006	Non mtl	-	Homofer
7	AST 007	Non mtl	-	Homofer
8	AST 008	Non mtl	+	Heterofer
9	AST 009	Non mtl	-	Homofer
10	AST 010	Non mtl	-	Homofer
11	AST 011	Non mtl	-	Homofer
12	AST 012	Non mtl	-	Homofer
13	AST 013	Non mtl	-	Homofer
14	AST 014	Non mtl	+	Heterofer
15	AST 015	Non mtl	-	Homofer

Notes: Fk. anae : Homofer : homofermentatif:
Heterofer : heterofermentatif

The result of macroscopic characterization cannot be used as the standard to determine each genus yet because the similarities n each isolate, whether the colony characters on seaweed plate or on oblique seaweed. Therefore, there should be advanced characterization, i.e. microscopic characterization in order to find the cells' morphology of each lactic acid bacteria isolate.

Before observing the cells, each lactic acid bacteria is painted by gram staining to see the nature of cells wall and cells form. Gram staining differentiates two group of bacteria, positive gram bacteria and the negative ones. The result of the observation (see appendix), shows that the lactic acid bacteria are red and purple. So, they can be grouped into positive and negative bacteria. Most all of the isolate are coccus or circle, and only a few that is bacil or bar. Lactic acid bacteria isolate shows chain cells arrangement, pairs, solitaire and tetrad, and non-motile.

The result of the observation on Table 4 shows that all bacteria isolate shows negative reactions toward catalyses test. It can be said so because there is no any bubbles. Fermentation type test is used to grouped lactic acid bacteria into homofermentative or heterofermentative group. In order to determine fermentation type, the gas production test is administered, by growing the culture in 10 ml liquid MRS for 2 -3 days with Durham hole which is put backward to catch the gas.

According to Borck et al., (1994) as cited by Soetanto (2004), the differences between homofermentative and heterofermentative lactic acid bacteria can be seen on its product and also on the existence of aldolase enzyme which is one the main key enzyme in glicolisis.A Heterofermentative bacterium does not have any aldolase enzyme so it cannot break biphospate fructose into biphospate triosa. Heterofermentative bacteria oxidate 6-phospat glucose into phosphate pentose and then reduce it into phosphate triose which is helped by phosfoketolase enzyme.

1. Lactic acid bacteria identification

Identification is a process in determining the group of bacteria obtained from isolate from any sources based on the bacteria's phenotypic characteristic. The identification to determine the bacteria's genus and species possibility is administered by *Profile Matching* method, through *Bergey's Manual of Determinative Bacteriology* (Holt *et al*., 1994) based on the result of phenotypic characterization.

The selected bacteria isolate show the nature of lactic acid bacteria with positive gram character, non-motile, non endospore and negative catalyze (Axelsson, 2004). The shape character and cells formation is the differentiator character in genus level. After the genus is known, the advanced identification is administered to find out the possible species of the bacteria isolate.

Based on the form characters, bacteria cells formation, and some phenotypic characteristics which is found out through *Bergey's Manual of Determinative Bacterilogy* (Holt, *et al*., 1994) that the 15 lactic acid bacteria isolate have the tendency of 4 lactic acid bacteria group, Lactobacillus, Pediococcus, Streptococcus, and Enterococcus.

Lactobacillus Genus

Isolate AST 03, AST 04, and AST 12 have bacil or bar cells so it includes in Lactobacillus genus. The member of this genus has relatively the same characteristics, negative catalyze, positive gram, bacil cell, 0.5 – 1.2 x 1.0 – 10.0 µm, homofermentative, non-motile, cells formation is solitaire/pair/chained, the color of the colony is white/milkywhite, the colony edge is entire, the shape of the colony is circle, the internal structure is opaque, the growth in oblique seaweed is dense/abundant and the shape is beaded. It can grow in 45ºC and also produce lactic acid from any carbon sources, galactose, lactose, maltose, sucrose, glucose, fructose, and sorbitol.

Based on the *Profile Matching*identification result all of the above phenotypic natures tend to show that the Lactobacillus genes are the member of Lactobacillus acidophilus species. However to make sure the correctness, some key characteristics are needed and they can be seen in *Bergey's Manual of Systematic Bacteriology* to determine the lactic acid bacteria isolate's species level.

Pediococcus Geneus

It includes isolate AST 01, AST 07, AST 08, and AST 15. The result of gram staining shows positive gram, negative catalyses, coccus cells, homofermentative, non-motile, the formation of the cells is tetrad/pair with diameter 0.5 – 2.0 µm, the colony color is white / milky white, colony edge entire, circle colony shape, opaque internal structure, the growth of oblique seaweed is moderate, and has beaded/filliform shape.

To determine the possibility of the lactic acid bacteria isolate, Profile Matching through *Bergey's Manual of Determinative Bacteriology* is used.

Enterococcus Genus

It includes isolate AST 06, AST 11, and AST 13. The characteristics are negative gram, negative catalyses, coccus cells, 0.6 – 2.0 x 0.6 – 2.5 µm, the formation of the cells is pair/chain, homofermentative fermentation type, non-motile, negative gas production (none), milky white colony color, circle colony shape, entire colony edge, opaque internal structure, the growth of oblique seaweed is thin/slight and has effuse/spreading/filliform shape.

Streptococcus Genus

The identification of Streptococcus includes isolate AST 09 and AST 10 shows that they have positive gram, coccus cell diameter 0.5 – 2.0 µm, chained/paired cells formation, negative catalyses test, non-motile, homofermentative, milky white colony color, circle colony shape, entire edge, and opaque internal structure, the growth of oblique seaweed is moderate/thin/slight and has filliform shape.

