# CHAPTER 1

## Introduction

Lactic acid is one of the most important organic acids widely used in many food and non-food industry. At the present, it is now being concerned as substrate for synthesis of poly lactic acid (PLA), an alternative biodegradable plastic but normally it is recognized as typical ingredient in food industry. Lactic acid can be produced either chemical or biological methods but the later has been intensively attended. Because of low energy consumption, advantage in reduction of hazardous reaction, timing and the optical purity of D-/or L-lactic acid, the fermentation of lactic acid by microorganisms is truly preferable. Most lactic acid producing microorganisms so far have been reported to be fungi, yeasts and bacteria as alternative resources. Lactic acid bacteria occur in nature overcome any other microorganisms such as *Enterococcus* sp., *Lactococcus* sp., *Streptococcus* sp. and particularly *Lactobacillus* sp., a main microorganism used for lactic acid production. Commonly different lactic acid bacteria influenced on selection of carbon source for lactic acid production. Various refine sugars have been used as carbon source including glucose, sucrose, maltose and other cheaper agricultural materials such as sugar cane juice, molasses, whey and etc. that are rich in fermentable sugars but these materials are not economical substrates and are increasingly being demanded therefore; the prices are increasing. The plenty, availability and cheapness of substrates are considerably concerned in order to reduce cost of lactic acid production particularly cost from production media. Starch containing materials are interesting and navigate to best solution of lactic acid production as mentioned reasons. However, most lactic acid bacteria lack of ability in starch degradation for lactic acid fermentation. Starch liquefaction and saccharification by enzymatic method is preferable and has been extensively reported. Throughout enzymatic hydrolysis, α-amylase and glucoamylase are two main enzymes play important role in production of fermentable sugars from starch to serve lactic acid bacteria for lactic acid production. Consequently, lactic acid fermentation stragtegy becomes at least two steps. Not only the complex procedures

but also the production cost is tremendously increased regarding the addition of starch degrading enzymes. Attempts avoid enzymatic methods and keeping on single step fermentation have been established when amylolytic lactic acid bacteria (ALAB) were isolated and used for lactic acid production leading to direct conversion of starch to lactic acid comes to be feasible and applicable. Fundamentally ALAB are capable of producing starch degrading enzymes to release fermentable sugars from starch for lactic acid production by themselves. Therefore, conversion of starch to lactic acid becomes single step as previously described for conventional lactic acid production. This fermentation strategy exactly reduces both complexities of fermentation processes and cost of lactic acid production and are interesting to apply this fermentation strategy to industry. The enzymes involving starch degrading system in ALAB have been reported up to now, are in group of amylolytic enzymes or amylases such as α-amylase, amylopullulanase and maltogenic amylase. These enzymes play important role on starch degradation and promote conversion of starch to lactic acid become available.

This research, *Lactobacillus* sp. S21 has been isolated and selected for direct lactic acid production from starch because of its potentiality in direct conversion of starch to lactic acid. Further species identification is performed in order to find novelty among the species but the main purposes are to purify extracellular amylolytic enzyme from this organism and to study on enzyme properties in accordance with rapid lactic acid production by this strain as well as enzyme classification. Starch degrading enzymes of this strain are also elucidated for well understanding about direct conversion of starch to acid and proposed starch degrading mechanisms involving lactic acid fermentation are introduced. The amylase gene is isolated and expressed in *E. coli* and *L. plantarum* for homologous and heterologous expression to investigate possibility in overexpression of the gene for over enzyme production and feasibility to express amylase gene in other *Lactobacillus* sp.. In addition, efficiency evaluation of direct conversion of starch to lactic acid by this strain is intensively determined with and without starchy wastewater for value adding and mitigating environmental impacts. Overall research means to high efficiency of direct conversion of starch to lactic acid by *L. plantarum* S21.