CHAPTER 1

INTRODUCTION

1.1 Background

In the present, health-promoting food or functional food has become a popular trend. Functional foods may be produced by adding bioactive compounds into the foods or using beneficial microorganisms in the food processing. In addition to the value added to the product, functional foods would be another choice of healthy diet for consumers. It would reduce consumers' risk of disease or boost consumers' chances of optimal health. Dietary fiber and probiotic food are the examples of functional foods in the market. Probiotic food is food that contains beneficial live microorganisms. These live microorganisms will help improve host's intestinal microbial balance (Gibson and Roberfroid, 1995), enhance nutrition, promote better health and reduce risk of diseases (Lannitti and Palmieri, 2010; Vasiljevic and Shah, 2008; Shah, 2007).

Probiotic is beneficial viable microbes that help promote health, balance microorganism in the digestive system and cure diarrhea. Probiotic also has therapeutic properties such as stimulating immune system, lowering blood cholesterol level, and decreasing the risk of colon cancer (Perdigeon et al., 1998; Kurmann and Rasic, 1991; Fooks et al., 1999; Kailasapathy and Chin, 2000; Kaur et al., 2002; Itsaranuwat et al., 2003; Marona and Pedrigon, 2004). A product that is considered to be probiotic food should contain the probiotic microbes at a concentration of more than 6 log CFU/mL at the time of consumption. To support good health, probiotic bacteria should survive during transit through the stomach until the end of intestine. For this reason, probiotic organism should be resistant to the environments in the digestive system such as acid in the stomach and bile salt. Beside survival in the intestinal tract, probiotic bacteria should have other health promoting characteristics such as producing useful enzymes, activating immune system, decreasing harmful enzyme production, reducing cholesterol level and inhibiting pathogenic bacteria. Probiotic bacteria that are mainly used in industries are lactic acid bacteria (LAB), for instance, *Lactobacillus*, *Bifidobacterium* and *Enterococcus* (Holzapfel and Schillinger, 2002; Vinderola and Reinheimer, 2003). LAB is used in fermented food industry, for example, fermented milk products,

fermented meat products and pickled vegetables. Probiotics are available in the market in some other forms such as capsules, tablets as well. *Lactobacillus* and *Bifidobacterium* are the example of LAB that is used in fermented milk products. LAB in the products helps to detoxify of some pathogenic bacteria in the large intestine and improve lactose digestion. In addition, LAB can prevent diarrheal disease; boost your immune system and lower blood cholesterol level. Several researchers indicated that using LAB as a starter culture in fermented milk production can enhance the nutritional value and therapeutic properties of products (Lannitti and Palmieri, 2010; Parvez et al, 2006). In healthy food industry, it tends to increasingly use probiotics to be the starter cultures in food and beverage productions.

Soya milk is a cheap source of protein compared to animal protein. It is low in saturated fatty acid no cholesterol and high in essential fatty acids such as linoleic and linolenic acid. Furthermore, soya milk contains important substances such as isoflavones and oligosaccharides. Isoflavones are natural flavonoids and act as estrogen which is an essential female hormone. It helps to prevent and cure heal cancer, coronary heart disease and osteoporosis. Many researchers studied the beneficial use of isoflavone to help treat menopause system in women (Dajanta et al., 2009; Phommalth et al., 2008). Oligosaccharides can enhance the growth of probiotic bacteria. Healthy products from soy bean have been developed and interested for a long time because they are not only good for health, but also can be alternative foods for lactose-intolerant person. Cereals and their products for examples, rice, wheat, corn, and soybeans should be kept and stored well. Otherwise, they may be contaminated by mycotoxin from mould, such as Zealarenone (ZEA) which is produced by *Fusarium* sp. (Richardson et al., 1985; Fandohan et al., 2003; Adejumo et al., 2007; Broggi et al., 2007; Zinedine et al., 2007; Ezekiel et al., 2008) ZEA causes brucellosis in animals, decreasing in milk production of mammals and this mycotoxin will be excreted into milk (Witte, 2003). ZEA can lower animals' growth rate and acts like Estrogen, a female hormone. It triggers hyper estrogenic syndromes and affects the hormone system in humans as well (Pfohl-Leszkowicz et al., 1995; Smith et al., 1997; Geraldo et al., 2006; Seeling et al., 2006; Zinedine et al., 2007). According to the reports of El-Nezami et al. (2002); Shetty and Jespersen (2006) and Zinedine et al. (2007) it is found that LAB were able to remove mycotoxin by binding process. Because of the reasons above, it is very important to select probiotic strains that have health-promoting properties and binding

ability to be used as a starter culture in probiotic soy drink production. The developments of health-promoting products from soybean are very beneficial for agro-industry in Thailand because these are not only value added to products, but also can increase choices or be alternative foods for vegetarian and lactose-intolerant consumers.

