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Potential of bacteriocin-producing lactic acid bacteria for safety improvements of traditional Thai fermented meat and human health

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ABSTRACT

Lactic acid bacteria (LAB) are very important in converting of agricultural products into safe, delicious and shelf stable foods for human consumption. The preservative activity of LAB in foods is mainly attributed to the production of anti-microbial metabolites such as organic acids and bacteriocins which enables them to grow and control the growth of pathogens and spoilage microorganisms. Besides ensuring safety, bacteriocin-producing LAB with their probiotic potentials could also be emerging as a means to develop functional meat products with desirable health benefits. Nevertheless, to be qualified as a candidate probiotic culture, other prerequisite probiotic properties of bacteriocin-producing LAB have to be assessed according to regulatory guidelines for probiotics. Nham is an indigenous fermented sausage of Thailand that has gained popularity and acceptance among Thais. Since Nham is made from raw meat and is usually consumed without cooking, risks due to undesirable microorganisms such as *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes*, are frequently observed. With an ultimate goal to produce safer and healthier product, our research attempts on the development of a variety of new Nham products are discussed.

1. Introduction

Lactic acid bacteria (LAB) are a non-taxonomic group of Gram positive, low G + C content, non-motile bacteria characterized by their capability to ferment sugar to lactic acid. The organisms are devoid of cytochromes and anaerobic in nature but can tolerate air. They are catalase-negative, although pseudo-catalase can be found in rare cases. Phylogenetically, they are a very diverse group of organisms and belong to the clostridial branch of Gram-positive bacteria (Thokchom & Joshi, 2012). LAB are widely used in the production of fermented food, and they constitute the majority of the volume and the value of the commercial starter cultures (Hansen, 2002). Members of LAB share the property of being Gram-positive bacteria that ferment carbohydrates into energy and lactic acid, which reduces the pH of the product and contributes to the product safety. They consist of a number of bacterial genera within the phylum Firmicutes. The genera *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Lactosphaera*, *Leuconostoc*, *Melissococus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* are recognized as LAB (Thokchom & Joshi, 2012). Lactic acid producing Gram-positive bacteria but belonging to the phylum Actinobacteria are genera such as *Aerococcus*, *Microbacterium*, and *Propionibacterium* (Sneath & Holt, 2001) as well as *Bifidobacterium* (Holzapfel, Haberer, Geisen, Björkroth, & Schillinger, 2001; Stiles & Holzapfel, 1997). Heterofermentative LAB of the *Carnobacterium*, *Leuconostoc* and *Weissella* genera are usually more involved in meat spoilage than the homofermentative *Lactobacillus* and *Pediococcus* genera. Therefore, commercially available meat starter cultures for dry-fermented sausage production exclusively belong to the latter two (Kröckel, 2013).

The growing concern of consumers today regarding the food health (referred to as functional food) and safety issues has led to the development of products that promote health and well-being beyond its nutritional effect (Työppönen, Petäjä, & Mattila-Sandholm, 2003). The known health promoting foods such as dairy products, especially sour milk and yoghurt, are fermented by viable probiotic lactic acid bacteria (LAB) with scientifically proven health effects and safety. Such cultures may promote health in different ways (Saxelin, 2000). Probiotics have a long history of human use, and cultured dairy products are traditional consumed in several parts of the world. The FAO/WHO defines probiotics as "Live microorganisms which when administered in adequate amount confer a health benefit on the host" (FAO/WHO, 2001). Across the globe, about 20 probiotic strains, singly or in combination, such as *Lb. acidophilus*, *Lb. casei*, *Lb. reuteri*, *Pediococcus pentosaceus*, and *Bifidobacterium longum* etc., are presently used in dietary supplements and functional foods or in mainstream food products. Similar in concept to consumption of probiotic cultured milk products, addition of probiotics to the fermented sausages could promote the health benefits (Lücke, 2000; Vuyst, Falony, & Leroy, 2008).

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In Thailand, there are many traditional fermented meat products which are consumed without heating after appropriate fermentation (pH lower than 4.6). Among them, Nham has gained the most popularity and acceptance among Thais and is widely consumed due to its unique texture, flavor, and color. The basic ingredients of Nham are minced pork (52% w/w) and cooked pork rind (35% w/w) which are mixed with fresh garlic (4.3% w/w), cooked rice (4.3% w/w), salt (1.9% w/w), sugar (0.3% w/w), whole bird chili (~2% w/w), sodium tripolyphosphate (0.2% w/w), monosodium glutamate (0.2% w/w), erythrobate (0.2% w/w) and potassium nitrite (0.01% w/w). The mixture is thoroughly mixed and then tightly packaged in banana leaves or plastic casing The product is allowed to ferment at ambient temperature over a 3 to 4 day period, during which time it attains a final pH of 4.6 (Phithakpol, Varanyanond, Reungmaneepaitoon, & Wood, 1995). Fermentation of Nham involves the successive growth of different microorganisms, dominated by lactic acid bacteria naturally present in Nham ingredients. Lactobacilli (*Lb. plantarum*, *Lb. pentosus* and *Lb. sakei*) and pediococci (*P. acidilactici* and *P. pentosaceus*) have been identified as the predominant microorganisms in Nham fermentations (Visessanguan, Plengvidhya, Chokesajjawatee, & Bakar, 2015). However, these fermented meat products, which are produced by naturally occurring LAB, often result in inconsistent qualities and unsafe products such as documented in reports on *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes* contaminations in Nham (Paukatong & Kunawasen, 2001). Thai fermented sausage producers are interested in improving the product stability and shelf life by inhibiting undesirable spoilage and pathogenic microorganisms.

