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SUMMARY OF DOCTORAL THESIS

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Title: Study on the respiratory chain of ethanologenic *Zymomonas mobilis* and characteristics of its thermotolerant strains

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Zymomonas mobilis is an alternative microorganism to *Saccharomyces cerevisiae* for ethanol production. The bacterium may acquire most ATP, about one mole of ATP per mole of glucose, via the Entner-Doudoroff (ED), glyceraldehyde-3-phosphate-to-pyruvate (GP) pathways and appears to maintain a high level of glucose flux through the pathways to compensate its low ATP yield, for which large amounts of enzymes are expresssed, constituting 30-50% of total soluble proteins of cells. Considering the fact that *Z. mobilis* has an incomplete TCA cycle, resulting in low productivity of NADH and ATP per glucose consumed, it is assumed that NADH oxidation by its respiratory chain becomes unfavorable for ethanol production due to the competition for NADH with the PE pathway.

In chapter 1, the respiratory chain of *Z. mobilis* was investigated. Membranes from cells cultivated under aerobic or anaerobic growth conditions showed dehydrogenase and oxidase activities for NADH, D-lactate and D-glucose and ubiquinol oxidase activity. NADH oxidase activity level of membrane fractions from cells grown aerobically was found to be higher than that of membrane fractions from *Escherichia coli* or *Pseudomonas putida*, which has a complete TCA cycle, indicating a crucial role of the respiratory chain in NADH oxidation in the organism. Cyanide-resistant terminal oxidase activity was observed and appeared to be due to a *bd*-type ubiquinol oxidase as the only terminal oxidase encoded by the entire genome. The terminal oxidase with a relatively strong ubiquinol oxidase activity exhibited remarkably weak signals of cytochrome *d*. Considering these findings and the presence of a type-II NADH dehydrogenase but not a type-I, a simple respiratory chain that generates less energy may have evolved in *Z. mobilis*.

In chapter 2, to find out a thermotolerant *Z. mobilis* strain, the growth and ethanol production of four isolates in Thailand were compared with those of the efficient strain

ZM4 (NRRL B-14023) at different temperatures. At 39˚C, TISTR 405, TISTR 548 and TISTR 550 were found to grow well, but only TISTR 405 was found to produce ethanol to an extent similar to that at 30˚C, and the growth and ethanol productivity at 39˚C were better than those of ZM4 at 30˚C, suggesting that TISTR 405 is suitable for ethanol fermentation at high temperatures. Analysis of genes directly related to ethanol formation or degradation, *adhA*, *adhB* and *pdc,* encoding alcohol dehydrogenase (Adh) A, AdhB and pyruvate decarboxylase, respectively, revealed that these genes were highly conserved in both strains. Comparison of their gene expression and activity of the products in both TISTR 405 and ZM4 at different temperatures or growth phases indicated that there was not a great difference at the transcriptional level, but the total activity of AdhA and AdhB in TISTR 405 was higher than that in ZM4. Both strains showed a significant increase in AdhB activity in the stationary phase.