

# The Pathway Analysis of *Tepidimonas taiwanensis* LMG 22826<sup>T</sup>, Polyhydroxyalkanoate and Alkaline Protease Producer

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**Abstract**—The complete genome sequence of thermophilic type strain *Tepidimonas taiwanensis* LMG 22826<sup>T</sup> confirmed the earlier statement of its high biotechnological potential. Thanks to its reported ability to produce alkaline proteases and to synthesize polyhydroxyalkanoates (PHA), *T. taiwanensis* is worth our attention. Here, we present further descriptive analysis based on pathways detection which provides us better understanding of the core of such significant properties. This information can be then used in *in silico* modeling approaches. In total, 108 biological pathways were detected, *phaCAB* operon and granule-associated proteins - phasins were found and butanoate pathway comparison with other PHA producing bacteria was provided. Finally, simplified PHA synthesis pathway was introduced.

**Keywords**—butanoate, metabolism, bioplastics, biotechnology

## 1. INTRODUCTION

In recent years, the aim is to find ‘eco-friendly’ alternatives to plastics that will not be derived from petrochemicals as they are now. Promising candidates for such purposes are biodegradable, biocompatible, and renewable polymers - polyhydroxyalkanoates (PHA) that have become popular and widely studied substances. PHA are produced by a wide range of bacteria, archaea, and even plants. Most interest is dedicated to synthesis of PHA by bacteria where the production relates to a response to a limited number of essential substrates. PHA then serve as the source of energy and help bacteria to survive in less prosperous conditions [1].

To decrease the costs of PHA production, one of the approaches is a synthesis by thermophilic bacteria. These bacteria are capable of living in higher temperatures and can produce PHA in these contamination free conditions [2]. The process can be then less cost demanding.

In this paper, we provide insight into such process in the thermophilic bacterium *Tepidimonas taiwanensis* LMG 22826<sup>T</sup>.

## 2. MATERIALS AND METHODS

The genome of type strain *T. taiwanensis* can be obtained from the GenBank under the accession number CP083911.1. Additional two bacteria, *Shlegelella Thermodepolymerans* DSM 15344 and *Aneurinibacillus Thermoaerophilus* CCM 8960, with accession numbers CP064338.1 and CP080764.1, respectively, were used for comparison. KEGG-related tools [3] were used for the analysis such as KEGG PATHWAY for identification of pathways by searching for homologous genes. Phasin proteins were found using KEGG GENES, the comparison of bacteria pathways was formed with Reconstruct tool in KEGG Mapper, and the final pathway of PHA synthesis was created in PathVisio software [4].

## 3. RESULTS AND DISCUSSION

Overall, 108 pathways were labeled as present in *T. taiwanensis*. As it was expected, ‘Metabolism’ derived pathways make approximately 80% of detected pathways. Other pathways were assigned to ‘Environmental Information Processing’, ‘Cellular Processes’ or ‘Genetic Information Processing’.

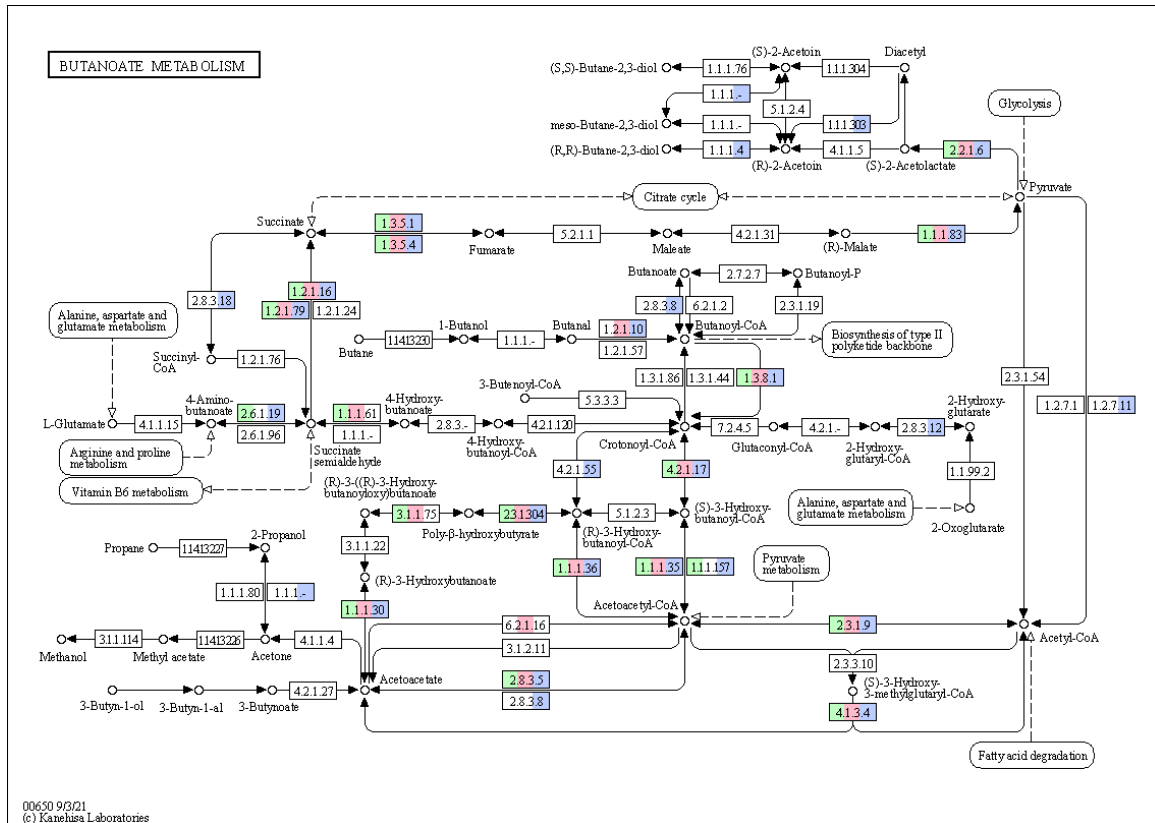
group’.