2. Determining the rate of lactic acid on each bacteria isolate

After some series of test on each isolate, the next test is a test to find out the ability of lactic acid bacteria in producing lactic acid. Lactic acid is product/compound which is produced by lactic acid bacteria during the carbohydrate fermentation process. The higher the lactic acid production the better the result compared to lactic acid bacteria isolate which has low ability in producing lactic acid. The measurement of the lactic acid is administered on 24 hours old isolate pure culture. Based on the measurement result, it is found out that isolate AST 04 has the highest lactic acid: 57.68 %. Therefore it can be used as probiotic because the lactic acid can be used to prevent the growth of pathogen bacteria. Organic acid like lactic acid and acetate acid produced by lactic acid bacteria as the result of lactose fermentation can help digestive and absorption system.

The observed and tested isolates whether tested morphologically, biochemical, and physiologically and those that have the nature or characteristics of lactic acid bacteria, are measured to find the rate of the lactic acid when the bacteria isolate are 24 hours old. The highest lactic acid is produced by AST 04: 57.68 %, while the least lactic acid produced by AST 05: 9.41 %.

Table 5. The rate of lactic acid on each 24 hours old lactic acid bacteria isolate

No.	Code	Absorbance Mark	Lactic rate (%)
1	AST 001	0,225	22,25%
2	AST 002	0,401	40,49%
3	AST 003	0,319	31,99%
4	AST 004	0,567	57,69%
5	AST 005	0,101	9,41%
6	AST 006	0,176	17,17%
7	AST 007	0,392	39,56%
8	AST 008	0.432	43,71%
9	AST 009	0,247	24,53%
10	AST 010	0,290	28,99%
11	AST 011	0,317	31,78%
12	AST 012	0,301	30,13%
13	AST 013	0,125	11,89%
14	AST 014	0,281	28,05%
15	AST 015	0,198	19,45%

On the other hand, the produced lactic acid of lactic acid bacteria during the fermentation process can change the taste and aroma of the food and at the same time the growth of harming bacteria can be prevented. It is because lactic acid bacteria compete with pathogen microbe or any unwanted organism (Calo-Mata *et al*,. 2008).

3. Fermentation Kinetic Test

The fermentation pattern of selected BAL isolate can be found by administering observation on the change of liquid medium density which is already inoculated by BAL in every

observing hour. The average mark of BAL growth density medium in any glucose concentration can be seen in table 3.

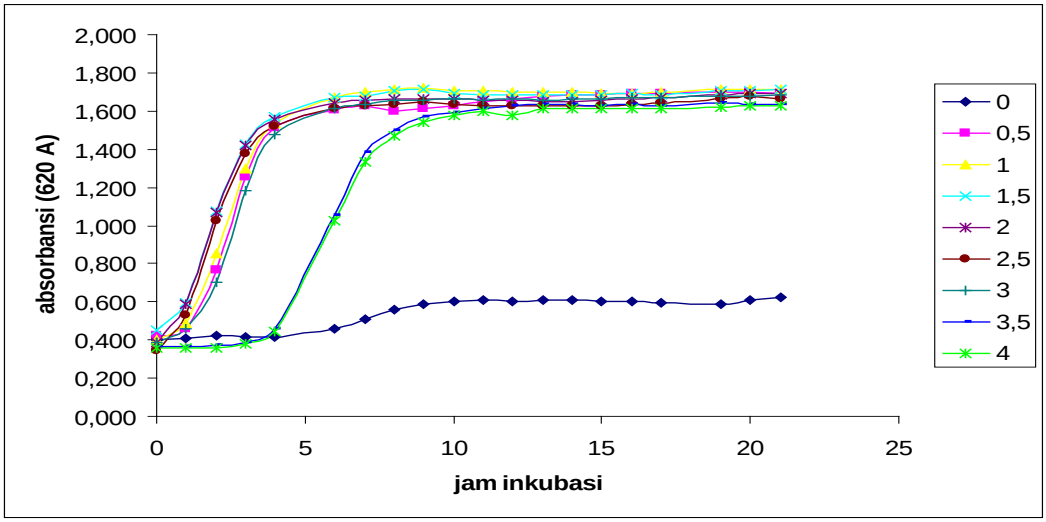
Table 3. Average BAL growth density medium in any substrate concentration

Hour	Substrate Concentration (%)								
	0	0,5	1	1,5	2	2,5	3	3,5	4
0	0,404^{a}	0,424^{a}	0,401^{a}	0,455^{a}	0,390^{a}	0,343^{a}	0,390^{a}	0,368^{a}	0,360^{a}
2	0,421^{b}	0,767^{b}	0,855^{b}	1,074^{b}	1,070^{b}	1,025^{b}	0,700^{b}	0,370^{a}	0,356^{a}
4	0,419^{b}	1,514^{c}	1,535^{c}	1,569^{c}	1,558^{c}	1,521^{c}	1,474^{c}	0,457^{b}	0,441^{b}
6	0,460^{c}	1,607^{d}	1,668^{d}	1,668^{d}	1,645^{d}	1,614^{d}	1,612^{d}	1,057^{c}	1,028^{c}
8	0,561^{d}	1,597^{d}	1,714^{e}	1,706^{d}	1,660^{d}	1,636^{de}	1,653^{e}	1,057^{c}	1,469^{d}
10	0,599^{e}	1,627^{d}	1,705^{e}	1,692^{d}	1,664^{de}	1,632^{de}	1,662^{e}	1,594^{e}	1,574^{e}
12	0,604^{e}	1,663^{e}	1,702^{e}	1,686^{d}	1,659^{d}	1,630^{de}	1,662^{e}	1,627^{f}	1,574^{e}
14	0,607^{e}	1,683^{ef}	1,697^{e}	1,684^{d}	1,652^{d}	1,629^{de}	1,664^{e}	1,635^{f}	1,614^{e}
16	0,600^{e}	1,690^{ef}	1,694^{de}	1,691^{d}	1,662^{de}	1,634^{de}	1,667^{e}	1,634^{f}	1,613^{e}
20	0,610^{e}	1,696^{f}	1,711^{e}	1,709^{d}	1,691^{e}	1,674^{e}	1,678^{e}	1,635^{f}	1,624^{e}