The present article provides an insight into the technology and microbiology of Nham with both probiotic and candidate probiotic LAB. Besides, the article also aimed at both safety and functional fermented meat products by using bacteriocin-producing strains as starter cultures.

2. Screening of bacteriocin-producing LAB for probiotic

Bacteriocins, the antimicrobial substances which are mostly produced by LAB, have gained tremendous attention as potential biopreservatives. These antimicrobial substances are ribosomally synthesized, extracellularly released bioactive peptides or peptide complexes, having a bactericidal or biostatic activity (De Vuyst & Leroy, 2007). Some bacteriocins appear to inhibit potential food borne pathogens including *Clostridium botulinum*, *Enterococcus faecalis*, *L. monocytogenes*, *S. aureus* and *Bacillus* spp. Bacteriocins produce by LAB are considered to be safe biopreservatives, since they are assumed to be degraded by proteases in gastrointestinal tract (Cleveland, Montville, Nes, & Chikindas, 2001).

Lactic acid bacteria originating from fermented meat have been considered for selection as starter cultures because of being well adapted to the ecology of meat fermentation and (Table 1). In addition, they should demonstrate antimicrobial activity against pathogenic Gram-negative bacteria which contains outer lipopolysaccharide membrane. Conventionally, in order to find the bacteriocin-producing strains, the isolated bacteria is cultured individually and the culture supernatant is assessed for its antimicrobial activity assay against the selected indicator strains by a spot on lawn method (Zendo et al., 2005). The amino acid sequences of purified peptides are analyzed by Edman degradation (Zendo, Nakayama, Fujita, & Sonomoto, 2008). However, the procedure to detect, purify and identify the novel type of antimicrobial peptides has shown to be tedious, time-consuming and expensive. With the recent technological advances in mass spectrometry, genomics and informatics, a new proteogenomic approach has been developed to enable us performing peptide identification with high sensitivity for the discovery of microorganisms that produce both known and putatively novel bacteriocins based on their molecular mass, antibacterial spectra and selectivity.

3. Development of safe, functional probiotic starter cultures for Nham

In order to be qualified as a candidate probiotic culture, other prerequisite probiotic properties of bacteriocin-producing LAB have to be assessed according to regulatory guidelines for probiotics which may vary in different countries. In general, it must be a strain that is generally recognized as safe (GRAS). They need to survive gastrointestinal (GI) transit in sufficient numbers to exert their beneficial effects when administered orally. Therefore, during GI passage, in order to colonize the intestinal mucus and offer an antagonistic effect against pathogenic microorganisms, cultures are required to tolerate the presence of pepsin and the pH of the stomach, the protease-rich conditions of the duodenum and the antimicrobial activity of bile salts in the upper part of the intestine. In addition, they should be able to adhere to intestinal mucus which is a prerequisite to exert beneficial effects, such as the exclusion of enteropathogenic bacteria and immunomodulation of the host (Osmanagaoglu, Kiran, & Ataoglu, 2010). The ability of a strain to adhere to intestinal mucus may prolong the time probiotics can remain in the GI tract and can influence the host.

3.1. Safety considerations

Presumption of safety of a microbe maybe based at the genus, species, or strain level, depending on the characteristics of the microbe and the end use. Lactobacilli and bifidobacteria have been considered to be safe due to their occurrence as normal commensals of the mammalian flora and their established safe use in a diversity of foods and health supplement products worldwide (Adams & Marteau, 1995). However, probiotics may theoretically be responsible for four types of side-effects including systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals, and gene transfer (Marteau, 2001). In recognition of the importance of assuring safety, even among a group of bacteria that are Generally Recognized as Safe (GRAS), FAO-WHO has recommended that antimicrobial resistance patterns and opportunistic virulence properties should be characterized (FAO/WHO, 2002). If the strain under evaluation belongs to a species that is a known mammalian toxin producer, it must be

Table 1
Screening of bacteriocin producing LAB with some probiotic potentials from various traditional Thai fermented meat products.

Strain	Source of isolate	Type of bacteriocin	Reference
Lc. lactis subsp. *lactis* N100&190	Nham	Nisin Z	Swetwiwathana (2005)
P. pentosaceus TISTR 536	Nham	Pediocin PA-1	Swetwiwathana (2005)
P. pentosaceus M13	Mum	Pediocin PA-1	Swetwiwathana, Zendo, Nakayama, and Sonomoto (2007)
Lb. plantarum KMITL-QU 54	Sai-krog Isan (fermented meat-rice sausage)	Pediocin like	Swetwiwathana, Pilasombut, and Sethakul (2008), Swetwiwathana, Sawa, Zendo, Nakayama, and Sonomoto (2008)
Weissella cibaria KMITL-QU 21	Sai-krog Isan	Under study	Swetwiwathana, Pilasombut, et al. (2008), Swetwiwathana, Sawa, et al. (2008)
Lactococcus garvieae BCC 43578	Nham	Garvieacin Q	Tosukhowong, Zendo, et al. (2011)
P. pentosaceus BCC 3772	Nham	Pediocin PA-1/AcH	Kingcha et al. (2012)
Weissella hellenica BCC 7293	Nham	Bacteriocin 7293A and B	Woraprayote et al. (2015)

No information on the study of bacteriocin.