論文の要旨
（Thesis Summary）

氏名　羅　宮臨風
（Name）Luo Gonglinfeng

論文題目(Thesis Title)
Basic and applied research on psychrophile-based simple biocatalysts for production of valuable chemicals
（低温菌シンプル酵素触媒を用いた有用化学品生産に関する基礎及び応用研究）

Whole-cell biocatalyst has broad applications in chemical and pharmaceutical industry. It is easier to be prepared than purified enzymes in a large scale. It also allows expensive cofactors to be regenerated through muti-step reactions, therefore decreases production costs significantly. However, there are two major problems which are crucial for development and application of whole-cell biocatalyst. First, the cell membrane and cell wall can behave as a barrier for substrate influx, thus enzymes in cells cannot be fully utilized, certain conversion processes can be completely disabled. Second, although whole-cell biocatalyst has advantage in the regeneration of expensive cofactors like $NAD(P)^+$ and NAD(P)H by multi-step reactions, these cofactors are also involved in a variety of other biochemical reactions, which means some undesirable side reactions may have negative effects to cofactor regeneration as well as the yield of target products.

Psychrophile-based simple biocatalyst is developed to solve these two major problems. psychrophiles are able to live and replicate in extreme cold, including temperatures below the freezing point of water. In psychrophile, the host metabolic enzymes could be inactivated at temperatures which have less effects on mesophile-origined heterologous enzymes, therefore undesirable side reactions caused by host enzymes can be eliminated. In contrast, the cell membrane of psychrophile is easy to be permeabilized through heat treatment, this will allow substrate to enter into the cells more efficient thus accelerating the conversion rate.

The current research involves two parts, the first part (chapter 2) is aiming to acquire an overall understanding about the organic acid related enzyme thermostability of a psychrophilic bacterium *Shewanella livingstonensis* Ac10, which is a promising host microorganism for Psychrophile-based simple biocatalyst. The results indicated that most of them had a reasonable limited thermostability and were inactivated at 50 °C, but a malic enzyme (SL-ME) was found with unexpected thermostability. SL-ME was then purified and characterized. SL-ME showed no activity loss under 60 °C treatment, similar to its mesophilic counterpart from *Escherichia coli* (MaeB). Consistently, SL-ME and MaeB irreversibly denatured at 71.9 °C and 64.5 °C, respectively. Therefore, SL-ME keeps robust catalytic activity over a wide temperature range.

The second part (chapter 3) described an attmpt to solve the permeability issue of whole-cell biocatalyst by heat treatment. A psychrophile-based simple biocatalyst was constructed to convert citric acid to itaconic acid, which is an unsaturated dicarboxylic acid with broad industrial applications. Two enzymes crucial for itaconic acid production from citric acid, aconitase B from *E. coli* and CAD from *Aspergillus. terreus*, were expressed in *S. livingstonensis* Ac10 cells which were then heated at a moderate temperature to increase permeability. The efficiency of the biocatalyst was increased by heat permeabilization at 45 °C for 15 min, and itaconic acid productivity of the cells after heat treatment (1.41 g/L/h) was increased around 6-fold in comparison with those without heat treatment (0.22 g/L/h). 67.3% of the productivity remained when the cells were recycled for 5 times (10 hours for each reaction). Therefore, the potential of this heat-permeabilized psychrophile host to increase the productivity of whole-cell biocatalyst was proved; however, further research failed to uncover the mechanism behind the heat permeabilization, as well as to obtain high final titer of itaconic acid through enzyme engineering.

In summary, both basic and applied researches are involved in current thesis. The results of investigation on key enzymes involved in organic acid metabolism support the basic concept of Psychrophile based simple biocatalyst, e.g., introducing heterologous enzymes from mesophiles into psychrophiles, and then inactivate homologous enzymes in hosts to eliminate side reactions thus improve the yield of target products. Potential of *S. livingstonensis* Ac10 as a host for Psychrophile based simple biocatalyst was also proved. The author is expecting to acquire a better understanding for the mechanism to increase the cell membrane permeability in order to further develop Psychrophile based simple biocatalyst and apply it to chemical industry.