# Identification of metabolic bottlenecks of microorganisms during cultivation at under high temperatures


## Yota TSUGE

### Institute for Frontier Science Initiative, Kanazawa University


**Research objective**

Each microorganism has its own temperature range for growth and is classified according to this range. However, factors that determine the upper limit of growth temperature in each classification have not yet been elucidated. At high temperatures, reactive oxygen species (ROS) are generated in cells, causing nucleic acid damage, lipid peroxidation, and protein inactivation. Although these are thought to be one of the causes of growth arrest at high temperatures, the molecules responsible remain unknown.

Many industrially used bacteria are mesophilic, and they can grow from around 25°C to 40°C. For industrial use, strains capable of growing at 40°C or higher are preferred to prevent contamination and reduce cooling costs. Therefore, mutant bacterial strains capable of growing at high temperatures have been obtained through laboratory evolution[1,2]. However, factors that determine the upper limit of growth temperature have not yet been clarified.

We investigated the metabolic state of amino-acid-producing *Corynebacterium glutamicum* at high temperatures. *C. glutamicum* only grows under aerobic conditions and ceases to grow under anaerobic conditions. However, its metabolism is maintained under anaerobic conditions, and it produces lactate and succinate. When the temperature exceeds 40°C, *C. glutamicum* stops growing under aerobic conditions, but we showed that the glucose consumption rate peaked at 43°C under anaerobic conditions and lactate and succinate productivities also increased[3]. These results show that the optimal temperature for glucose metabolism in glycolysis and the reductive branch of the TCA cycle was higher than the upper limit of the growth temperature. Therefore, we considered that one of the reasons why cells cannot proliferate at high temperatures is because certain cellular components cannot be synthesized because of the inhibition of metabolic reactions downstream of glycolysis.

In this study, we investigated metabolic alterations in *C. glutamicum* under aerobic conditions at high temperatures to identify metabolic bottlenecks under high-temperature stress.

**Methods**

*C. glutamicum* wild type (ATCC 13032) was cultured in a synthetic medium with glucose as a sole carbon source in a flask, and cells in the exponential phase were collected.

Then, the cells were transferred to a new synthetic medium with initial $OD_{600}$ of 0.8 and 10 for cultivating at 30 and 43°C, respectively, to start aerobic cultivation in a jar fermenter with pH at 7.0 and aeration rate of 1 vvm. The dissolved oxygen level was maintained above 20% of the saturated oxygen concentration at 30°C. Extracellular metabolites were analyzed using high-performance liquid chromatography, and $CO_2$ concentration was measured using an exhaust gas analyzer.

**Results**

First, to investigate the upper limit of the growth temperature of *C. glutamicum*, the wild-type strain was cultured in a nutrient medium and a synthetic medium in flasks. Growth stopped at 43°C and 40°C in the nutrient and synthetic media, respectively. Therefore, in this study, the non-growth temperature of *C. glutamicum* for investigating the metabolic state at high temperature was set as 43°C. Next, the cells were cultured at 30°C or 43°C using a jar fermenter to investigate the metabolic state. Although no cell growth was observed at 43°C, glucose was consumed during the reaction (Fig. 1). However, unlike under anaerobic conditions, the biomass-specific glucose consumption rate at 43°C was 0.45 g-glucose/g-CDW/h, which was 31% lower than that at 30°C. Next, we investigated the conversion products of the consumed glucose. At 30°C, 47.6% was converted to biomass and 46.4% to $CO_2$, whereas at 43°C, 27.7% was converted to $CO_2$ and several metabolites such as lactate, dihydroxyacetone, glutamate, α-ketoglutarate, and pyruvate (Fig. 2). Lactate production was unexpected because it is produced only

