論文の要旨
（Thesis Summary）

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論文題目(Thesis Title)
Development of psychrophile-based simple biocatalysts for simultaneous biosynthesis of 1,3-propanediol and 3-hydroxypropionic acid
(低温菌シンプル酵素触媒による 1,3-プロパンジオールと 3-ヒドロキシプロピオン酸の共生産)

1,3-Propanediol (1,3-PDO) and 3-hydroxypropionic acid (3-HP) are three-carbon organic molecule and have a vast range of applications in the cosmetics, food, pharmaceutical, textile industries. Approximately 90% of these compounds are used to synthesize the new polyester polytrimethylene terephthalate (PTT). *Klebsiella pneumoniae* is a well-known bacterium that can convert glycerol to both compounds. The production pathway in *K. pneumoniae* consists of two reductive routes: first, upon activation with vitamin B_{12}, glycerol dehydratase catalyzes a dehydration reaction, converting glycerol to 3-hydroxypropionaldehyde (3-HPA). 3-HPA is then used as a substrate from which 1,3-PDO and 3-HP are produced. 1,3-PDO dehydrogenase requires NADH to reduce 3-HPA to 1,3-PDO. The reduction NAD^+ to NADH is coupled with an oxidation reaction by aldehyde dehydrogenase. This study aims to evaluate the abilities of the Psychrophile-based Simple bioCatalyst (PSCat) reaction system to co-biosynthesize 3-HP and 1,3-PDO. To achieve that goal, first, the PSCat was developed to increase the biosynthesis potential of this method. Then, applying the developed PSCat to produce both chemicals simultaneously.

Development a new method of psychrophile-based simple biocatalyst

The production of 1,3-PDO was considered to develop the PSCat. In the conventional PSCat, one gene cassette with two or more gene was prepared. In a new method, each gene cassette has only one gene. The two enzymes (glycerol dehydratase (DhaB) and 1,3-PDO dehydrogenase (DhaT)) involved in the metabolic pathway were expressed together and individually in the two psychrophilic host bacterial strains. The intracellular metabolic flux was inactivated using heat treatment at 45°C for 15 min. The enzymatic activity and production of 1,3-PDO were examined at 37°C.

The enzymatic activity for DhaT was measured for both individual and group expression of this enzyme. The recombinant *Shewanella livingstonensis* Ac10 with *dhaT* (Ac10-T) and *Shewanella frigidimarina* DSM 12253 with *dhaT* (Sf-T) strains expressed only DhaT enzyme. The DhaT activity in AC10-T and Sf-T were 172.7±35.2 and 121.6±13.8 μmol/min/mg protein at 37°C, respectively. The activity of DhaT was decreased when DhaB and DhaT expressed together in one host.

After individual gene expression (25.0 mM), 1,3-PDO productivity of the cells increased by approximately 2.5 times at 37°C, in comparison to that when genes were expressed together (10.2 mM). The best combination of 1,3-PDO production at 37°C was Ac10-B and Sf-T, with a 2:1 ratio. This combination was selected to examine the cofactor regeneration system for the new strategy PSCat approach. Productivity was boosted (31.1 mM) when the cofactor regeneration system was included in the biocatalyst. Hence, both the ability of individual gene expression and the cofactor regeneration system were verified in the new strategy PSCat approach.

Simultaneous production of 1,3-PDO and 3-HP

The genes derived from *K. pneumoniae*, encoding DhaB, DhaT, and PuuC were introduced into *S. livingstonensis* Ac10 and *S. frigidimarina* DSM 12253. These genes were expressed individually as a new strategy of PSCat in psychrophile host cells to examine the PSCat method on 3-HP and 1,3-PDO production. The enzymatic activity, production of each chemical individual, and together were examined at 40°C.

The DhaT activity was measured at 40°C temperature and the activity decrease with increasing temperature. The DhaT activity in the recombinant Ac10-T and Sf-T strains was 158.2±38.9 and 104.5±14.2 μmol/min/mg protein at 40 °C, respectively.

Despite the slight decrease in DhaT activity, the rise in temperature did not significantly affect 1.3-PDO

production. The 1,3-PDO productivity after individual gene expression at 40°C was around 25.8 mM. The combination of Ac10-B and Sf-T, with a 2:1 ratio of DhaB:DhaT, was the best combination at 40°C.

The DhaB and aldehyde dehydrogenase (PuuC), involved in the metabolic pathway of 3-HP production, were expressed individually in the psychrophilic host bacterium. The psychrophilic host enzymes were inactivated using heat treatment at 45°C for 15 min. The PuuC activity was 46.1±8.5 and 39.4±6.2 μmol/min/mg protein at 40°C in the recombinant *S. livingstonensis* Ac10 with *puuC* (Ac10-U) and *S. frigidimarina* DSM 12253 with *puuC* (Sf-U) strains, respectively. The productivity of 3-HP was 60.6 mM after individual gene expression in the psychrophilic host bacterium. The combination of Ac10-B and Sf-U, with a 2:1 ratio of DhaB:PuuC, was the best combination to produce 3-HP.

Three enzymes (DhaB, DhaT, and PuuC) were expressed individually in both *S. livingstonensis* Ac10 and *S. frigidimarina* DSM 12253 as host bacteria. The same condition (45°C for 15 min) was used to inactivate the psychrophilic hosts' intracellular metabolic flux. The ratio of 4:2:1 was chosen for Ac10-B:Sf-U:Sf-T, respectively. We also added 30 mM glycerol as a substrate and adjusted the vitamin B_{12} concentration to the new volume. After one hour, for the control group, we added only more cell suspensions. After 60 min and mixing, 3-HP and 1,3-PDO production reached approximately 30 mM and 16 mM in both control and reaction tubes, respectively. Production increased for both compounds, reaching 48.3 mM for 3-HP and 24.3 mM for 1,3-PDO in the reaction tube to which we added more substrates and adjusted vitamin B_{12} concentration after 120 min. Simultaneously, no increase was observed in the control group. Following this, the production level stabilized.