The BAL growth is shown by the increasing medium density in accordance with the increasing rate of the substrate and the length of incubation. Significant growth ($P<0.05$) starts at the 2nd hour on concentration 0.5 %, 1.0 %, 1.5 %, 2.0 %, 2.5 %, and 3.0 % until at the 6th hour. Meanwhile, on concentration 3.5 % and 4.0 % there are still some adaptation toward the new growing media. At the 8th hour, there is a significant growth ($P<0.05$) on substrate concentration 0 %, 1.0 %, 3.0 %, and 4.0 %, while there is no significant growth on concentration 0.5 %, 1.5 %, 2.0 %, 2.5 %, and 3.5 %. Starting at the 10th hour, there is no more significant growth on all substrate concentration. According to Madigan et al., (1998) the microbe growth pattern consists of 4 phases, i.e. slow phase (lag phase) which is often called as adaptation phase because the microbe is adapting with the new environment where the BAL grows. The second phase is exponential phase which is marked by the very fast growth of the fermentation cells and product. The third phase is stationary phase where the growth of the cells is steady, or it also can be said as the decreasing of the growth and the primary product. The last phase is death phase. It happens when the incubation is continued after the growth phase already reaches stationary phase, it is marked by the lyses process of the bacteria.

Based on the explanation above, it can be concluded that lag phase only happens until the 2nd hour. It is only short because the nutrient in the enrichment and growth medium are almost the same (Fardiaz, 1988). Exponential phase happens for around the 8th hour while the stationary phase starts at the 10th hour.

BAL growth pattern can be seen in the picture below.



Picture 4. BAL kinetic growth in glucose substrate.

The data and picture of growth kinetic above shows that adaptation phase happens quickly in glucose concentration 0.5 5 – 3.0 %. Meanwhile, the adaptation phase in 0 %, 3.5 %, and 4.0 % glucose concentration happens quite a while. According to Fardiaz (1998) the length of the adaptation is caused by some factors, the growth medium and environment and also the number of inoculums.

Fardiaz (1998) also states that exponential phase is very affected by pH and nutrient of the medium, includes its environment and temperature. In this phase, the microbe needs more energy than in the other phases. In stationary phase, the cell population number is steady because the number of growing cells is the same with those that die. The cells size in this phase is smaller because the cells keep splitting although there are no more nutrients left. Because of the lack of nutrient, it is possible that the cells will have different composition with those that grow in exponential phase. In death phase, half of the microbe population starts to die because there are no more nutrient in the medium or there are no more spare energy in the cells. The speed of the death depends on the condition of the nutrient, the environment, and the microbe species.

Picture 1 shows that the higher glucose substrate concentration, the slower the BAL growth speed. It is shown by the long duration of adaptation phase on substrate concentration above 3.0 %. While the long adaptations phase in 0 % substrate concentration is caused by the inexistence of the needed substrate for growth. BAL growth is affected by the rate of nutrient in its environment. The medium and environment growth are the same as the previous medium and growth environment so adaptation phase is not needed (Fardiaz, 1987). The medium used for BAL enrichment is MRS while the medium used for growth kinetic is basalt medium.

The growth speed will increase due to the increasing of substrate rate until critical substrate rate. If the substrate rate is increased, the growth speed will decrease (Wibowo et al., 1987). According to Wang *et al.*, (1978) in increasing substrate concentration there will be growth blocking effect by the substrate. It is related to the specific component blocking effect in the enzyme or cells structural component.

Specific growth speed. BAL specific growth speed in the glucose contained medium is obtained byanalyzing the regression between the density and the incubation time in exponential phase on each substrate concentration treatment so the equation y = a + bx is obtained. B is slope that shows specific growth speed. According to Wang *et al.*, (1979) the increasing of medium density shows the increasing of cells number where OD + 1 equal to 1 – 1.5 g BK cells. After counting through estimation that OD = 1 equal to 1 g BK cells, the specific growth for each treatment (0.5 %, 1.5 %, 2.0 %, 2.5 %, 3.0 %, 3.5 %, and 4.0 %) continuously is 0.13 g BK cells/hour, 0.14 g BK cells/hour, 0.11 g BK cells/hour, 0.12 g BK cells/hour, 0.15 g BK cells/hour, 0.17 g BK cells/hour, and 0.16 g BK cells/hour. 0.5 g BK cells/hour, 0.17 g BK cells/hour, and 0.16 g BK cells/hour.

