AU J.T. 9(4): 217-221 (Apr. 2006)

# Analysis of Chemical and Microbiological Changes During Over-fermentation of *Naem* by Using Biochemical and Molecular Biology Methods

**Trilert Chaicherdsakul, Worrawalan Phoonsawat and Vethachai Plengvidhya***

**Faculty of Biotechnology, Assumption University**
**Bangkok Thailand[1]**

## *Abstract*

*'Naem' is a traditional Thai fermented sausage regularly fermented at room temperature for approximately 48-72 hours. However, many producers and consumers prefer keeping the products at room temperature. This practice can lead to unacceptable products due to continuous fermentation. This period can be described as over-fermentation. Previous studies using traditional biochemical and molecular biology methods to study the ecology of commercial Naem fermentations revealed that various lactic acid bacteria species such as Leuconostoc mesenteroides, Lactobacillus plantarum, L. pentosus, L. curvatus, L. sakei, L. bravis and Pediococcus pentosaceus were involved (Kunawasen 2000). However, no attention has been paid to the over-fermentation period (more than 3 days after the start of the fermentation). The objective of this research was to study the microbial diversity and community structure of Naem during the over-fermentation period by using PCR of rRNA genes methods. Naem samples from commercial processing plant were sampled during the over-fermentation period of 120 and 168 hours after the start of the fermentation. The samples were plated on MRS agar and selected numbers of isolates for each time point was recovered. DNA was isolated for PCR fingerprinting and 16S rDNA sequence analysis. A total of two hundred isolates were collected for analysis. A database of PCR fragment pattern has been generated by amplifying the intergenic transcribed spacer (ITS) regions between the 16S and 23S rDNA genes. The 16S rDNA gene has been sequenced to further identify selected isolates. According to ITS-PCR banding patterns, the number of bacterial species involved in the over-fermentation was four on days five and five on days seven. The use of ITS-PCR and 16S rDNA analysis indicated that L. plantarum was the dominant species in over-fermented Naem. We recovered only a small number of isolates belonging to L. brevis, L. paracasei, L. curvatus, L. fermentum and P. pentosaceus. The combination of ITS-PCR for isolate grouping and further characterized by 16S rDNA sequencing can offer and alternative tool for describing the important bacterial species involving in over-fermented Naem samples. This study reveals that minimizing the number of L. plantarum might be one alternative in prolonging the shelf life of Naem when stored at room temperature by delaying the over-fermentation period.*

***Keywords:*** *Polymerase chain reaction (PCR), Over-fermented Naem, Lactic acid bacteria, rRNA genes, intergenic transcribed spacer (ITS), 16S rDNA sequencing, restriction digestion*

---

* Food Biotechnology Laboratory, National Center for Genetic Engineering and Biotechnology, Pathum Thani, Thailand