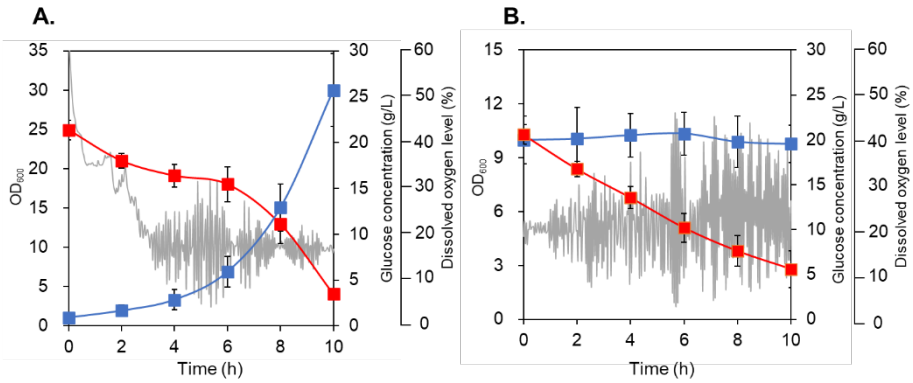


Fig. 1 Cell turbidity (blue), glucose concentration (red), and dissolved oxygen concentration (gray) at 30°C (A) and 43°C (B)

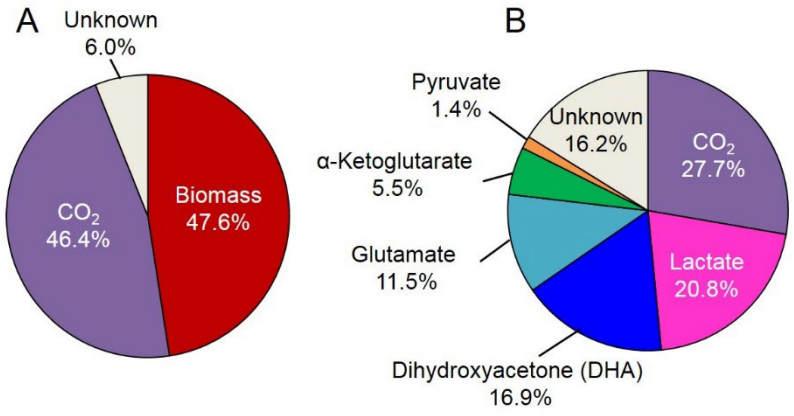


Fig. 2 Conversion products of glucose at 30°C (A) and 43°C (B)

under oxygen-deprived conditions. However, succinate, which is also produced under oxygen-deprived conditions, was not detected, indicating that lactate production was not due to decreased oxygen availability. These results suggest that pyruvate dehydrogenase (PDH) may be inactivated at 43°C. Therefore, we investigated the activities of pyruvate-related enzymes. The specific activity of PDH was not observed at 43°C as expected, whereas the specific activity of lactate dehydrogenase (LDH) at 43°C was comparable with that at 30°C. These results suggest that a cause of metabolic alterations at high temperatures in *C. glutamicum* is the inactivation of PDH.

Metabolic alterations caused by an increase in temperature in *Escherichia coli* BL21(DE3) were also investigated. As observed in *C. glutamicum*, the specific activity of PDH disappeared at above the upper limit of growth temperature (50°C), whereas the specific activity of LDH at 50°C was comparable with that at the growth temperature (37°C). These results suggest that PDH may be an enzyme with a low tolerance to high temperatures, which is common in mesophilic bacteria.

## Conclusion

In this study, we showed that *C. glutamicum* maintained glucose metabolism under aerobic conditions at above the upper limit of the growth temperature by altering its metabolic state through the inactivation of PDH. In the future, we will replace the genes encoding PDH that function even at high temperatures to eliminate metabolic bottlenecks and search for further downstream bottlenecks. Moreover, in this study, 16.2% of glucose was converted to unknown compounds other than metabolites in glycolysis and TCA cycle at 43°C. At high temperatures, the metabolic pathways that do not function at the growth temperature may function. Therefore, we will search for metabolites converted from glucose at 43°C, other than those identified in this study.

## References


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