# COMPARISON OF MICROBIAL DIVERSITY DURING NATURAL AND STARTER CULTURED NHAM FERMENTATION

## INTRODUCTION

Traditional Thai food fermentation is one of the oldest biotechnological processes. It not only extends the shelf-life but also enhances the flavor, texture and nutritional quality of the products. Among a variety of Thai fermented meat products, Nham or fermented pork sausage is common product popular consumed throughout Thailand, with great diversity in production methods and organoleptic characteristics between regions of country. This kind of product is characterized by high acidity and the final pH of approximately 4.2-4.6 (Phithakpol *et al.*, 1995 and Valyasevi and Rolle, 2002). Fermentation of Nham is a well-known microbial process and ecological studied during fermentation was date back to the 1970s (Techapinyawat, 1975, Tanasupawat and Daengsubha, 1983, Wiriyacharee *et al*., 1990 Tanasupawat *et al.*, 1992 and Krairojananan *et al*., 1997, Kunawasen, 2000). The dominant microorganisms have already been isolated from Nham. They belong to lactic acid bacteria (LAB) including lactobacilli (*Lb. acidophilus, Lb. cellubiose, Lb. graminis, Lb. plantarum, Lb. pentosus, Lb. curvatus, Lb. sakei, Lb. delbruckei, Lb. paracasei* and *Lb. brevis*) and pediococci (*P. acidilactici* and *P. pentosaceus*). Other microorganisms, *Micrococcus* sp. and *Staphylococcus* sp. that are capable of reducing nitrate to nitrite and ensure color development were also detected.

Starter cultures are widely used for producing various kinds of fermented meat products including Nham to shorten the fermentation time, ensure proper acid production, helping color development, enhance the flavor, drip loss improvement, and inhibition of undesirable microorganisms. The development of starter culture should involve intense research on the roles of microorganisms during the fermentation. The biochemical and physiological roles of these microorganisms in the development of flavor and aroma of the Nham product must be elucidated (Smitinont, *et al*., 1999). This information can be used as the criteria in the selection of starter

microorganism for used in the fermentation. The starter culture finally selected would be the one giving a satisfactory performance in the process and also giving an acceptable organoleptic evaluation of the Nham product. The development of starter cultures for Nham fermentation was initiated in the 1975 (Techapinyawat, 1975). The first commercial Nham production using starter culture technology has been successfully used by Wanasanun Co. Ltd, Thailand since 1990. Furthermore, developments of starter formula for Nham have been carried out by Valyasevi *et al*. (2001) to improve the microbial fermentation processes and product diversity. Selected isolates from the dominant microorganism in good quality Nham were evaluated for their ability to use as starter culture for Nham fermentation as well as the sensory quality of the final product. Among the isolate selected, *Lactobacillus plantarum* is one of the effective strains suitable for use as Nham starter culture.

Over the past decade, many studies have been focused on the effect of using starter culture on organoleptic properties, sensorial characteristic, biochemical and physico-chemical properties, and the food-borne pathogens of Nham (Petchsing and Woodburn, 1990, Svetvivadhana, 1990, Wiriyacharee *et al*., 1990, Rakphoa, 1996, Twichatwitayakul, 1996, Smitinont *et al.*, 1999, Valyasevi *et al*., 2001, Kwanmuang, 2003 and Visessanguan *et al.*, 2005 and 2006a, b). However, no previous studied have evaluated the effect of starter culture on the microbial diversity during Nham fermentation. It is interesting to notice how the starter culture influenced the dynamics of the different bacterial species, especially LAB isolated from Nham which may in turn lead to the better understanding of fermentation ecology.

The study of the effect of the starter culture on LAB ecology in fermentation processes, are crucial since they are the information that will have allow a better understanding of starter culture's role in Nham ecosystem in order to increase the confidence of manufacture to decide the use of this microorganism in Nham production. Sensitive and reliable detection and identification methods are important for monitoring population changes of both natural and starter cultures used in the fermentation process. Traditional methods for bacterial identification based on several morphological, physiological and biochemical characteristics are laborious, time

consuming, sensitive to growth conditions for expression of the characteristics, and often lacking sensitivity to detect small differences among closely related strains. Recently, molecular technique, based on detection of differences in the genetic materials is an alternative method for a faster and more reliable microbial differentiation. Furthermore, many genotypic methods which are based on the principle of polymerase chain reaction (PCR) has been recognized as a simple PCR-based technique with rapid, high discriminatory power, low cost, suitable for high-throughput analysis, and provide reliable results. The technique has gained popularity and is widely used in molecular typing of wide range of bacteria.

In this study the focus is on studying changes of microbial population as well as chemical changes of Nham fermented with and without added starter culture. The PCR based typing techniques, ITS-PCR was used to identify each microbial isolates collected during the fermentation process. In addition, 16S rDNA sequencing was used to identify selected isolates from each microbial group. Moreover, rep-PCR was used to monitor changes of *Lb. plantarum* strains that present during starter cultured Nham fermentation

# OBJECTIVES

1. To group and identify lactic acid bacteria that present during natural and starter cultured Nham fermentation by using ITS-PCR and 16S rDNA sequencing.

2. To assess the growth and survival of *Lb. plantarum* BCC 9546 from starter cultured Nham fermentation using rep-PCR fingerprint technique.

3. To compare the microbial diversity and chemical profiles in natural and starter cultured Nham fermentation.