Quality control of *Saccharomyces* yeasts: differentiation of species level and strain grouping using COX 2 gene analysis and MALDI-TOF MS analysis

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The genus *Saccharomyces* is the most important and the most extensively used microorganism in industry, as production of food, alcohol, vitamins, and other growth factors. In NITE Biological Resource Center (NBRC) 363 *Saccharomyces* strains have been preserved and distributed to researchers and industrial users. The quality management of microbial strains is the essential function in the culture collection. The molecular identification method based on the 26S rDNA D1/D2 domain sequences is applied to the quality control of all of the yeast strains in NBRC. This is a powerful method for the species identification of yeast, but it is very difficult to distinguish below the species level, especially *Saccharomyces* species.

Therefore we have applied two methods, the cytooxygenase-2 (COX 2) gene analysis and the matrix-assisted laser description/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis, to the quality control of *Saccharomyces* species. The result of COX 2 gene analysis for the more than 300 *Saccharomyces* strains show that *Saccharomyces cerevisiae, Saccharomyces mikatae, Saccharomyces paradoxus* are divided into five, two, two clades, respectively. The strains of *Saccharomyces bayanus* are divided into two groups and one is clustered with *S. pastorianus*. The strain grouping based on the COX 2 gene analyses seems to correlate with the strain habitat and sources.

Recently, MALDI-TOF MS has emerged as a new technique for microbial species identification. This is a rapid, sensitive and simple method for identification of microorganisms, but the conventional mass spectral fingerprint analyses are frequently influenced by mass spectral variability. To solve this problem, the ribosomal protein was

used as a biomarker in the MALDI-TOF MS analysis in this study. We have characterized the expressed ribosomal proteins of genome sequenced yeast *Saccharomyces cerevisiae* NBRC 1136 to investigate the ribosomal subunit proteins as reliable biomarker. Approximately 43 ribosomal subunit proteins could be selected as a biomarker and were used for the MALDI characterization in *Saccharomyces* species. At present, only limited numbers of ribosomal proteins show the mass spectral variation, but some ribosomal proteins might be used to distinguish the groups of strains in *S. cerevisiae* and *S. mikatae.*