CHAPTER I

INTRODUCTION

Milk coagulation is a basic step in cheese manufacturing obtained by clotting milk casein using enzymes either from animals, microbial and plants sources. For long time calf rennet, extracted from the calf's fourth stomach, is the most widely used coagulant in cheese making all over the world to manufacture a variety of cheeses. These products are important part of a well-balanced diet and also important source of calcium (Kashket & DePaola, 2002). A variety of cheese can be made depending on the type of milk, the milk clotting enzymes (MCEs) used and the starter organisms which could be lactic acid bacteria (LAB), molds and other microorganisms (Sato et al., 2004). MCEs are the primary active agent in cheese making, which involves the enzyme-mediated cleavage of *kappa*-casein (κ -casein) which covers the protein micelles at the peptide bond Phe 105-Met 106 that renders the casein micelles unstable and eventually causes aggregation that yields a clot or a gel (Mariela et al., 2010)

The production of dairy products continues to increase considerably but the number of ruminants is decreasing because rennet is obtained from complex stomach of ruminant animals (Reps et al., 2006). To meet the demand for dairy products worldwide it has become necessary to find other sources of milk clotting enzyme (MCE) particularly from microbial sources. Additionally, the animal rennet used in cheese making has become controversial due to religious reasons such as the non-Halal slaughtering of the animals (Nagodawithana & Reed, 1993). MCE are proteolytic enzyme, which are

essential for cheese production and one of the most important enzymes in the food industries. Rennet which is widely used in cheese making is normally extracted from calf stomach. The enzyme basically consists of chymosin and pepsin. Alternative enzymes should have specific properties with high ratio of milk clotting activity (MCA) to proteolytic activity (PA).

Several microbial enzymes show activity similar to animal rennet and are suitable for cheese making. Many researches have detected microbial MCE such as *Rhizomucor miehei* (Merheb et al., 2010), *Thermomucor indicae-seudaticae* (Reps et al., 2006), and *Aspergillus oryza* (Shata, 2005) which produce proteinase by fermentation. These enzymes specifically cleave the Phe105–Met106 bond of bovine κ-casein (Horne & Banks, 2004). The above researchers all observed that MCE from plants and fungi showed high proteolysis leading to a weak body, bitter flavor defects, and reduced cheese yield during storage (Hynes et al., 2001; Reps et al., 2006; Yegin, et al., 2010).

Efforts to study production of MCE from bacteria have concentrated on the genus *Bacillus* spp. such as *B. subtilis* natto (Shieh et al., 2009) and *B. sphaericus* (El-bendary et al., 2007) genus of *Streptomyces* spp. (Kathiresan & Manivannan, 2007) and *S. clavuligerus* (Keila et al., 2001). However, there are few reports on the MCE derived from lactic acid bacteria. Sato et al. (2004) reported that three isolates of *Enterococcus faecalis* have ability to clot milk similar to enzymes obtained from animal origin. They further stated that LAB can be used to clot milk in order to make camembert cheese. Lactic acid bacteria are Gram-positive, non-sporulating, catalase negative, aero-tolerant, acid tolerant, nutritionally fastidious, strictly fermentative

microorganisms that lack cytochromes and produce lactic acid as the major end product of carbohydrate metabolism. It has a long history of safe use in fermented foods and it is generally recognized as safe (GRAS) (Mohd Adnan & Tan, 2007). LAB produce proteolytic enzymes that hydrolyze milk proteins providing the amino acids that are essential for growth of LAB cells.

In general, MCEs are the primary active agents that result in casein coagulation. The temperature and pH for the milk and enzyme are very important as well as concentration of calcium chloride ($CaCl_2$) have significant effect on the MCA yield and rheological characteristics of the product (Najera et al., 2003). Rheological characterization of milk gel is important in determining the body and textural characteristics and also for examining how these parameters are affected by milk composition, processing techniques and storage conditions (Najera, et al., 2003). The purpose of this study was to investigate the activity of extracellular milk clotting enzyme from LAB isolates obtained from different sources for making soft gel from goat's and skim milk.

Therefore, the objectives of this study were to:

i. Determine the milk clotting activity (MCA) and proteolytic activity (PA) of LAB isolated from different food sources.

ii. Evaluate factors affecting MCA and proteolytic activity of crude enzyme on skim milk.

iii. Characterize Milk Clotting Enzyme (MCE) and protein degradation of milk casein by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of purified enzymes from selected LAB.

iv. Evaluate the rheological properties of soft gel made from the extracellular enzyme extracted from selected LAB.