Selection of Indigenous Lactic Acid Bacteria from Indonesia for Cheese Starter

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Abstract

Screening of lactic acid bacteria (LAB) from *Indonesia Culture Collection* (InaCC) that potential as a starter culture and their application for cheese making at laboratory scale has been conducted. These isolates were selected based on some properties as cheese starter, such as produce acid, protease and coagulant potential. The result of this research were out of 39 InaCC isolates, DR 1-7-1 was the high acid potential isolate with Lactic Acid Index 3.25, DSB 4-2 was the high protease potential isolate with Protease Index 3.82, DR 2-2-3 and DSB 4-2 were the high coagulant potential with form coagulum time on 24 hours.

Keywords: lactic acid bacteria, cheese, starter, Culture collection, rennin

INTRODUCTION

Production of cheese is essentially achieved by bringing four ingredients together: milk, rennet, microorganisms (culture starter), and salt. The cheese making process includes the following steps: gel formation, acid production, whey expulsion, salt addition, and finally ripening period (Beresford *et al.*, 2001). Lactic acid bacteria (LAB) are generally recognized as a safe (GRAS microorganism) and play the important role in various traditional food fermentation. Among these traditional food fermentation, cheese are known to be essentially fermented by LAB. Starter strains in industrial terms can be defined as isolates which produce sufficient acid to reduce the pH of milk to <5.3 in 6 h at 30- 37 °C (Beresford *et al.*, 2001). The starter cultures commonly used in cheese manufacture include *Lactococcus*, *Leuconostoc* *Lactobacillus* species and *Streptococcus thermophilus*.

Microorganisms which is essential in cheese starter strains have some properties, such as high ability to produce acid and predictable rate (Stadhouders, 1986; Sandine, 1985; Farrow, 1980), flavour and texture formation, decrease in the pH, formation of curd, expulsion of whey (Beresford *et al.*, 2001), has proteolytic activity.

The ability to produce lactic acid from lactose is probably the most important property of dairy LAB. It helps to reduce pH, which in turn increases the expulsion of whey from the curd, thus lowering the moisture content (Caridi, 2003). The proteolytic activity exerted by most LAB is not only a prerequisite for growth, but also affects product texture and flavour, especially in dairy products (Law and Kolstad, 1983; Thomas and Pritchard, 1987). Proteolytic activity encompasses proteinases that degrade proteins, such as caseins, into relatively large protein fragments (oligopeptides), and peptidases that break down protein fragments into small peptides and free amino acids. The total proteolytic activity consists of a complex mixture of a proteinase and a number of peptidases (Kamaly and Marth, 1989; Olsen, 1990).

Starter cultures are essential for industrial production of all kinds of cheese. Before adding to milk, cultures have been pre-grown in milk or milk-based media. Depending on the cheese type, the inoculation volume varies from 0.2% to 2% of volume of milk (Cogan,1996). Cheese industry usual use defined single or multiple culture starters to obtain cheese with high and constant quality. Starter cultures used in cheese making in Indonesia come from defined strain starter obtainable from import (commercial suppliers). *Indonesia culture collection* (InaCC) has the LAB isolate that suspected potential used for cheese starter. Because of that, need to screening indigenous lactic acid bacteria from InaCC that has superior natural quality potential using for cheese starter. Technological characterization of LAB strains for preparation of the experimental starter could include, in particular, growth, acidifying and proteolytic activity (Madrau *et al.*, 2006).

The object of this research was to obtain the LAB isolates originally Indonesia that potential used for cheese starter.

MATERIALS AND METHODS

Microorganism

The strain lactic acid bacteria (LAB) totally 39 isolates belonging to *Indonesia Culture Collection* (InaCC) are used. All of isolates has been previously isolated from dadih (Palembang, Riau and West Sumatra) and donkey (South Sulawesi). The Isolates was growth at MRSA+$CaCO_3$ 0.5% media.

