CONCLUSION AND RECOMMENDATION

Conclusion

Based on the ITS-PCR database and 16S rDNA sequencing it can be concluded that, the microbial diversity of cultivable microorganisms in the natural and starter cultured Nham were different during fermentation. However, both types of Nham showed a dramatic succession of LAB species during fermentation. In natural fermentation, the dominant LAB species were *Lc. garvieae, Lc. lactis, Lb. plantarum* and *P. pentosaceus*. Other species that were found at low proportion were *Ec. faecium, Lb. brevis, Lb. curvatus, Lb. farciminis, Ln. citreum, Ln. fallax, Ln. pseudomesenteroides, W. cibaria, Weissella* sp. In contrast, starter cultured Nham, were found to contain only 3 dominant LAB species during fermentation. Three species of LAB belonged to *Lc. garvieae, Lc. lactis* and *Lb. plantarum*. Other species that were found at low proportion were *Enterococcus* sp*, Vc. carniphilus, Ln. citreum, Ln. fallax, Ln. pseudomesenteroides, W. cibaria, Weissella* sp., *W. paramesenteroides* and *P. pentosaceus.*

From our results, the diversity of LAB species is clearly different when comparing microbial diversity of natural and starter cultured Nham at each sampling time of fermentation. In addition, starter cultured Nham displayed the lower diversity than in natural fermentation especially after *Lb. plantarum* became the predominant species at 24 h of fermentation until the end of fermentation. However, the genetic diversity of major LAB species fairly similar in both type of Nham fermentation during the early phase (0-6 h) of fermentation but clearly different when the fermentation proceeded to 12 h and continue to differ until the end of fermentation. We can broadly categorized Nham into 3 possible phases of LAB succession steps. At the early phase (0-6 h) of fermentation, *Lc. garvieae* were the predominant species in both types of Nham fermentation. At the middle phase (12-24 h) of fermentation, *Lc. lactis* became the dominant species in natural Nham while *Lc. lactis* became the dominant species followed by *Lb. plantarum* at 12 h of fermentation in starter cultured Nham. After 24 h of fermentation, *Lc. lactis* remained the dominant species

followed by *P. pentosaceus* in natural Nham while *Lb. plantarum* became the dominant species in starter cultured Nham. At the later stage (36-72 h) of fermentation, *Lb. plantarum* were the predominant species in both types of Nham fermentation while *P. pentosaceus* remained the second dominant species in natural Nham.

The characterization by rep-PCR was performed on the *Lb. plantarum* strains isolated from starter cultured Nham. The results obtained underlined how distributive *Lb. plantarum* BCC 9546 strains that conducted the Nham fermentation in order to confirm potentially the role of unmarked *Lb. plantarum* BCC 9546 in the development of Nham and, in consequence, to decide for the use of this strain in the Nham processes for the manufacture. The result of this study confirms importance of the starter culture as the bacterium responsible for Nham fermentation and subsequent characteristics since it is successfully established and dominated over other lactic acid bacteria throughout the fermentation period.

This study has further contribute to our understanding of the effect of *Lb. plantarum* starter culture have on the microbial diversity and community structure as well as chemical changes of Nham fermentation. *Lb. plantarum* BCC 9546 has the potential to be used as starter culture for Nham production. This strain had an ability to adapt to the complex environment of fermented Nham, can acidify its environment rapidly, which resulting in efficient acid production and the accompanying pH decrease which has been considered to provide an additional tool for preventing the outgrowth of spoilage microorganisms and food-borne pathogen as well as enhancing the competitiveness of the desired microorganisms. For this reason, it can be concluded that the use of *Lb. plantarum* BCC 9546 as starter culture to initiate the production of Nham had play a role in accelerating the fermentation process by increasing lactic acid production which subsequently resulting in decreasing the diversity of LAB species.

Recommendations

1. According to the results obtained in this research, the $(GTG)_5$ genomic fingerprinting was used to monitoring of *Lb. plantarum* isolates from starter culture inoculated Nham. Further studies should be carried out to monitor *Lb. plantarum* isolates in natural Nham for complete data with the final goal to comparison the distribution of *Lb. plantarum* strains between natural and starter cultured Nham. In addition, it also could be interesting to find a new strain of *Lb. plantarum* isolates to use as starter cultures that can completely eliminate undesirable microorganism for Nham fermentation in order to obtain products with high good hygienic quality and safety from spoilage microorganism or some serious food borne pathogens.

2. The molecular methods based on PCR technique, namely ITS-PCR and 16S rDNA sequencing in this study offered important tool regarding the differentiation and identification of LAB isolates at the species level. In the case of the development of a starter culture, the use of rep-PCR allowed to understand not only which strains are able to perform well or not, but also could be select new strains that might be candidate strains to use as starter cultures in manufacturing of Nham. Therefore, these molecular methods are an important tool to take consideration in developing LAB as starter cultures for fermented Nham and other traditional Thai fermented products.

3. Culture-dependents are applicable in the dynamics of specific and cultivable microorganisms. However, alternative technique is required in order to more complete picture of the microbial diversity and its ecology in fermented sausage. In the last few years, the dynamic of the LAB populations during sausage fermentations, have clarified using the culture-independent technique, such as PCR-DGGE. To us this is an important aspect led to complete understanding the fermentation process of Nham, and it should be investigated further. Nevertheless, the application of culture-dependent methods remain valuable as it follows isolation of cultures which may be used as starter to improve the technological properties for the preparation of Nham and other traditional fermented product.