博士（工学）　宋　　育阳

学位論文題名

Development of Coryne Factory for the Production of Lactate-based Polyesters

（乳酸ベースポリマー生産のためのコリネ菌微生物工場の開発）

学位論文内容の要旨

Biopolymers such as poly(lactic acid) (PLA) can be used as plastics replacing the need for petrochemical-based plastics which cause the global warming and petroleum depletion problems. Thus, the aim of this study was to produce useful lactate (LA)-based polyesters in termed "Coryne Factory". The platform organism *Corynebacterium glutamicum* has advantages over *Escherichia coli* which is a typical model system for PLA production in that *C. glutamicum* does not contain the harmful substrates, (i.e., endotoxin) and it has a high cell density during fermentation. These benefits make *C. glutamicum* an attractive candidate as a host for the productions of biopolymers used in food-grade and biomedical applications. PLA is representative biocompatible and biodegradable plastics in various applications. Recently, a whole-cell biosynthesis system for LA-based polyester production has been constructed using engineered *E. coli*. The discovery of LA-polymerizing enzyme (LPE) was a key to develop the first microbial system.

In this context, general introduction including the research purpose is described in Chapter 1.

In Chapter 2, based on this prototype system, the *C. glutamicum* was engineered as a host strain for LA-based polyester production. The metabolic pathways were designed in *C. glutamicum* to generate monomer substrates, lactyl-CoA (LA-CoA), and 3-hydroxybutyryl-CoA (3HB-CoA), for the copolymerization catalyzed by the LPE. A key factor in the construction of the metabolic pathway to produce LA-based polyesters in *C. glutamicum* was the stereochemistry of LA, because it has been demonstrated that LPE has strict stereospecificity toward D-LA-CoA. Therefore, *C. glutamicum* was remodeled to be a D-LA overproducer by introduction of D-lactate dehydrogenase (D-LDH). The polymer synthesized in the engineered strain was determined by GC/MS and NMR analyses which showed that the strain produced P(LA-*co*-3HB) with the LA fraction of 99.3 mol%. The result had a striking contrast to the result obtained by the *E. coli* system, which produced the copolymer with up to 47 mol% LA under the similar conditions. This research indicated that the engineered *C. glutamicum* serves as a useful platform to produce PLA-like polyester. Compared to the current chemical process for PLA production using harmful heavy metal catalyst, this complete one-step bioprocess is preferable to produce LA-based polyester for food-contacting and medical uses.

In Chapter 3, because the LA-based polymer was successfully produced by recombinant *C. glutamicum*, I aimed at using this endotoxin-free platform to produce various kinds of LA-based copolymers in the "Coryne Factory". For this purpose, precursor strategy was investigated. The addition of the related precursors such as propionate and 2-hydroxybutyrate in the medium successfully achieved the production of copolymers containing 3-hydroxyvalerate and 2-hydroxybutyrate respectively.

In Chapter 4, I next addressed to utilize less expensive feedstock to decrease the production cost. To meet this goal, the α-amylase cell-surface displaying system was introduced into the "Coryne Factory" that achieved single step polymer production from starch. The remodeled recombinant strain exhibited a significant α-amylase activity (1.4 U/mL) and the utilization of starch, consequently produced 6.3 wt% polyhydroxybutyrate. Notably, this polymer content was higher than that obtained using glucose (4.9 wt%). This result demonstrated that the introduction of α-amylase cell-surface display system into the "Coryne Factory" achieved single step polymer production from starch.

Chapter 5 describes the summary of all studies. In short, for the first time, I succeeded in establishing the "Coryne Factory" that can produce LA-based polyesters. This new endotoxin-free platform should be suitable for wider range of applications, especially food and medical related uses. The precursor strategy indicated the possibility for LA-based copolymer production. This beneficial system will facilitate further next-generation research studies such as those on the biosynthesis of LA-based copolymers with desirable properties and the application of other sugars that give pyruvate as a metabolized product. Thus this process could be more cost effective by using low grade, low cost sugars as a carbon source.

学位論文審査の要旨

主 査	教 授	大 利	徹
副 査	教 授	高 木	睦
副 査	教 授	及 川	英 秋
副 査	教 授	坂 口	和 靖
副 査	准教授	田 島	健 次
副 査	教 授	田 口	精 一

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In this context, general introduction including the research purpose is described in Chapter 1.

In Chapter 2, based on this prototype system, Ph.D candidate describes the engineering of C. glutamicum as a host strain for lactate-based polyester production. The candiadte designed metabolic pathways in C. glutamicum to generate monomer substrates, lactyl-CoA (LA-CoA), and 3-hydroxybutyryl-CoA (3HB-CoA), for the copolymerization catalyzed by the LPE. A key factor in the construction of the metabolic pathway to produce LA-based polyesters in C. glutamicum was the stereochemistry of LA, because it has been demonstrated that LPE has strict stereospecificity toward D-LA-CoA. Therefore, C. glutamicum was remodeled to be a D-LA overproducer by introduction of D-lactate dehydrogenase (D-LDH). The polymer synthesized in the engineered strain was determined by GC/MS and NMR analyses which showed that the strain produced P(LA-co-3HB) with the LA fraction of 99.3 mol%. The result had a striking contrast to the result obtained by the E. coli system, which produced the copolymer with up to 47 mol% LA under the similar conditions. This research indicated that the engineered C. glutamicum serves as a useful platform to produce PLA-like polyester. Compared to the current chemical process for PLA production using harmful heavy metal catalyst, this complete one-step bioprocess is preferable to produce LA-based polyester for food-contacting and medical uses.

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Based on the review and interview of the doctor thesis, this study can be judged to be very significant and valuable from the viewpoints of academic research and industrial potential. Ph.D degree of Engineering should be awarded to the candidate.