4. BAL AST-04 Isolate Endurance Test toward Bail Salt

It is only administered on selected main BAL isolate (AST 04 isolate) by using broth MRS liquid medium (Pronadisa) in batch fermentation system. The active BAL isolate is grown in enrichment medium by using broth MRS for 24 hours and then 1 % (v/v) is inoculated into broth MRS medium treated with bail salt concentration (Oxoid bile salt) 0 %, 0.1 %, 0.2 %, 0.3 %, 0.4 %, and 0.5 %. Each treatment is done three times, and then incubated in 39ºC and observe the growth by measuring the density (OD) by using 650 nm spectrophotometer (Pereira and Gibson, 2002). The observation is held every hour until a constant density is reached. Then the pH of the sample is measured by using pH-meter (Nahm, 1992). On the peak of exponential phase, the production of lactic acid is determined to make sure that the decreasing of pH medium is caused by the high production of lactic acid. The measurement of lactic acid production is done by using Baker and Summerson method (Hawk's, 1976).

Table 6. Average BAL growth density in some bail salt concentration.

Hour-	0	0.1	0.2	0.3	0.4	0.5
0	0.13 ^{a}	0.14 ^{a}	0.14 ^{a}	0.14 ^{a}	0.13 ^{a}	0.14 ^{a}
0.5	0.16 ^{a}	0.14 ^{a}	0.16 ^{ab}	0.16 ^{ab}	0.13 ^{a}	0.14 ^{a}
1	0.22 ^{b}	0.16 ^{b}	0.19 ^{b}	0.20 ^{b}	0.15 ^{a}	0.14 ^{b}
2	0.44 ^{c}	0.25 ^{c}	0.33 ^{c}	0.35 ^{c}	0.20 ^{b}	0.17 ^{c}
3	0.82 ^{d}	0.51 ^{d}	0.61 ^{d}	0.69 ^{d}	0.36 ^{c}	0.25 ^{d}
4	1.04 ^{e}	0.79 ^{e}	0.81 ^{e}	0.89 ^{e}	0.56 ^{d}	0.62 ^{e}
5	1.22 ^{f}	1.05 ^{f}	0.99 ^{f}	1.05 ^{f}	0.78 ^{e}	0.84 ^{f}
6	1.23 ^{f}	1.12 ^{g}	1.05 ^{g}	1.08 ^{fg}	0.88 ^{f}	0.92 ^{g}
7	1.26 ^{fgh}	1.19 ^{h}	1.08 ^{gh}	1.10 ^{fg}	0.92 ^{g}	0.94 ^{h}
8	1.26 ^{fgh}	1.20 ^{gh}	1.09 ^{gh}	1.12 ^{g}	0.94 ^{gh}	0.97 ^{i}
9	1.26 ^{fgh}	1.21 ^{ij}	1.11 ^{hi}	1.19 ^{h}	0.97 ^{h}	1.04 ^{j}
10	1.26 ^{fgh}	1.23 ^{jk}	1.13 ^{ijk}	1.21 ^{h}	1.03 i	1.09 ^{k}
11	1.27 ^{ghi}	1.23 ^{jk}	1.20 ^{l}	1.32 ^{i}	1.08 ^{j}	1.16 ^{l}
12	1.25 ^{fgh}	1.24 ^{kl}	1.25 ^{m}	1.35 ^{ij}	1.13 ^{k}	1.23 ^{m}
13	1.32 ^{ij}	1.26 ^{lm}	1.34 ^{m}	1.40 ^{j}	1.17 ^{l}	1.30 ^{n}

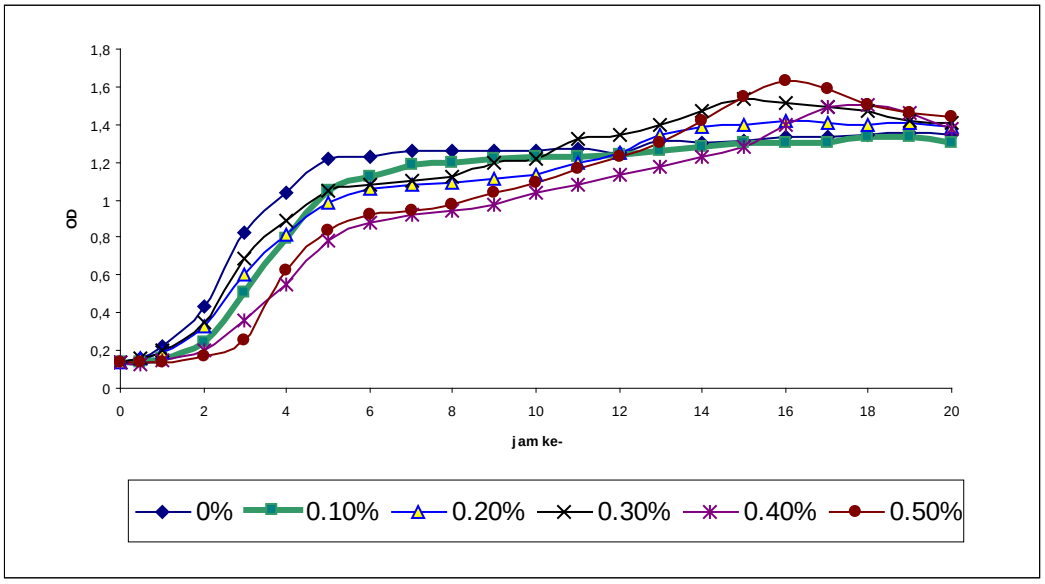
14	1.30 ^{hij}	1.28 ^{mn}	1.39 ^{m}	1.47 ^{mn}	1.23 ^{m}	1.42 ^{q}
15	1.32 ^{ij}	1.30 ^{no}	1.40 ^{m}	1.53 ^{o}	1.28 ^{n}	1.54 ^{s}
16	1.33 ^{j}	1.31 ^{op}	1.42 ^{m}	1.52 ^{no}	1.40 ^{o}	1.63 ^{k}
17	1.34 ^{j}	1.31 ^{op}	1.40 ^{m}	1.49 ^{no}	1.49 ^{pq}	1.58 ^{p}
18	1.35 ^{j}	1.33 ^{p}	1.40 ^{m}	1.47 ^{lmn}	1.51 ^{q}	1.50 ^{o}
19	1.35 ^{j}	1.33 ^{p}	1.41 ^{m}	1.42 ^{klm}	1.46 ^{p}	1.46 ^{n}
20	1.35 ^{j}	1.30 ^{p}	1.39 ^{m}	1.41 ^{kl}	1.38 ^{o}	1.44 ^{d}

