

CHAPTER I

INTRODUCTION

1. Rationale and Background

The isolation and screening of microorganisms from natural sources has always been the most powerful means for obtaining useful and genetically stable strains for industrially important products. Lactic acid bacteria (LAB) are important in the food and dairy industries because the lactic acid and other organic acids produced by these bacteria act as natural preservatives as well as flavour enhancers. LAB find increasing acceptance as probiotics which aid in stimulating immune responses, preventing infection by enteropathogenic bacteria, and treating and preventing diarrhea (Reid, 1999). LAB is a diverse bacterial group consisting of 11 genera. These bacteria are Gram-positive, non-spore-forming cocci or rods which produce lactic acid as their main metabolic product. Lactobacilli are found in a variety of habitats, also in the gastrointestinal tract of humans. Lactic acid bacteria have a long history as GRAS (generally recognized as safe) organisms and especially members of genus *Lactobacillus*, *Lactococcus* and *Streptococcus* are widely used in the fermentation industry. Several species of the genus *Lactobacillus* have been used in food products as probiotic organisms. Probiotic strains are selected for potential application on the basis of particular physiological and functional properties. Since generalizations about the probiotic performance of species cannot be yet made based on scientific evidence, it is more reliable to assume that probiotic properties are strain specific (Sanders et al., 1999).

LAB, particularly in the genera of *Lactococcus*, *Lactobacillus*, *Pediococcus* and *Leuconostoc* are involved in the production of fermented foods representing virtually all commodity groups including dairy, vegetable, fruit, meat, and cereal products (Marrug, 1991). The primary role of these microorganisms in fermented foods is one of the preservation methods. The lactic acid bacteria are also used to provide variety in the food supply. They carry out this function by altering the flavor, texture, and appearance of raw commodities in a desirable way (Davidson and Hoover, 1992).

Preservation of fermented foods by lactic acid bacteria is due primarily to sugars being converted to organic acids (lactic, acetic) causing a reduction in pH and removal of carbohydrates as nutrient sources. In addition, lactic acid bacteria produce antimicrobial substances including hydrogen peroxide, diacetyl, secondary reaction products, and bacteriocins which have potential to inhibit a variety of other microorganisms (Davidson and Hoover, 1992). With the emergence of foodborne pathogens i.e. *Escherichia coli* O157: H7 and *Staphylococcus aureus*, the discovery of new food processes and the desire by consumers for “natural” food products, these microorganisms have been recognized as a potential source of biopreservatives for foods. Substantial research on these microorganisms in recent times has focused on such an application (Davidson and Hoover, 1992).

Nowadays, the need for safe products with standard and desirable technological properties has resulted in the use of starter cultures for the production of dry fermented sausages, to control the fermentation and ripening process, inhibiting the growth of other undesirable microorganisms. The most promising bacteria for starter cultures are those which are isolated from the indigenous microflora of traditional products. These microorganisms are well adapted to the meat environment and are capable of dominating the microflora of products. The strains selected as starter or protective cultures must have the most important technological properties and/or bacteriocin production capabilities.

A starter culture can be defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process. The LAB group occupies a central role in these processes, and has a long and safe history of application and consumption in the production of fermented foods and beverages (Caplice & Fitzgerald, 1999; Ray, 1992; Wood, 1997; Wood & Holzapfel, 1995). They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes is of importance. In this way they enhance shelf life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product. The earliest production of fermented foods was based on spontaneous fermentation due to the development of

the microorganisms naturally presented in the raw material. The quality of the end product was dependent on the microbial load and spectrum of the raw material. Spontaneous fermentation was optimised through backslopping, i.e., inoculation of the raw material with a small quantity of a previously performed successful fermentation.

With the different traditional formulation of the dry-fermented sausage process, physico-chemical modifications occur, especially dehydration, fermentation of carbohydrates and acidification, development of a typical colour, lipolysis and oxidation of lipids, and proteolysis (Ordonez et al., 1999). These changes, which are responsible for the organoleptic characteristics of the final products, are due to the activity of the different microbial groups (above all lactic acid bacteria) which develop in sausages during the fermentation or ripening stage (Ordonez et al., 1999).

Mhom is a Thai traditional dry-fermented sausage made from meats including pork or beef as a principal component along with internal organs i.e. liver or spleen and other ingredients such as salt, fried or cooked rice, garlic, and pepper. The raw material and ingredients are mixed, casing stuffed and tied. Then they are kept about 2-3 days for fermentation. Usually, Mhom has been consumed raw or cooked. This fermented meat product has been developed and widely consumed in the northeastern region but the technology of fermentation is still mediated by indigenous bacteria rather than added starter cultures (Thiravattanamontri et al., 1998). The bacteria found most commonly in Thai fermented meat products are lactobacilli, pediococci and micrococci, but the precise role of these bacteria in the quality of the products is not known (Thiravattanamontri et al., 1998). Developments in meat fermentation have been focusing on application of defined starter cultures to process control and product consistency (Kastnera et al., 2006). In addition to difficulties in obtaining product consistency, pathogens in raw fermented sausage still cause food safety problems.

This research will be conducted on the isolation and identification of lactic acid bacteria involved in Thai fermented meat sausage (Mhom), screening of their antimicrobial activity, growth rate, and acidifying capacity, and also their application as starter cultures for Mhom production.

2. Objectives of the Study

2.1 To isolate, characterize and group microorganisms from naturally fermented Mhom Product

2.2 To identify and make a phylogenetic tree of Mhom isolated lactic acid bacteria through the API System, RAPD-PCR and 16s rRNA amplification and sequence analysis

2.3 To determine the antimicrobial inhibitory activity of identified LAB against *Escherichia coli* O157:H7 and *Staphylococcus aureus*

2.4 To determine the growth rate and acid-producing capability of high potential antimicrobial producing LAB identified

2.5 To determine the appropriate starter culture combination for Mhom production through flavor and texture profile along with sensory analysis of the products

2.6 To monitor the dynamic microbial profile during fermentation of the product

3. Scope and Limitation of the Study

Lactic acid bacteria are isolated and characterized from traditional fermented Mhom. Then, these bacteria are identified and a phylogenetic tree is made via the API System, RAPD-PCR and amplification of 16s rRNA and analysis of the sequence. Their antimicrobial inhibitory activity against pathogens, growth rate and acidifying capacity are determined and considered for starter application in Mhom production. The dynamic appropriate starter culture profiles during fermentation of the product are also monitored.

4. Anticipated Outcomes

4.1 To attain lactic acid bacteria isolated and identified from Thai traditional dry sausage (Mhom)

4.2 To obtain the phylogenetic tree of Mhom isolated LAB

4.3 To attain LAB having antimicrobial activity against potential bacterial pathogens

4.4 To achieve microbial profile of the appropriated starter culture during Mhom fermentation