論文の内容の要旨

応用生命工学専攻

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論文題目 **Studies on the carbon and energy metabolism of the thermophilic hydrogen-oxidizing bacterium *Hydrogenophilus thermoluteolus* TH-1**
(高温性水素細菌*Hydrogenophilus thermoluteolus* TH-1の炭素並びにエネルギー代謝に関する研究)

Chapter 1. *Hydrogenophilus thermoluteolus* TH-1 a facultatively chemolithoautotrophic, hydrogen-oxidizing bacterium

The facultative chemolithoautotroph has an ability to grow autotrophically by using the inorganic carbon and inorganic electron donor. Moreover, this kind of microorganism can grow heterotrophically when organic carbon is available or it can grow mixotrophically when inorganic carbon, inorganic electron donor and the organic carbon source are available at the same time.

This chapter describes the ability of strain TH-1 to grow autotrophically, heterotrophically and mixotrophically with different kinds of substrate. The specific growth rate was recorded in detail.

Specific enzymatic activities for autotrophic growth, hydrogenase and ribulose-1,5-biphosphate carboxylase/oxygenase, were measured. The utilization of each substrate was discussed in detail.

Chapter 2.　The operation of glyoxylate cycle in acetate/butyrate metabolism

Glyoxylate cycle is an important pathway for acetate fixation or it can play an important role as an anaplerotic pathway to replenish immediate substrates for TCA cycle. Pyruvate: ferredoxin oxidoreductase (POR) also functions as an acetate accumulation pathway by fixing CO_2 with actetate to generate pyruvate. The interesting thing was that genes involved in two acetate accumulation pathway (glyoxylate cycle and pyruvate: ferredoxin oxidoreductase) were found in the genome of strain TH-1. Malate synthase activity was high in cell-free extract when strain TH-1 utilized acetate or butyrate for heterotrophic growth. This result implied that in strain TH-1 glyoxylate cycle was operative in acetate or butyrate metabolism.

This chapter describes the function of glyoxylate in acetate or butyrate metabolism. The enzymatic activities of glyoxylate cycle were measured for isocitrate lyase and malate synthase. In addition, some enzymes belonging to TCA cycle and gluconeogenesis were also measured. The functions of POR and glyoxylate cycle were analyzed and discussed.

Chapter 3. Toxicity caused by photorespiration and the detoxification of glycolate by function of glyoxylate cycle under mixotrophic growth on acetate/butyrate of *Hydrogenophilus thermoluteolus* TH-1

Calvin-Benson-Bassham cycle (CBB cycle) is an important pathway for carbon fixation in the biosphere. CBB cycle is popularly distributed in plants and many autotrophic microorganisms. In bacteria domain, totally six carbon fixation pathways were reported to date: CBB cycle, Acetyl CoA pathway, reductive TCA cycle, 3-hydroxypropionate bicycle, 3-hydroxypropionate/4-hydroxybutyrate cycle, 2-hydroxycarboxylate/4-hydroxybutyrate cycle. CBB cycle is the most popular among autotroph.

The main enzyme of CBB cycle and also the most abundant protein on the Earth is ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO). This enzyme catalyses the first step of carbon fixation that fixes CO_2 to ribulose-1,5-biphostphate when CO_2 is a substrate. However, RubisCO can also incorporate O_2 to ribulose-1,5-biphosphate by oxygenation activity via a process so-called photorespiration. Photorespiration is a waste process that decreases carbon fixation efficiency to approximately 25% by producing 2-phosphoglycolate. Furthermore, 2-phosphoglycolate was reported as an inhibitor of carbon fixation process and other by-products such as ammonia and peroxide were found to be toxic to the cells.

This chapter describes the operation of CBB cycle under autotrophic or mixotrophic conditions with different carbon sources. The toxification caused by photorespiration was investigated and the detoxification system was discussed.

Chapter 4. Transcriptome and metabolome analyses of central carbon metabolism under autotrophic, heterotrophic, and mixotrophic conditions in *Hydrogenophilus thermoluteolus* TH-1

Nowadays, microarray is a new and good molecular method with highly potential application in many fields such as academia, medical science, pharmaceutical, biotechnological, agrochemical, and food industries. Microarray is a powerful tool to research about the expression of genes because with one single experiment, we can get over 10,000 of blotting data, which makes monitoring of the genome activity possible.

This chapter describes the expression of whole genes of TH-1 under autotrophic condition, heterotrophic or mixotrophic conditions with butyrate as substrate. The expression profiles of all central carbon metabolic pathways were evaluated and discussed.

Chapter 5. The production of poly-3-hydroxybutyrate in *Hydrogenophilus thermoluteolus* TH-1 under autotrophic and heterotrophic conditions.

This chapter describes the ability of strain TH-1 to accumulate PHB under autotrophic or heterotrophic induction condition. The roles of three PHA synthase genes were analyzed; phylogenetic tree was constructed and discussed. After induction by nitrogen starvation, PHB rapidly accumulated under autotrophic and heterotrophic conditions. PHB granules accumulated in globular form with a diameter of 0.2-0.5 μm. They located close to or in contact with the membrane under autotrophic condition, but were randomly distributed in cytosol under heterotrophic condition. The highest percentages of PHB

accumulation under autotrophic and heterotrophic conditions were 38.6% after 360 minutes and 53.8% after 180 minutes, respectively.