E333

Localization of NADH-semidehydroascorbate reductase and correlation between NADH-semidehydroascorbate reductase and NADH-cytochrome b_5 reductase in *Saccharomyces cerevisiae*

Sung-Wook Hwang*, Seong-Tae Kim, Won-Ki Huh, Byung-Hoon Lee and Sa-Ouk Kang
Department of Microbiology, College of Natural Sciences, and Research Center for Molecular Microbiology, Seoul National University

In *Saccharomyces cerevisiae*, NADH-cytochrome b_5 reductase is localized in the mitochondrial outer membrane. Subcellular and submitochondrial localization of NADH-semidehydroascorbate (SDA) reductase activity was identified and NADH-SDA reductase activity was found out to be localized in the mitochondrial outer membrane. In order to investigate the correlation between NADH-SDA reductase activity and NADH-cytochrome b_5 reductase, the gene encoding NADH-cytochrome b_5 reductase (mcr1) was disrupted in α-type cell of *S. cerevisiae*. NADH-SDA reductase activity was not detected by spectrophotometric method in haploid mcr1::URA3 disruptant. Therefore, NADH-SDA reductase activity can be correlated to NADH-cytochrome b_5 reductase in *S. cerevisiae*

E334

Purification and Characterization of a Catalase-Peroxidase from *Pleurotus ostreatus*

Seung-Jae Lee, Seung-Rock Lee and Sa-Ouk Kang
Department of Microbiology, College of Natural Sciences, and Research Center for Molecular Microbiology, Seoul National University

Catalase-peroxidase was purified 80-fold in four steps to an electrophoretic homogeneity from the oyster mushroom, *Pleurotus ostreatus*. The enzyme was composed of four subunits with a molecular mass of 62 kDa and contained four molecules of protoheme IX per tetramer. It showed a typical high-spin ferric heme spectrum with a Soret band at 408 nm and a peak at 632 nm and binding of cyanide caused a shift of Soret band to 428 nm, the appearance of a peak at 557 nm, and abolition of the peak at 632 nm. Reduction with dithionite resulted a shift of Soret band to 425 nm, and appearance of a peak at 529 and 559 nm. The enzyme lost all of its activity after incubation at 5℃ after illumination for 6 hours. The optimal pH was 6.8 and 5.5 for the catalatic and peroxidatic activities, respectively. The enzyme was inactivated by ethanol/chloroform, and was inhibited by salicylic acid, KCN, NaN_3 and NH_2OH, but not by the monofunctional catalase inhibitor, 3-amino-1,2,4-triazole.