The growth pattern of BAL AST-4 in medium that contains bail salt is measured by observing the density change in liquid medium every hour. The microbe growth in liquid medium is shown by the increasing of density and the emerging of sediment in the base of the tube (Gupte, 1990). The average density of BAL AST-4 growth medium in some bail salt concentration is shown in table 6 and graph 2.

Growth Pattern. The average observation result of BAL growth pattern observation in the medium that contains bail salt in different concentration can be seen in table 6. The data in table 6 shows that the density increases significantly ($P<0.05$) in accordance with the incubation length. It shows that the BAL grows in the medium that contains bail salt.

In table 6, it can be seen that the density is increasing on all treatment of fermentation. It shows that BAL AST-4 are able to grow in medium that contains bail salt. The growth is shown by the density increasing for all treatments. BAL AST-4 are still able to grow in medium with 0.5 % bail salt. In table 3, it can be seen that the growth of BAL AST-4 in bail salt containing medium, it is shown by the increasing of the density in accordance with the increasing duration of the incubation. The density increases insignificantly until at the 2nd hour, and then keep increasing significantly ($P<0.05$) until at the 20th hour. It shows that the BAL is growing. According to Harjo et al., (1988), the microbe growth consists of 3 phases, slow phase (lag phase) which is often called as adaptation phase because the microbe is adapting with its environment, in this case is the medium where the lactic acid bacteria grow. The second phase is exponential phase which is shown by steady growth cells and as the start of the decreasing growth process and its primary product.

The adaptation phase happens until the 2nd hour and followed by exponential phase until the 20th hour. The stationary phase cannot be seen at the 20th hour because the density is already increasing. It is because the BAL AST-6 that shows any growth is decreasing.



PPicture 2. BAL growth graphics in MRS media by adding bail salt.

Based on the obtained result in picture 2, it can be seen that in lag phase the growth of BAL AST-4 only happens until the first 30 minutes. It happens in a short time because what is used in the activation process is the same as those used in growth medium. The contents of MRS medium nutrient can be seen in the appendix. Exponential phase starts at the 1st hour and after the exponential phase, microbe enters stationary phase. The increasing bail salt concentration in BAL AST-4 growth medium causes the decreasing density significantly ($P<0.01$).

Bail salt tend to block BAL growth so the higher the concentration of bail salt and added in the medium, the BAL growth will be blocked. According to Brand et al., (1976) as cited by Oh et al., (2000), bile acid shows blocking effect toward microbe growth and this blocking activity is larger compared to the other organic acids.

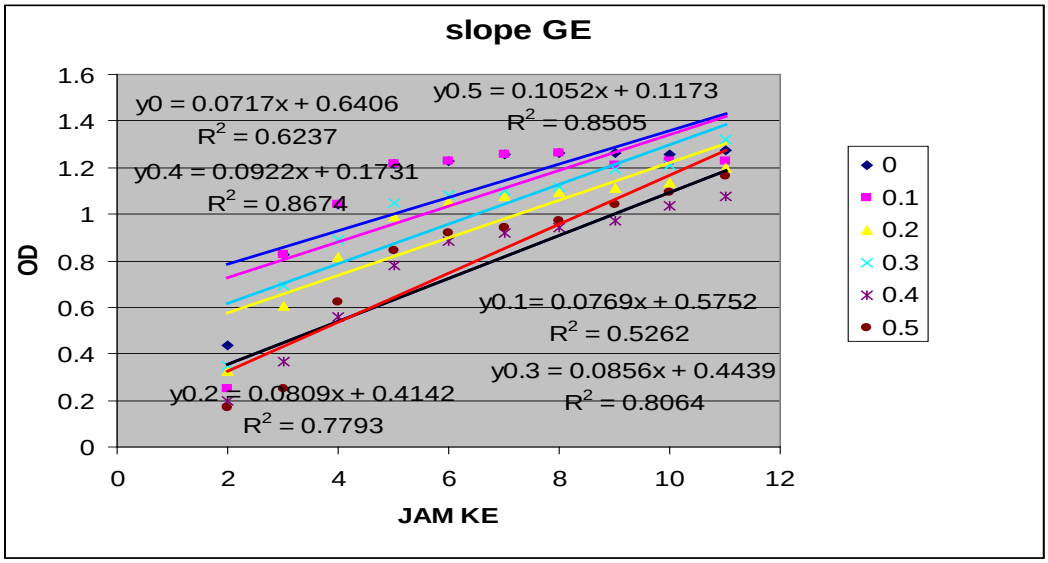
Dune (2001) states that bail salt shows antibacterial activity which block the growth of *Eschericia coli* strain, *Klebsiella* sp, and *Entercoccus* sp *in vitro*. Brand *et al*,. (1976) as cited by Oh *et al*., (2000) states that bail acid shows blocking effect toward the

growth of microbe and the blocking activity is much larger than the other organic acids.