This research aimed to study health-promoting properties of probiotic bacteria in order to be used as a starter culture for a probiotic soya beverage production. The basic probiotic characteristics of the bacteria in this study were already assessed for the persistence in the gut environment, antagonism against pathogenic bacteria (Kansandee, 2010) and safety in food (Generally Recognized as Safe; GRAS).

1.2 Research objectives

1.2.1 To investigate health-promoting properties of the probiotic bacteria strains, *Lactobacillus pentosus.*

1.2.2 To produce a probiotic soya beverage and study its chemical properties, physical properties, microbial properties, evaluate its sensory properties, and product's shelf life.

1.3 Research hypothesis

1.3.1 *Lb. pentosus* has health-promoting properties such as the reduction of cholesterol level, the binding of mycotoxin. Thus, it is suitable as a starter culture in the production of soya beverage.

1.3.2 The soya beverage product that is produced by probiotic bacteria *Lb. pentosus*, has all good characteristics which are chemical, physical, and microbial properties as well as customers' acceptance.

1.4 Significance of the research

Be able to use potential probiotic bacteria *Lb. pentosus* as a starter culture in the production of the probiotic product with is a health-promoting benefits to be accepted by consumers. This product will be suitable for vegetarians and health-conscious consumers such as lactose-intolerant, ageing and menopauses women**.**

1.5 Scope of study

The scope of this research as the following:

1.5.1 Eight strains of probiotic bacteria were used in the research; *Lb. pentosus* DM068, JM0812, JM085, UM054, UM055, VM095, VM096, and YM122. These bacteria were received from the laboratory of Asst. Prof. Dr. Pariyaporn Itsaranuwat, Department of Biotechnology, Mahasarakham University. Basic probiotic properties were assessed in these bacteria (Kansandee, 2010).

1.5.2 The study of characteristics tested of probiotic bacteria in this work.

1.5.2.1 Study of health-promoting properties of probiotic bacteria;

1) Capacity of carbohydrates utilization by determine of growth pattern, pH changes, sugar contents, and short chain fatty acid (SCFAs) production

2) Bile Salt Hydrolase activity.

3) *In-vitro* cholesterol binding activity.

4) *In vitro* adhesion capacity.

1.5.2.2 *In-vitro* ZEA binding activity.

1.5.3 Study on the use of probiotic *Lb. pentosus* strains with health-promoting properties as a starter culture for probiotic soya beverage production.

1.6 Experimental plan

Completely randomized design (CRD) and analysis of variance were conducted by using Duncan's Multiple Range Test (DMRT) for comparison of the means. All experiments were carried out in triplicates. The statistics used in data analysis are as the following:

1.6.1 Descriptive Statistics

Descriptive statistics used to describe the basic features of a collection of data in the study are:

$\overline{x}$ Mean

% Percentage

SD Standard Deviation

1.6.2 Inferential Statistics

Pair comparison according to DMRT, significant test at P-value $\leq .05$

1.6.3 The research was conducted from May 2013 until March 2014

1.6.4 The research was conducted at the laboratory of:

1) Department of Biotechnology, Faculty of Technology, Mahasarakham University

2) Central Laboratory of Mahasarakham University, and

3) Chemical Engineering Laboratory, Department of Bioprocess & Microbial System, UMR CNRS/INPT/UPS 5503, University of Toulouse – ENSAT, France

1.7 Definitions

1.7.1 Probiotic is a live microorganism or group of microorganisms that can survive in the intestinal tract with beneficial properties to human and animal hosts. Probiotic bacteria can help improve host's intestinal microbial balance and reduce the amount of pathogenic bacteria. A product that is considered to be probiotic food should contain the probiotic bacteria more than 6 log CFU/mL at the time of consumption.

1.7.2 Lactic acid bacteria (LAB) are the bacteria belonging to the family *Lactobacilli Bifidobacteria* that can produce lactic acid. LAB are Gram-positive, non-spore forming cocci or rods. The shape of cell can change depending on the environment. They mostly are microaerophile. Some types are anaerobes because they obtain energy only from the metabolism of sugars in the absence of oxygen. LAB are tolerant to acidic conditions.

1.7.3 The fermented milk is a dairy product that has been fermented with non-pathogenic, non-toxic bacteria which mostly are LAB. The bacteria produce lactic acid during the fermented process by converting lactose sugar into lactic acid. This process will lead to a drop in pH of the product and cause the denaturing of milk proteins to form curds. The product will have a sour taste. The fermented milk is suitable for the person who cannot drink the regular milk because cannot digest lactose sugar (lactose intolerance). The samples of fermented dairy product that use LAB are yoghurt, drinking yoghurt, and cheese.

1.7.4 Starter culture is a microbiological culture, such as fungi, bacteria, or yeast, which has been isolated and purified. It can be one single culture or mixed cultures to perform or start the fermentation process.

1.7.5 Health-promoting properties are the properties of microorganism in the host's gastrointestinal tract that can produce useful substances which are health beneficial to the host, for instance, to help improve the microbial balance in the intestine, reduce blood cholesterol level, and reducing of mycotoxin.