### 3.1. PHA PRODUCTION

The ability to produce PHA by *T. taiwanensis* LMG 22826<sup>T</sup> was already experimentally proved with the cultivation of bacterium on grape pomace extract with the highest use of glucose and fructose as sources of carbon [5]. A member of the PHA family, polyhydroxybutyrate (PHB) was found to be synthesized, precisely 3-hydroxybutyrate (3HB) [5] which has potential to be used in a wide range of applications from plastics to the medical industry.

### 3.2. BUTANOATE METABOLISM PATHWAY

Main steps of the PHA production are included in a butanoate metabolism pathway. To compare the PHA synthesis apparatus on genome level, butanoate metabolism pathways of three PHA producing bacteria were merged into a single pathway, see Figure 1. The pathway consists of rectangles being enzymes, arrows expressing metabolic reactions, and dots standing for other molecules. White rectangles are gene products that weren’t found in bacterial genomes and the colored ones were present. Green, pink, and blue rectangles define enzymes found in *T. taiwanensis* LMG 22862<sup>T</sup>, *S. thermodepolymerans* DSM 15344, and *A. thermoaerophilus* CCM 8960, respectively.



**Figure 1:** Butanoate metabolism pathway comparison of three PHA producing bacteria. Green, pink, and blue color define enzymes found in *T. taiwanensis* LMG 22862<sup>T</sup>, *S. thermodepolymerans* DSM 15344, and *A. thermoaerophilus* CCM 8960, respectively.

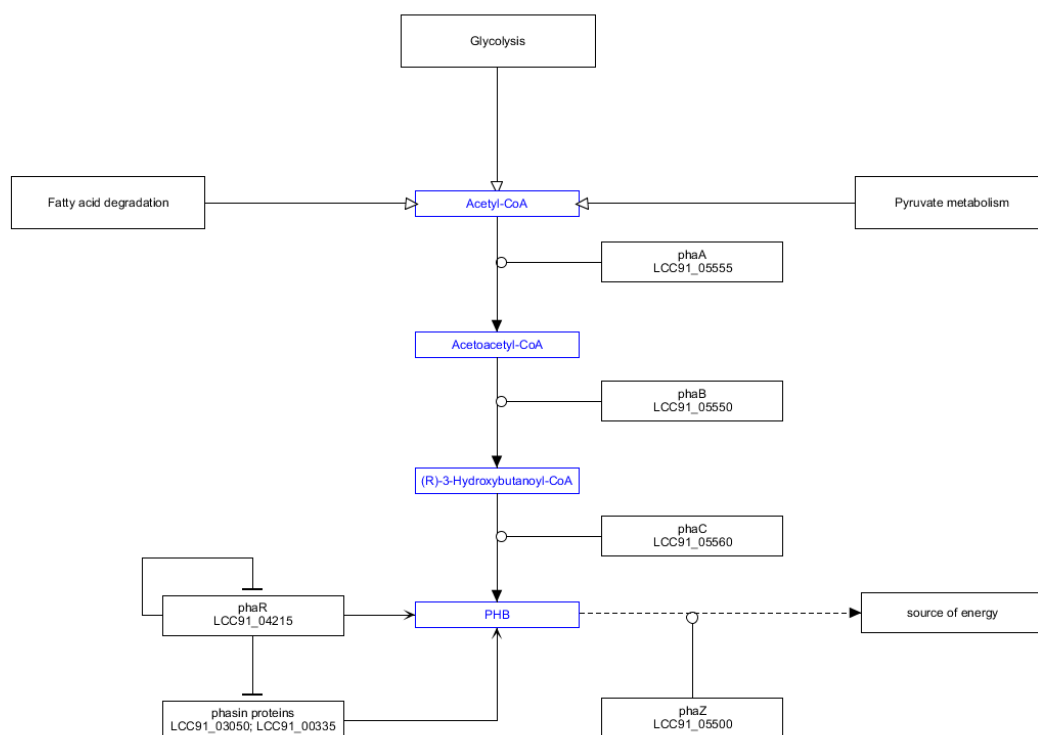
At first sight, the difference between the third mentioned bacterium is significant in comparison to the difference between the first two bacteria. As the main variation can be considered the lack of *phaZ* (Enzyme Commission number - EC:3.1.1.75) gene encoding PHA depolymerase enzyme. This enzyme has been reported to have a significant impact on PHA maintenance in a cell [6], because it generally serves as a decomposing machinery of PHA granules in case of a starving bacterium. In fact, *A. thermoaerophilus* CCM 8960 owns gene coding PHA depolymerase as was detected recently [unpublished results]. Similarly, for the first two bacteria, it seems that the only source of *acetyl-CoA* and/or *acetoacetyl-CoA*, the main chemical compounds in PHA synthesis process, is pyruvate metabolism and fatty acid degradation, respectively. However, deeper insight revealed that there is an

