CHAPTER 1

INTRODUCTION

In recent years, there has been an increase in the use of natural products from plants as therapy, diseases prevention, disease symptoms decrement and food supplements (also known as functional foods, designer foods, pharmafoods or nutraceuticals). Fermented product containing plant is one of interesting natural products. Over the past five years a large number of medicinal plants have been produced and marketed in fermented juice form or as beverage for health; namely, "Fermented Medicinal Plant Juices" (FMPJs). FMPJs are non-alcoholic beverages produced from different kinds of plants such as vegetables, fruits, medicinal plants and cereals. The FMPJ product is altered to lactic acid by lactic acid bacteria (LAB). Nowadays it is still a household product distributed in various parts of Thailand (Chaiyasut et al., 2004; Kantachote and Charernjiratrakul, 2004). People believe that these products are able to relieve disease symptoms and are promoted as supplemented beverages that are said to have anti-aging properties, anti-cancer properties, anti-inflammatory properties, anti-microbial properties and immune enhancing effects (McClatchey, 2002; Peerajan, 2006).

Soybean (*Glycine max* (L.) Merr., *Leguminosae*), the most important source of protein for human nutrition, is one of the most widely used plants. It serves as an interesting example how people from different cultures prepare foods and functional foods. Soybean is identified as one of macrobiotic food. It consists of 40% protein, small amount of saturated fat and no cholesterol. Interestingly,

soybean is the richest source of isoflavones. It is widely used for producing fermented food and healthful soymilk. It is popularly regarded as a healthy food, partly owing to the isoflavones contained in their seeds (Deshpande et al., 2000). However, some nutritional values and phytonutrients such as digestible protein and absorbed isoflavone are lacking (Yongsmith et al., 1997). Moreover, the finished products of natural fermented plants, including fermented soybean milk have no safety and quality control for every batch. It is possible that in some cases, undesired microbes and metabolites may be presented due to unsuitable conditions of fermentation. Because during the fermentation process, there are both desired microorganisms such as lactic acid bacteria, and undesired microorganisms such as microbial pathogens and spoilage microorganisms. This is due to the natural fermentation with non pasteurized and sterilized manufacturing to maintain nutritional quality and labile useful components. The spontaneous fermentation by lactic acid bacteria in fermented product containing plant stringent conditions inhibits subsequent growth of other microorganisms. However, the problem due to the growth of spoilage microorganisms and food-borne pathogens still occurs, thus there are still the risk of consumption of the product (Chaiyasut et al., 2004; Kantachote and Charernjiratrakul, 2004).

In order to solve these problems, it is necessary to use probiotic bacterial starter to eliminate the possibility of microbial contamination and to control the safety and quality of the fermented product. Also, the quality of product as functional properties are needed for consumer, especially, people are facing with a lot of health problems caused by the living style in this era. Human are under high risk of getting harmful chemical agents or pathogens into bodies such as antibiotic substances used and remained in food products. Those effect on the balance of

intestinal microorganisms and cause many disorders or serious diseases such as diarrhoea, allergy, high cholesterol and low immunity (Wongputtisin, 2003). Starter cultures of bacteria with an important functionalities, benefits and health advantages are developed for functional products. They can contribute to the microbial safety or offer one or more organoleptic, technological, nutritional, or health advantages. Recently, probiotics brought a lot of scientific attention by their functionality in the digestive tract (Ouwehand et al., 1999; Holzapfel et al., 2001; Kaur et al., 2002; Leroy and Vuyst, 2004). Hence, one of the objectives of this research was to screen for the probiotic properties of isolated bacteria.

The useful enzymes such as β-glucosidase enzyme related with digestive property and antioxidant activity needed to study. Several plants and medicinal plants compose of phytoestrogen or other natural compounds such the glycosides, which are compounds containing a sugar and a nonsugar residue (aglycone) in the same molecule. Glycoside hydrolysis may occur enzymatically through glucosidases or via acid hydrolysis. β-glucosidase is an interesting enzyme that hydrolyses a bond between glucose and aglycone. This aglycone may be an aroma compound or health related compound (Pyo et al., 2005; Yin et al., 2005; Otieno et al., 2006). Therefore, β-glucosidase has a potential to hydrolyse some medicinal plant such as the bitter glucoside (oleuropein of olives) to debitter by naturally fermented process (Leal-Sanchez, 2003; Randazzo, 2003). Moreover, β-glucosidase activities had a potential to enhance antioxidant acitivity of nutraceutical product containing medicinal plants and cereals.

Furthermore, one of the health-promoting benefits of probiotics is their ability to reduce blood cholesterol via several actions (Gilliland, 1985; Lim et al.,

2004; Liong and Shah, 2005a; Begley et al., 2006) such as cholesterol assimilation by the bacteria, cholesterol binding to the bacterial cell wall, and bile salt hydrolase deconjugate of bile salt (Brashears et al., 1998; Pereira and Gibson, 2002; Kim et al., 2008).

The aims of this study were to isolate and select probiotic lactic acid bacteria from food origins and to examine in *vitro* probiotic with β-glucosidase producing and cholesterol-lowering property for safety and quality improving of fermented soybean milk. The result of this study can lead to further development of fermented soybean milk and other fermented plant products as the functional foods.

The objectives of this study were as follows:

1. To investigate key probiotic properties of LAB which were isolated from Thai non human origin sources such as pickle, fermented plant beverages and fermented pork or fish.

2. To investigate β-glucosidase enzyme activity of isolated LAB.

3. To investigate bile salt hydrolase and in *vitro* cholesterol lowering property of isolated LAB.

4. To produce and investigate soybean milk fermentation with a selected probiotic lactic acid bacterium which was used as a starter culture.

5. To study effects of a selected probiotic and fermented soybean milk on cholesterol profiles in *vivo*.