CO_2-based biopolymer synthesis and elucidation of a novel biopolymer degradation pathway

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Research objective

Hydrogen-oxidizing bacteria, which utilize CO_2 and H_2 as their sole carbon and energy sources, respectively, have attracted significant attention in recent years owing to their potential as biosystems for CO_2-based biomanufacturing. Several strains of hydrogen-oxidizing bacteria produce polyhydroxybutyrate (PHB) and other biopolymers. A successful example of practical application of the biopolymer production is exhibited by *Ralstonia eutropha*, a representative hydrogen-oxidizing bacterium. In these bacteria, PHB serves as an energy storage system, and its biosynthesis and degradation switch in response to the nutritional status of the cells. However, the detailed mechanisms underlying this switch remain largely unelucidated, even in *R. eutropha*.

Hydrogenophilus thermoluteolus TH-1 is a hydrogen-oxidizing bacterium that exhibits exceptionally high proliferative ability, with a doubling time of merely one hour under autotrophic conditions. Owing to its remarkable proliferation ability, TH-1 holds considerable promise for applications in CO_2-based biomanufacturing. While this strain accumulates intracellular PHB under certain conditions, it appears to lack the genes responsible for PHB degradation present in other microbes, suggesting the existence of a novel PHB degradation pathway in this strain (Fig. 1). This study was aimed at investigating the mechanisms underlying PHB biosynthesis and biodegradation in *H. thermoluteolus* TH-1.

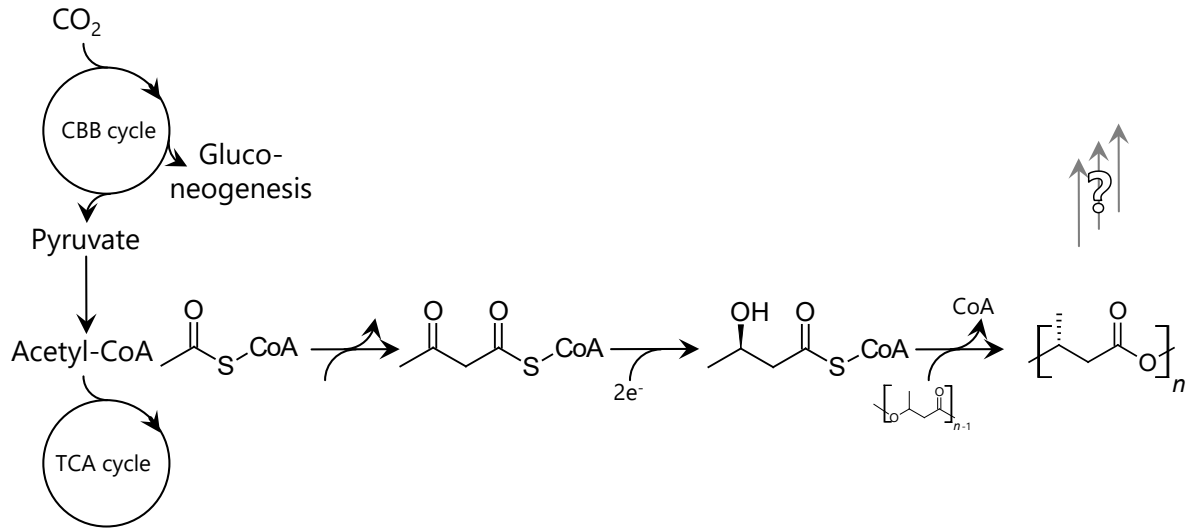

Fig. 1. PHB biosynthesis and biodegradation pathways in *H. thermoluteolus* TH-1.

Methods

Transcriptome analysis

PHB accumulation in TH-1 cells was induced under nitrogen starvation conditions and PHB degradation was induced by transferring the cells to carbon starvation conditions. RNA was extracted from the cells and used for transcriptome analysis.

Enzymatic analysis

The activity of enzymes presumably involved in PHB synthesis and degradation was assayed. Cell-free extracts of TH-1 and purified fractions of the heterologous enzymes were used as enzymatic solutions to assess their activities.

Evaluation of intracellular redox balance

TH-1 cells cultured under various conditions were harvested and used to prepare cell-free extracts. Intracellular NADP (H) and NAD (H) levels were measured using commercially available assay kits.

Acquisition of mutants

A random mutagenesis library of TH-1 cells was constructed by treatment with the mutagenic compound 1-methyl-3-nitro-1-nitrosoguanidine. The mutants that produced no PHB and those that accumulated but did not degrade PHB were screened using Nile red staining and cell sorting.

Results

Previous studies have reported that *R. eutropha* represses genes encoding enzymes in the TCA cycle during PHB accumulation, thus directing carbon flux toward PHB synthesis. Contrary to these findings, transcriptome analysis of TH-1 cells suggests that such repression does not occur in TH-1 cells, suggesting an alternate mechanism for PHB accumulation. Induction of one of the *phaB* homologs was observed, suggesting that this enzyme contributes to PHB accumulation. Under PHB-degrading conditions, the induction of several unexpected genes was observed, and the relationship between these genes and PHB degradation was verified.

Unlike most previously reported PhaB homologs, the PhaB homolog in TH-1 cells exhibited NADH-dependent activity. Moreover, our measurements indicate that the intracellular NADH levels increased during PHB accumulation. These results suggest the existence of a novel, unidentified mechanism that results in PHB accumulation in TH-1 cells, as illustrated in Fig. 2.

Screening for mutants that did not produce PHB and those that accumulated but did not degrade PHB was performed after random mutagenesis. The obtained mutants were examined for phenotypic analysis and identification of mutations that are required for PHB synthesis and degradation.

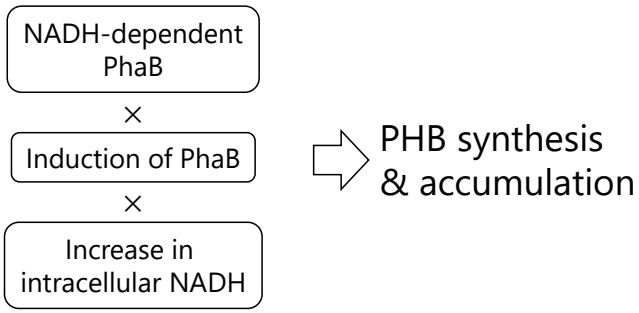

Fig. 2. Presumed mechanism of PHB accumulation in *H. thermoluteolus* TH-1.

Conclusion

The present study proposes a novel mechanism to induce PHB accumulation, identifies candidate genes essential for PHB production, and provides mutants useful for elucidating the PHB degradation pathway. Future studies based on these outcomes will reveal the detailed mechanisms of PHB synthesis and degradation in TH-1 cells, leading to useful insights and technological advancements that facilitate CO_2-based manufacturing and efficient degradation of biopolymers.

References