Screening Starter Cultures Properties

1. Acid potential screening

Screening for isolate that produce acid by qualitative using media MRSA + $CaCO_3$ 0.5% (Khunajakr *et al.* 2008). Incubation has been done for 68 hours at room temperature (26 °C). The measurement of acid index has been done by quantitative analysis.

2. Protease potential screening

Screening for isolate that has protease activity potential by qualitative using media SMA (*skim milk agar)*. Incubation has been done for 68 hours at room temperature (26°C) (Taheri *et al.* 2009). The measurement of protease index has been done by quantitative analysis (Akhdia, 2003).

3. Coagulum potential screening

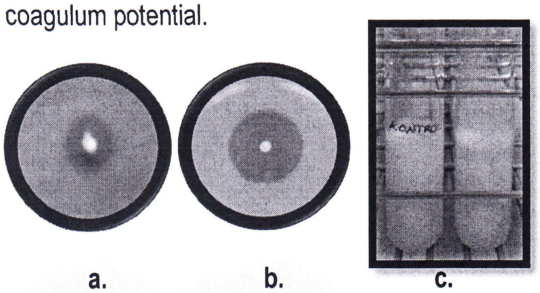
LAB coagulum screening has been done by using media of pasteurization of the milk. Incubation has been done for 24 hours at room temperature (26°C) until LAB candidate has produced the coagulum form (the curd).

4. The growth Measurement

The growth of curve from the both of candidate isolates to know the optimum growth time. The both isolate was grown on media MRSB, at room temperature, 150 rpm. Sampling has been done for 3-4 times per day with interval each two hours. The cell result then was measured absorbance on 660 nm wavelength.

RESULTS AND DISCUSSION

Screening LAB isolates which isolated from local product milk derivates, such as dadih from Sumatera Island and donkey from Sulawesi Island has been done using three parameters, such as acid, protease, and coagulum potentials. Flores (2008) says that there are life bacteria in yoghurt and dadih which can be used as cheese starter. As Figure 1, shown clear zone that indicated lactic acid and protease potential and the curd for coagulum potential.



a. b. c.

Figure 1. BTCC microbial collection of lactic acid bacteria (BAL) (a. clear zone which indicates an acid potential b. clear zone which indicates protease potential, and c. coagulation screening)

1. Acid potential screening

From 39 isolates indicated that all of the positive produce acid/clear zone (Figure 1b) at medium MRSA+$CaCO_3$ 0.5% with difference of acid index (Table 1). The formed of acid because neutral condition from $CaCO_3$ to acid that was produced. The acid index shows that the isolate has potential to produce acid which functioned to decrease of the milk pH become of protein isoelectric pH. If the protein has reached its isoelectric pH, It would be helping in the formation of curd (Hartoyo *et al.* 2010).

The best acid index value is above 3, the value 2-3 index which has the ability to moderate and the index was below the 2 that have less ability to produce the lactic acid (Said and Ningrum 2009). In this study, isolates that have acid potential ability in the top 3, namely isolates DR 1-7-1 with acid index of 3:25.

Table 1 The Acid index value from 39 candidate isolates as cheese starter potential