According to Bezkorovainy (2001), probiotic resistance toward bail salt *in vitro* can be divided into two types, survival power (survivel) and growth. The variation of survival power depends on the concentration and the length of the interaction between the microbe and bail salt. The other researches on microbe growth in medium that contains bail salt connects other variables, i.e. the existence of unconjugate bail acid in the medium.The unconjugatebail acid is the agent that can lyses bacteria (bacterial lysing) better than conjugate bail acid. The decomposing bail salt (deconjugation) is administered by hydrolyses bile salt enzyme. The enzyme is produced by Lactobacilli and Bividiobacteria. Therefore, the increasing concentration of bail salt will increase the forming of unconjugate bail acid which can block the growth of BAL.

The existence of bail salt in the BAL medium growth is as an inhibitor. BAL in 3 % bail salt concentration can still show some growth even though it is significantly different with those without any control (without any bile salt).

If the density in endurance test of the bile salt is compared to the density of fermentation kinetic in glucose substrate, it can be seen that the average density of endurance test on bile slat is much higher. It is caused by the differences on the medium used. In endurance test on bile salt using MRS medium shows that is contains 2 % dextrin. Meanwhile on fermentation test, the used medium is basalt medium with glucose substrate. Rumen liquid in basalt medium in this research is substituted by H20 so that the used medium is clear and the density can be read. According to Hobson (1988), there are a lot of nutrient and growth factor in rumen liquid. The growth factor can be derived from the food or microbe recycling. By the replacement of rumen fluid with H20, the concentration of nutrient in basalt medium is decreasing and affects the growth of BAL. Fardias (1988) states that the growth of BAL is affected by the number of nutrient in the medium.



Picture 03. Bail Salt Slope Graphics.

Exponential phase for bail salt rate treatment are 0 %, 0.1 %, 0.2 %, 0.3 %, 0.4 %, and 0.5 % at 1-5, 1-7, 1-6, 1-14, 2-16, 1-15 hour. Exponential phase tend to be longer in accordance with the increasing rate of bail salt. However, if the growth slope is observed, the higher the bail salt rate in the slope medium, the lower the growth (See Picture 03).

The slower slope shows that there is a growth obstacle in the medium that contains bail salt. Noh et al., (2000) explains that bail salt shows a blocking effect toward the growth of microbe and this blocking activity is bigger than the other organic acids.

5. Degree of Acidity

The pH measurement is administered at 20th hour to find out the acid produced by BAL AST 4. In table 6s, it can be seen that in 20th hour observation, the pH medium is decreasing from the starting pH 6.5. The pH decreasing during the fermentation process is caused by the accumulation of fermentation products like acid lactic and other organic acids like acetate and propionate acid. Those organic acids are the final result of BAL glucose hydrolysis (McDonald, 1991).

Table 7. BAL fermentation pH in medium that contains 20th hour bile salt

Repetition	Bail Salt Rate					
	0%	0,1%	0,2%	0,3%	0,4%	0,5%
1	4,64	4,75	4,94	5,01	5,04	5,13
2	4,59	4,72	4,93	5,00	5,06	5,17
3	4,6	4,74	4,90	4,99	5,07	5,15
4	4,59	4,75	4,91	5,01	5,06	5,15

5	4,59	4,74	4,91	5,01	5,04	5,17
Average	4,60[a]	4,74[b]	4,92[c]	5,00[d]	5,05[e]	5,15[f]
±	0,022	0,012	0,016	0,09	0,013	0,016

[a,b,c,d,e,f]different superscript in different column shows there is a very significant difference (P<0.01)

The result of this research shows that bail salt give real effect (P<0.01) toward pH final fermentation. The pH of final fermentation is higher in accordance to the increasing concentration of the bail salt in the medium. The differences of pH is related to the blocking of bail salt toward the BAL growth. The inhibition toward the growth causes the ability of the BAL in producing organic acid decreasing. The organic acids that produced during fermentation determine the decreasing of pH. In a higher concentration of bail salt, the pH of final fermentation is also higher. The effect of bail salt inhibition toward BAL growth causes the decreasing BAL ability in producing organic acid especially lactic acid. The existence of these organic acids really affects on the decreasing of pH medium. By the increasing concentration of bail salt in medium growth causes the inhibition effect toward BAL growth is also increasing. It can be seen in table 6. The increasing of bail salt is followed by the increasing of pH medium.

Even though there is an inhibiting activity by the bail salt, BAL can still grow in medium that contains bail salt up to 3 % concentration. It can bee seen by the increasing of the density and the decreasing of pH at the last observation hour although it shows significant differences on the control (without bile salt). At the last observation, pH medium can reach critical pH, i.e. around 4. At this critical pH, pathogen microbe can stand any longer (Gilliland, 1990). According to Pereira and Gibson (2002), the parameter ability and grow in Ph 3.0 for 2 hours and grow in medium that contains 1.000 mg/l bail is used as the standard in determining the resistant power of microbe culture toward acid and bail.

Jack et al., (1995); Montville and Kaiser (1993) as cited by Oh et al., (2002) state that BAL produces some anti-microbe compound, i.e. organic acids (lactic acid, acetate, propionate, and format). The ability of BAL in producing organic acids is shown by the decreasing of pH medium, at the 20th hour pH medium in average reaches 4.74 and on 0.5 % bail salt concentration, BAL can still reduce the pH until 5.15.