alternative pathway in the glycolysis which can convert *pyruvate* to *acetyl-CoA*, but it isn't included in this pathway. This fact can be somehow limiting, and we should be still skeptical when we are using such predictive tools.

Further, when we looked for the class of PHA synthases of these bacteria, *T. taiwanensis* and *S. thermodepolymerans* possess a gene that encodes the PHA synthase class I. On the other hand, *A. thermoaerophilus* has PHA synthase class III. Both classes should be producing scl-PHA (short-chain-length) [1] but as recent research showed, *A. thermoaerophilus* can synthesized mcl-PHA (medium-chain-length) [unpublished results]. This can be also the reason the butanoate pathway of this bacterium is different in comparison with other two bacteria.

Besides already reported *phaC* gene encoding PHA synthase, *acetyl-CoA acetyltransferase* (*phaA* gene – LCC91\_05555) and *acetoacetyl-CoA reductase* (*phaB* gene – LCC91\_05550) was found in *T. taiwanensis* genome. These genes together form an operon marked as *phaCAB* which is responsible for the chain of reactions that leads to PHA production. Further, *T. taiwanensis* genome possesses depolymerase encoded in *phaZ* gene and *phaR* gene known as transcriptional repressor. The *phaR* gene can be also found as a subunit of PHA synthase class IV where serves as an essential part of synthesis machinery [2]. The role of *phaR* gene seems to be also important for the PHA synthesis of other classes because the lack of this gene was shown to lead to increased or even stopped production of these granules [1]. Basically, *phaR* gene autoregulates itself in the mean of repressor and it also represses the production of granule-associated proteins known as phasins [1]. With this knowledge, the genome was additionally searched for phasins. Two proteins were successfully detected as phasin family proteins (LCC91\_00335, LCC91\_03050).

To simplify the pathway that provides the production of PHA granules and to also include the present *phaR* gene and found phasins, the pathway for the PHA synthesis in *T. taiwanensis* was created and is shown in Figure 2. Blue rectangles are products whose conversion is catalyzed with enzymes with rounded arrows, two arrows that lead from *phaR* and phasins standing for binding and blue PHB rectangle symbolizes PHB granules.



**Figure 2:** Simplified pathway of PHA synthesis in type strain *Tepidimonas tawanensis* LMG 22826<sup>T</sup>.

### 3.3. ALKALINE PROTEASES

Another industrially utilizable property of this bacterium is alkaline protease production. The ability of alkaline proteases production by *T. tawanensis* LMG 22826<sup>T</sup> was first found experimentally [7], and later was confirmed based on a prediction of molecular weights from protein sequences derived from

genome assembly [8]. The genome sequence revealed the presence of genes coding many alkaline proteases but only three of them encoded for extracellular proteases that are used in many industries, such as detergent or medical industry [9].

Only one of these three alkaline proteases was detected in a pathway. Serine protease (EC 3.4.20.107), as a product of *degP/htrA* gene (LCC91\_09805), manages proteolysis of misfolded proteins in periplasm. This gene is a part of Two-component system pathway which provides a connection of an outer environment with an intracellular one. Unfortunately, connection of serine protease with the outer environment is missing. In that case, further understanding of the process responsible for producing the serine protease by *T. taiwanensis* remains hidden and additional experiments will be needed.

Apart from serine protease, the genome of *T. taiwanensis* also has the gene encoding lysyl endopeptidase (LCC91\_10460, EC:3.4.21.50) and protease IV (LCC91\_01535, EC:3.4.21.-). The first mentioned is produced by many bacteria and has been already selling as a commercial product and protease IV has suitable properties for usage in the detergent industry [8].

#### 4. CONCLUSIONS

This paper aimed to provide deeper insight into mechanisms that induce such biotechnological potential of *Tepidimonas taiwanensis* LMG 22862<sup>T</sup>. The pathway detection revealed 108 pathways, the most of them belonging to 'Metabolism' pathway group. Further, phasin proteins were found in the genome, as well as genes that completed the *phaCAB* operon and corresponding locus tags were reported. Finally, the simplified pathway of PHA synthesis by this bacterium was introduced and can also serve as the knowledge base for possible modeling purposes.

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