No	Code	Source	Acid index
1	DP 1-1-1	Dadih Palembang	2.20
2	DP 1-1-2	Dadih Palembang	2.00
3	DP 1-2	Dadih Palembang	2.20
4	DP 1-3-1	Dadih Palembang	-
5	DP 1-3-2	Dadih Palembang	-
6	DP 1-4-1	Dadih Palembang	1.80
7	DP 2-1-2	Dadih Palembang	2.00
8	DR 1-1-1	Dadih Riau	1.75
9	DR 1-1-2	Dadih Riau	2.33
10	DR 1-2-1	Dadih Riau	1.50
11	DR 1-2-2	Dadih Riau	2.25
12	DR 1-3-1	Dadih Riau	1.63
13	DR 1-3-2	Dadih Riau	2.00
14	DR 1-6	Dadih Riau	1.67
15	DR 1-7-1	Dadih Riau	3.25#
16	DR 1-7-2	Dadih Riau	2.60
17	DR 1-7-3	Dadih Riau	2.25
18	DR 1-7-4	Dadih Riau	2.25
19	DR 1-8-3	Dadih Riau	1.83
20	DR 2-1-2	Dadih Riau	1.71
21	DR 2-2-3	Dadih Riau	3.00
22	DR 2-3-1	Dadih Riau	2.33
23	DR 2-3-2	Dadih Riau	1.75
24	DR 2-4-1	Dadih Riau	1.75
25	DR 2-4-2	Dadih Riau	1.67
26	DR 3-1-2	Dadih Riau	1.50
27	DR 3-1-3	Dadih Riau	2.50
28	DR 3-2-1	Dadih Riau	2.00
29	DR 3-3-2	Dadih Riau	2.50
30	DR 4-1	Dadih Riau	1.67
31	DR 4-2	Dadih Riau	1.00
32	DSB 1-1	Dadih Sumatera Barat	1.33
33	DSB 4-2	Dadih Sumatera Barat	1.75
34	DSB 6-3	Dadih Sumatera Barat	2.25
35	DSB 6-5	Dadih Sumatera Barat	2.75
36	DSB-1	Dadih Sumatera Barat	1.71
37	DSK-1		-
38	DSS 2-2	Donkey Sulawesi Selatan	2.50
39	DSS 2.1	Donkey Sulawesi Selatan	2.50

2. Protease potential screening

There were 18 positive isolates that producing protease clear zone on medium Skim Milk Agar (Figure 1c) and the index value is not the same. The formation of the clear zone is due to the protease enzyme produced by LAB and react to break casein substrate contained in the medium.

In this study, the candidate isolates were which have the potential of protease above 3 is isolates DSB4-2 with protease index 3.82. The value of protease index was show the capacity of isolates to produce proteases which functioned to break the milk protein. The breakdown of milk protein and aided also by the Calcium ion in the milk will make the pieces of the milk protein will precipitate to form the curd.

Table 2 The protease index and coagulum from 39 of candidate isolates as cheese starter potential

No	Code	Source	Protease index	Coagulum H1/H2
1	DP 1-1-1	Dadih Palembang	1.50	-/+
2	DP 1-1-2	Dadih Palembang	2.00	-/+
3	DP 1-2	Dadih Palembang	-	-/+
4	DP 1-3-1	Dadih Palembang	1.86	-/+
5	DP 1-3-2	Dadih Palembang	1.56	-/+
6	DP 1-4-1	Dadih Palembang	2.50	-/+
7	DP 2-1-2	Dadih Palembang	-	-/+
8	DR 1-1-1	Dadih Riau	-	-/+
9	DR 1-1-2	Dadih Riau	-	-/+
10	DR 1-2-1	Dadih Riau	1.33	-/+
11	DR 1-2-2	Dadih Riau	1.50	-/+
12	DR 1-3-1	Dadih Riau	-	-/+
13	DR 1-3-2	Dadih Riau	3.00	-/+
14	DR 1-6	Dadih Riau	-	-/+
15	DR 1-7-1	Dadih Riau	1.67	-/+
16	DR 1-7-2	Dadih Riau	1.30	-/+
17	DR 1-7-3	Dadih Riau	2.67	-/+
18	DR 1-7-4	Dadih Riau	-	-/+
19	DR 1-8-3	Dadih Riau	-	-/+
20	DR 2-1-2	Dadih Riau	-	-/+

