

Saowalak Dansakul 2009: Study of Molecular Techniques for the Identification of Yeast Strains from Thai Fermented Food and Alcoholic Beverages. Doctor of Philosophy (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Associate Professor Vichien Leelawatcharamas, Ph.D. 144 pages.

Twenty-five yeast isolates were taken from Loog-pang (mold bran starter), Kao-mag (alcoholic sweetened rice) and during Satho (traditional Thai rice wine) and coconut toddy fermentation process. Yeast isolates were identified by carbon compound assimilations using the API ID 32 C kit and by sequencing of their D1/D2 domain of 26S rDNA region. Fourteen isolates of *Sacharomyces cerevisiae* were identified whereas 5 isolates of *Saccharomycopsis fibuligera* could not be identified by this kit however, this species shows high homology when identified by sequence analysis. The 6 remaining isolates were identified as *Candida tropicalis* (2), *Issatchenkia orientalis* (1), *Pichia caribbica/Candida fermentati* (2) and *Pichia farinosa* (1). For most sequenced isolates homologies of 98-99% were obtained with sequences in GenBank. D1/D2 domain sequencing yielded approximately 600 bp region for alignment using ClustalX program. Bootstrap values from a phylogenetic analysis using neighbor joining method showed strong support. All *Sc. cerevisiae* isolates clustered together as the same result of the *Sm. fibuligera* group.

*Sc. cerevisiae* was found to be the dominant species for Kao-mag and the fermentation of Satho and coconut toddy, whereas *Sm. fibuligera* was found to be the dominant species for Loog-pang and Kao-mag. The intraspecies variations were examined by molecular DNA markers. Twenty-six isolates of *Sc. cerevisiae* and 11 isolates of *Sm. fibuligera* including referent strains were screened for polymorphism of DHPLC analysis of D1/D2 domain. Three and 4 different profiles were obtained for *Sc. cerevisiae* and *Sm. fibuligera* isolates, respectively. *Sc. cerevisiae* isolates were further differentiated by microsatellite and mtDNA-RFLP. Both markers produced 19 polymorphic profiles among the 26 isolates. ISSR marker was used to differentiate *Sm. fibuligera*. The patterns obtained by eight UBC (University of British Columbia) primers gave 80 reproducible amplified bands ranging from approximately 300 to 2000 bp. This marker could classify 11 isolates of *Sm. fibuligera* into 7 groups with clear profiles. This study provides addition evidence of a genetic separation. DNA markers used in this study could be successfully applied to study intra-specific variation and genetic variations in the yeast species from different kinds of food products in Thailand. This research also included a small survey for study of ethyl alcohol fermentation and amylolytic activity for *Sc. cervisiae* and *Sm. fibuligera*, respectively.

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Thesis Advisor's signature