IV. CONCLUSION

Based on the result of the research, it can be conclude that:

1. There are 15 isolate lactic acid bacteria in 34 days old Broiler Strain Lohman chicken feces.
2. Lactic acid bacteria isolate have these characteristics: positive gram, negative catalyses, non motile, coccus cells shape, chained cells formation, paired, solitary, and tetrad, homofermentative fermentation type, can form acid without producing any gases.
3. The identification of lactic acid bacteria results in 4 lactic acid bacteria genera group, i.e. Lactobacillus, Pediococcus, Streptococcus, and Enterococcus.
4. The addition of bail salt in BAL isolate growth medium obtained from chicken feces causes different biomass (optical density) production until 1.0 5 concentration. However, the speed of specific growth starts at 0.2 % concentration. BAL isolate obtained from chicken feces are still able to grow until the concentration of bail salt is 0.5 %.

REFERENCES

[1] Astuti, M. 1980. Rancangan Percobaan dan Analisis Statistik. Fakultas Peternakan.UGM. Yogyakarta.

[2] Axelsson, L. T. 1998. Lactic Acid Bacteria Classification and Physicly. Dalam : Lactic Acid Bacteria. Seppo Salminen and Atte Vin Wright (Eds). Marcel Dekker Inc. New York.

[3] Barnes, E. M. dan G. C. Mead. 1986. Anaerobic Bacteria in Habitats Other than Man. 1st edition. Black well publications. Oxford.

[4] Bezkorovainy, A. 2001. Probiotic: Determinant of Survival and Growth in the Gut. American Journal of Clinical Nutrition. 73 : 399s-405s.

[5] Borris, R. 1987. Biology of Enzime. Dalam: Biotechnology. Rehm. M. J. and Reed. G. (eds) Verlag Chemic. Weinheim. 7a: 37-62

[6] Brock, T. dan M. T. Madigan. 1988. Biology of Microorganism. 8th ed. Prentice Hall. Inc., Englewood Cliffs. New Jersey.

[7] Dune, C. L. O'Mahany., L. Murphy., G. Thorton., D.Morrissey., S. O'Halloran., M. Feeney., S. Flynn., G. Shanahan and J.K. Collins. 2001. In Vitro Selection Criteria for Probiotic Bacteria of Human Origin : Correlation With in vivo Finding. American Journal of Clinical nutrition.73:2

[8] Fardias, S. 1988. Microbiologi Pangan. PAU Pangan dan Gizi. UGM. Yogyakarta.

[9] Frost, G. M and D.A Moss. 1987. Produktion Enzyme by Fermentasi. Dalam: Biotekchnology Rehm, M. J and Reed, G. (eds). Vol. 7a. Verlag Chemic, Weinheim.

[10] Gilliland, S. E. 1990. Bacterial Starter Culture for Food. 5th ed. CRC Press Inc. Florida.

[11] Hardjo, S. N., S, Indrasti dan T, Banttacut, 1989. Biokonversi: Pemanfaatan Limbah Industri Pertanian. IPB. Bogor.

[12] Harper, H. A. 1983. Review of Physiological Chemistry. 17th eds. Lange Medical Publication Los Altos California.

[13] Havenaar, R. and J.H.J. Hulis in'Veld. 1992. Probiotic : A general Viev, In : B. J. B. Wood (ed) The Lactic Acid Bacteria. Elsevier Applied Science. London.

[14] Jaminez-Diaz, R., J. L.,Ruiz-Barba, D,P., Cathcart. H., Hollo, I, F., Nes, K. H., Stetten, dan P. J. Warmer 1993. Purification and Partial Amino Acid Sequence of Plantaricin S. a Bactericin Produced by Lactobacillus Plantarum LPCO 10. The Activiti of Which Depend on the Complementary Action of Two Peptides. Appl Environ. Microbial. 61 : 4459-4463

[15] Jeni, B.S.L dan S. E. Rini. 1995. Aktivitas Antimikrobia dari Beberapa Spesies Lactobacillus terhadap Mikrobia Patogen dan perusak Makanan. Buletin Teknologi dan Industri Pangan. 6:2

[16] McDonald, P. 1981. The Biochemistry of Silage. John Weley and Sons, New York, USA

[17] McDonald. P., R. A. Edward. J . F. D. Greenhalgh and C. A. Morgan. 1995. Animal Nutrition Fifth edition. Longman Singapore Published (Pte) Ltd. Singapore.

[18] Merchen, N,R. 1079. Digestion. Absorbtion and Excretion in Ruminant. Dalam The Ruminant Animal Digestive Phisiology and Nutrition, Prentice Hall, New Jersey

[19] Mortvedt-Abiguard, C.L..J. Nissen-Meyer. B. Jelle. B Grenov. M. Skaugen dan I. F. Nes. 1995 Production On pH Independent Bactericidal Activity Of Lactocin as Antibiotik From Lactobacillus sake L-45. APPL. Eviron. Microbial. 61: 175-179.

[20] Nahm, K. H. 1992. Pracical Gide To Feed Forage and Water Analysis (Accurate Analysis With Minimal Equepment). Yoo Han Publishing Inc.. Korea,

[21] Oh, S. S.H. Kim. R. W. Wirobo. 2000. Characerization and Purification of Bacteriocin Produced by a Potential Probiotic Cultur Lactobacillus Acidophillus 305C. J Dairy Sci. 83 : 2747-2752.

[22] Oh, T dan B. Yoshimi. 1998. A new Probitic From Pig Waste and its Effect on Pig . Korea Research Institute of Bioscience and Biotechnology. Korea.

[23] Paat, P. C. 1999. Inokulasi Bakteri Asam Laktat yang Diperoleh Dari Kultur Campuran Effective Microorganism Pada Proses Silase Rumput Raja. Tesis Fakultas Peternakan. UGM. Yogyakarta.