21	DR 2-2-3	Dadih Riau	-	(+/+)
22	DR 2-3-1	Dadih Riau	-	-/+
23	DR 2-3-2	Dadih Riau	-	-/+
24	DR 2-4-1	Dadih Riau	-	-/+
25	DR 2-4-2	Dadih Riau	-	-/+
26	DR 3-1-2	Dadih Riau	1.13	-/+
27	DR 3-1-3	Dadih Riau	1.13	-/+
28	DR 3-2-1	Dadih Riau	1.67	-/+
29	DR 3-3-2	Dadih Riau	-	-/+
30	DR 4-1	Dadih Riau	1.33	-/+
31	DR 4-2	Dadih Riau	-	-/+
32	DSB 1-1	Dadih Sumatera Barat	1.33	-/+
33	DSB 4-2	Dadih Sumatera Barat	3.82#	(+/+)
34	DSB 6-3	Dadih Sumatera Barat	-	-/+
35	DSB 6-5	Dadih Sumatera Barat	-	-/+
36	DSB-1	Dadih Sumatera Barat	1.56	-/+
37	DSK-1		-	-/+
38	DSS 2-2	Donkey Sulawesi Selatan	-	-/+
39	DSS 2.1	Donkey Sulawesi Selatan	-	-/+

3. Coagulum potential screening

There are 2 isolates (DSB 4-2 and DR 2-2-3) that positive can form coagulum on the first day after inoculation of pasteurized milk medium (Figure 1c). Then, after the second day of incubation, all the milk has had coagulum. The formation of coagulum (curd) can be due to acidification of lactic acid and protease enzymes that was produced by LAB. This step is the final decision to select the best isolates will be tested in cheese making. Based on two-stage screening before we selected that isolates will be tested in the process of cheese making is DSB 2-2-3 and DR 4-2. We choose the DR 2-2-3 not DR 1-7-1 because of the DR 2-2-3 has the coagulum potential in the first day (former curd) and it's also has the acid potential still in the maximum range which is 3, the second highest after the DR 1-7-1. Although DR 1-7-1 has the potential of high acid, but it was not selected because this isolate has capability to form coagulum (the curd) on the second day.

5. The growth Measurement

The growth of curve from both candidates isolates for cheese starter have been made to know when optimum growth of both isolates were selected (Figure 2). From the time of optimum growth, can be seen also when optimum production of primary metabolites from each isolate. The candidate isolates will be mixed as cheese starter into the milk in cheese making at the optimum time of growth. So the cheese-making process will be faster. From Figure 2 can be seen that the DR 2-2-3 and 4-2 DSB has the same optimum time, it was 24 hours. So at the 24th hour to acid and enzyme production is also high, and that's when the testing will be done as a starter in the cheese making.

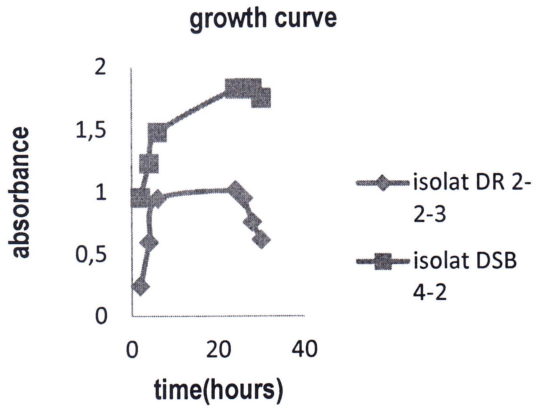


Figure 2 Growth curve from candidate isolates for cheese starter (DR 2-2-3 and DSB 4-2)

CONCLUSIONS

From 39 LAB isolates, DR 1-7-1 was the high acid potential isolate with Lactic Acid Index 3.25, DSB 4-2 was the high protease potential isolate with Protease Index 3.82, DR 2-2-3 and DSB 4-2 were the high coagulant potential with form coagulum time on 24 hours; and DR 2-2-3 and DSB 4-2 were selected for candidate isolate for cheese starter.

ACKNOWLEDGEMENTS

This research was funded by DIPA PN MEAT-PRO FY2012. Thank to *Indonesia Culture Collection* (InaCC) for providing the LAB collections.

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