Studies on novel high-temperature fermentation for L-lactic acid production

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https://hdl.handle.net/2324/1544030

出版情報：九州大学, 2015, 博士（農学）, 課程博士
バージョン：

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論文題目　：Studies on novel high-temperature fermentation for L-lactic acid production
（L-乳酸生産のための新規な高温発酵に関する研究）

区　　分　：

論 文 内 容 の 要 旨

The sustainable production of fuels, chemicals and polymers from the biomass resources within integrated biorefinery is very important for minimizing the dependency on limited fossil fuels. Waste biomass such as food waste generated from every household is a big burden to our community, especially in their recycle system.　Utilization of available biomass resources via the microbial fermentation process is one solution for developing environmental-friendly and sustainable society.　In this study, a noble mixed culture system (MCS) and its microorganisms in high temperature fermentative production of L-lactic acid (LA), a well-known material for the synthesis of fine biodegradable bioplastics, was investigated.

In chapter II, new LA fermentation of food waste with MCS, named as 'meta-fermentation' was investigated. When temperature was set as a primary controlling factor, high L-LA yield with 100% optical purity was achieved at temperatures 50–55ºC. The microbial community structure at 50–55ºC, analyzed by denaturing gradient gel electrophoresis, showed the existence of six major bacteria (*Bacillus coagulans*, *B. smithii*, *B. humi*, *B. thermoamylovorans B. thermocloaceae* and *Corynebacterium sphenisci*) in the MCS. In chapter III, to further control and understand such MCS, a systematic isolation method of all the major bacteria involved in efficient production of L-LA was designed and named as systematic feedback isolation technique. This includes feedback of literature informations and high throughput colony screening by direct colony MALDI-TOF-MS. As a result, six major targeted bacteria and other several unexpectedly isolates were successfully obtained. In chapter IV, identification of the unexpectedly isolated thermotolerant bacterial strain MO-04 was performed with comparing to the closest bacterial species *Bacillus thermolactis* R-6488^T. Other than their DNA-DNA relatedness (44.5%), the strain MO-04 showed many distinguishable characteristics from strain R-6488^T, particularly in sugar assimilation, growth pH and growth temperature. Based on its morphological feature, novel species *Bacillus kokeshiiformis* MO-04^T was proposed. In chapter V, characterization of one of the targeted major bacteria in MCS, isolate MC-07 (99.2% 16S rRNA gene similarity with *B. thermoamylovorans* LMG18084^{T}) was carried out. The isolate MC-07 showed abilities to make a clear halo zone in starch containing media, and to produce optically pure L-LA directly from starch. In particular, isolate MC-07 successfully produced L-LA with 100% optically purity and yield of 0.977 g/g at 50°C under pH-swing control at 7.0 in a simple mineral salt medium without external enzymatic liquefaction of starch.

In conclusion, a new concept, meta-fermentation was proposed for efficient utilization of food waste by mixed culture seed. To elucidate this kind of MCS, whole systematic isolation technique was developed. During the studies on MCS, a novel thermotolerant species *Bacillus kokeshiiformis* MO-04^{T} was identified and proposed, and characterized a thermotolerant bacterial species directly fermenting starch without enzymatic liquefaction. These new findings would be helpful for sustainable utilization of waste biomasses to value added fine chemicals.