Fresh Cheese Probiotic with Local Isolate *Lactobacillus casei* 2.12 as starter in fermentation

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**Abstract.** The local isolates of Lactic Acid Bacteria (LAB) have not been optimally developed in the cheese making process. The aim of this research was to know the clotting activity and the percentage and characteristics of curd produced during milk fermentation using local isolate of BAL *L. casei* 2.12. The results showed that during 12 hours incubation the value of milk clotting activity (MCA) was 8.33 SU / mL, the curd percentage was 12.5% (w / v) with curd firmness (++++) very good and Total Plate Count 1.14. 10⁹ cfu / g. These results indicate the use of *L. casei* 2.12 as a starter LAB probiotic capable of causing coagulation of casein and produce fresh cheese probiotic.

1. Introduction

Cheese is a dairy product that is very popular with Indonesian people, even though it is not an authentic Indonesian food. Cheese was found in the fertile region of the Euphrates and Iraq Tigris rivers 8,000 years ago at Revolution Agriculture. Many kinds of food products are added to cheese to add flavor, so the demand for national cheese is quite fantastic reaching 8 million tons per year. National cheese production has not fulfilled this demand with various obstacles faced, especially the availability of milk clotting enzymes namely rennin or rennet which is a proteolytic enzyme.

Protease is an enzyme that is able to hydrolyze peptide bonds in proteins. Based on the Nomenclature Committee of The International Union of Biochemistry, proteases are classified as hydrolase enzymes (EC.3.4). Protease is a very important enzyme in the food and non-food industries. Utilization of proteases in the food industry is to reduce turbidity in the beer industry, reduce gluten in the bread industry, and to agglomerate milk in the cheese industry. Protease enzymes can be obtained from plant, animal and microbial tissue. The limitations of the ability of animals and plants to fulfill protease requests have led to the development of microbial proteases.

The production of rennin from calves is decreasing in the world, resulting in high prices of these enzymes, so efforts are made to find alternative enzymes (substitute). Protease enzymes other than rennin which has rennin-like activity, namely milk clotting activity (Milk Clotting Activity), are known as Rennin Like Protease (RLP). RLP sources can be obtained from plant and microbial sources. Plant sources have characteristics with high proteolytic activity.

There are proteases produced by microbes that have characteristics similar to rennin from the stomach of the calf and produce curd products from milk as produced by animal rennin so that the protease is known as Rennin Like Protease (RLP). *Rhizomucor miehei, Rhizomucor pusillus, Endothia parasitica, Aspergillus oryzae, and Irpex lacteus* have been commercially produced to produce RLP [1]. The Milk Clotting Enzymes (MCE) was produced from *Bacillus* [2,3,4]. *Bacillus stearothermophilus* showed the milk-clotting activity of 24.23 SU/ml [5].
Microbes that are native Indonesian germplasm are still not optimally developed into biological agents in industries based on biotechnology, so this study is expected to contribute to the development of local microbial utilization, especially Lactic Acid Bacteria (LAB) for the production of Rennin Like Protease enzymes (RLP) needed by the national cheese industry and reducing the import of these enzymes. Local Lactic Acid Bacteria (LAB) isolated from traditional Indonesian fermented food products have not been developed and mass produced to produce rennin.

2. Materials and Methods
2.1. Screening Skim Milk Agar
*L. casei* 2.12 was screened using MRS (deMan Rogosa and Sharpe) agar medium containing 3% skim. *L. casei* 2.12 colonies capable of coagulating casein.

2.2. Milk Clotting Activity (MCA)
As much as 2.5 mL of the substrate (10% skim milk in 10 mM CaCl₂) was incubated for 5 min at 37 °C followed by adding of 0.5 mL enzyme extract. Measurement of time length was started from the addition of enzyme extract to the formation of the first particles. MCA was calculated as: \( SU = 2400 \times 5 \times D/T \times 0.5 \) (1) as described by Kawai and Mukai (1970), in which T is milk-clotting time (s), and D is dilution of the enzyme. One Soxhlet unit (SU) of milk-clotting activity was defined as the amount of enzyme required for clot formation of 1 mL of substrate in 40 min at 35 °C.

3. Results and Discussion
The use of local LAB isolates in the process of curd formation in cheese making is still not widely used. Isolate *L. casei* 2.12 which was isolated from the milk of Ettawa goat had the ability to coagulate milk (casein) with the MCA activity of the crude enzyme produced was 18 SU/mL. This activity is not much different from the MCA activity of *Enterococcus faecium* 1.15 which is 20 SU/mL [6]. MCA testing is quantitative testing of quantitative enzyme activity. The main factor that determines the value of MCA is the velocity of milking (skim). The faster coagulation occurs, the higher the MCA value. A total of 5 mL (10% skim solution in 0.01 M CaCl₂·2H₂O) was added an enzyme to be tested as much as 0.5 ml. Next, incubate at 37 °C in the water bath and record the formation time of curd (t).

| No | Isolate | Sources            | Clotting zone (cm) | Milk Clotting Activity (SU/mL) |
|----|---------|--------------------|--------------------|-------------------------------|
| 1  | 2.12    | Ettawa Goat Milk   | 1.5                | 18                            |

Coagulation of casein can occur due to acids or enzymes, namely rennin. Enzymatic coagulation of milk is caused by the breakdown of the peptide bond between phenylalanine and methionine (105th sequence with 106) from the kapa casein polypeptide, resulting in k-casein and macropeptide [7,8]. Macropetides produced from these cuts are soluble in water, while the casein will settle. Instability in casein miselae results from the breakup of Phe and Met bonds so that other casein fractions settle. The mechanism of casein clotting due to enzymatic activity (rennin) is different from casein clotting due to acid. Clumping because rennin in the initial phase (primary) occurs the interaction between rennin and k-casein (a), then the peptide bond breaks between phenylalanine and methionine (105th sequence with 106) of the casein kapa polypeptide, producing para-k-casein and macropeptide (b), and then macropeptide aggregation

3.1. Making Fresh Cheese with Fermentation
Isolate *L. casei* 2.12 is a class of LAB which is a safe bacteria, so that in the process of forming curd can be used in the form of a starter with a fermentation process.
The results showed that during 12 hours incubation the value of milk clotting activity (MCA) was 8.33 SU / mL, the curd percentage was 12.5% (w / v) with curd firmness (++++) very good and Total Plate Count 1.14. 10^9 cfu / g.

4. Conclusion
The use of L casei 2.12 as a starter in the process of making cheese is very potential with curd production reaching 12.5% (w/v).

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