## Importance of salt concentration and long-term fermentation in the quality of salty-fermented freshwater fish paste in Thailand and Laos

The salty-fermented freshwater fish paste known as *pla-ra* and *pa-daek* in Thailand and Laos is universally used in daily meals as a storage-stable almighty seasoning or protein-rich cooking ingredient. It is traditionally made at home and also sold on the market nowadays. For the preparation, raw fish is mixed with salt and rice bran or roasted rice powder, and then fermented at tropical temperatures for at least 6 months. It is generally believed that longer fermentation makes the products taste better. Moreover, consumers often think the taste of the products varies from place to place. Scientific evidences regarding such characteristic features and microbes involved in the fermentation are expected to be useful in promoting quality improvement and consumption of *pla-ra / pa-daek* products, which are traditional high-value-added products that highly utilize indigenous fishery resources particularly in the inland region of Southeast Asia.

Regional characteristics of the pH and salt concentration are observed in the samples examined (Fig. 2A). The product pH is positively correlated with the salt concentration (Fig. 2A). Multiple species (2-8 species) of lactic acid bacteria are detected by the culture-independent method (summarized in Fig. 1) in 10 pla-ra / pa-daek products collected from central, northern, and northeastern Thailand, and Vientiane in Laos (Fig. 2B). The products can be classified into two groups by the representative genus of lactic acid bacteria, such as Tetragenococcus or Lactobacillus. Salt concentration of approximately 10% is the dividing point between these two groups (Fig. 2A). Products in the latter group contain relatively higher amount of lactic acid. The amount of glutamic acid, which is the source of delicious taste, increases in a fermentation-time dependent manner by 4 to 6 months in products prepared by following a common recipe (Fig. 3). Glutamic acid is one of the amino acids generated by the digestion of fish protein during fermentation.

Taken together, salt concentration is a key factor in determining the activity of lactic acid bacteria and the resulting lactic acid amount that relates to the regionality in taste preference. The producers can utilize a simple tool, such as a test paper, to monitor the salt concentration and pH for improving product quality and productivity. The overgrowth of Lactobacillus species that possibly results in acidification of the product by excess acid production can be prevented by controlling the salt concentration accordingly. It is recommended that the fermentation be continued long enough to generate a sufficient amount of glutamic acid.

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separation by Denaturant Gradient Gel Electrophoresis (DGGE)

DNA

D

DNA extraction

C

pla-ra / pa-daek

Measurements of pH, salt, lactate, glutamate

Polymerase Chain Reaction (PCR

bacterium A









Fig. 3. Time-dependent change of the glutamic acid (source of delicious taste) during fermentation of *pla-ra* prepared by following a common recipe

16S rRNA gene