[24] Pereira, D.I.A and G. R. Gibson. 2002. Cholesterol Assimilation by Lactic Acid Bacteria and Bifidobacteria Isolated from the Human Gut. J. App and Evironmental Microbiology 68 : 4689-4693

[25] Poedjiadi,A. 1994. Dasar-Dasar Biokimia. Universitas Indonesia Press. Jakarta.

[26] Rachman, A. 1989. Pengantar Teknologi Fermentasi. IPB. Bogor.

[27] Ray, B. 1993. Cell Off Lactic Acid Bacterial as Food Biopreserfatives of Microbial Origin Ray. B. dan Daeschel. M. (Eds). CRC Press. Inc.. Germany.

[28] Schved, F., A.Lalaazar, Y. Henis. dan B. J. Juven, 1992. Purification Partial Characteristization and Plasmic Linkage of Pediosin SJ-1. a Bacteriocin Produced by Pediococcus Acidilactidi. J. APPL. Bacterial. 72:267-273

[29] Soetanto, H., M.C. Padaga., Y.N. Angraeni. 2001. Pola Pertumbuhan BAL Asal Usus Halus Sapi Perah Pada Berbagai Suhu Lingkungan. J. Ilmu-ilmu Hayati (life science) 13:1

[30] Sudarmo. S. M. G.R. Reza. S. Pitono, S. P. Like. Kontribusi Prebiotik pada Formula Untuk Memelihara Ekosistem Mikrobiota Normal pada Usus. Laboratorium/SMF Ilmu Kesehatan Anak. Rumah Sakit Dr. Soetomo. Fakultas Kedokteran. Unair. Surabaya.

[31] Suyanandana, P., P, Budhaka, S. Sasanarakkil.. P. Saman.. P. Disayaboot.. Y. Cai. Dan Y. Benno. 1998. New Probiotic lactobacilli and Enterococci from Fish Intestine and Their Effect on Fish Production. Thailand Institute of Scientific and Technological Research. Thailand.

[32] Wang. D. I. C.. C. L Cooney.. A. L. Demaina.. P. Dunnil.. A. E. Humphrey and M,D, Lilly, 1978, Fermentation and Enzyme Technology A Wiley, Intercience Publication, New York

[33] Wibowo, J. 1990. Biokimia Proses Fermentasi. PAU Pangan Dan Gizi. UGM. Yogyakarta.

[34] Wibowo, J. 1993. Produksi Antibiotik Dengan Proses Fermentasi. Kumpulan Hand-Out Lokakarya. 11 sampai 26 Januari 1993. Pusat Antar Universitas Biotek. Universitas Gadjah Mada. Yogyakarta

[35] Winarno dan J.Wibowo, 1993. Produksi Antibiotika dengan Proses Fermentasi. Kumpulan Hand Out Lokakarya. 11 sampai 26 Januari 1993. PAU Biotek. UGM. Yogyakarta.

[36] Winarno, F. G. 1997. Probiotik. Seminar Probiotik dan Keamanan Pangan. Universitas Gadjah Mada. Yogyakarta. 12 Juni 1997.

[37] Agerbaek, M., L.U. Gerdes and B. Richelsen. 1995. Hypercholesterolemic effect of a new fermented milk product in healthy middle age men. Eur. J. Clin Nutr 49 : 346 – 352.

[38] Akalin, A.S., S. Gone and S. Duzze;. 1997. Influence of yogurt and acidophiles yogurt on serum cholesterol levels in mice. J. Dairy Sci. 80 : 2721-2725

[39] Anonim. 1996. Profil Statistik Kesehatan di Indonesia. Departemen Kesehatan RI.

[40] Fernandes. C.F., K.M. Shahani and M.A. Amer. 1987. Therapeutic roel of dietary Lactobacilli and lactobacilli fermented dairy product. FEMS Microbiol. Rev 46 : 343 – 356

[41] Gilliland, S.E. and C.R. Nelson and C. Maxwell. 1985. Assimilation of cholesterol by Lactobacillus acidophilus. Appl. Environ, Microbial. 49 : 377 – 381.

[42] Gilliland, S.E., T.E and M.L. Speck. 1977. Deconjugation of bile acids by intestinal Lactobacilli. Appl. Environ. Microbial. 49 : 377-381.

[43] Grunewald, K.K. 1982 Serum Cholesterol levels in rats fed skim milk fermented Byobacillus acdopilus. J of Food Sci, 47 : 2078-2079

[44] Harrison, V.C. and Peat. 1975. Serum Cholesterol and Bowel flora in the new born. Am. J, Clint Nutr. 28 : 595-618.

[45] Harsono, A. and T. Tono Oka. 1995. Binding of cholesterol with lactic acid bacteria cells. Milchwissenschaff 50 : 556-560.

[46] Hepner, G.R. Fried. S.St. Jear, L. Fasetti and R. Morin, 1979. Hypercholesterolemic effect of yogurt and milk. Am. J, Clint Nutr. 32 : 19-24.

[47] Jasper, D.A., L.K. Massey and L.O. Leudoke. 1984. Effect of Consuming yogurt prepared with three culture strains on human serum lipoprotein. J. Food Sci.

[48] Kociubinski, G., Perez and G de Antoni. 1999. Sceening of bile salt and bile precipitation in lactic acid becteria and Bifidobacteria. J. Food Prot 62 (8) : 905-912.

[49] Lehninger, A.I. D.L. Nelson and M.M. Co. 1993. Principles of Biochemistry 2nd ed. Wosta Publisher.

[50] Mann, G.V. 1977. A factor in yogurt which lower cholesterolemia in man Atherosclerosis 26 : 335 – 340

AUTHORS

First Author – Dr. Ir. Astuti, M.P * Department of Biology, Yogyakarta State University, Indonesia