Production of 1,5-diaminopentane and succinate in a single *Corynebacterium glutamicum* strain via a bidirectional metabolic switch controlled by the oxygen level

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Report

In this study, we successfully constructed a *Corynebacterium glutamicum* strain that can produce 1,5-diaminopentane under aerobic conditions and succinate under anaerobic conditions by metabolic engineering. In this strain, the oxygen level controls a bidirectional metabolic switch, which channels the carbon flow into different metabolic pathways.

Research aims

With the growing concern over global warming, the bio-production of polyamide monomers has attracted increasing attention in recent years. *C. glutamicum*, which is mainly known as an amino acid producer, has been used as a host strain capable of producing the polyamide monomers 1,5-diaminopentane and succinate[1-2]. This study aimed to construct a *C. glutamicum* strain that can produce 1,5-diaminopentane under aerobic conditions, and succinate under anaerobic conditions. In this strain, changes in the oxygen level can trigger a metabolic switch directing the carbon flow between the Embden-Meyerhof pathway (EMP) and the Pentose Phosphate pathways (PPP).

Methods

The *C. glutamicum* ATCC 13032 strain was genetically engineered to construct a strain that produces, as the main product, 1,5-diaminopentane and succinate under aerobic and anaerobic conditions, respectively. In the parental strain used for this study, the genes encoding lactate dehydrogenase, menaquinone oxidoreductase, acetyl-CoA transferase, acetate kinase, and phosphotransacetylase were inactivated to minimize the production of lactate and acetate as by-products under anaerobic conditions. Moreover, the lysine carboxylase gene from *Escherichia coli* was introduced in the genome of *C. glutamicum* to promote the biosynthesis of 1,5-diaminopentane from lysine. A single mutation in the aspartokinase gene was also introduced to prevent product inhibition by lysine. Furthermore, to facilitate the accumulation of oxaloacetate, a precursor of both 1,5-diaminopentane and succinate, a single mutation was introduced in the pyruvate carboxylase gene, which was

overexpressed. We further engineered this parental strain to shut off the carbon flux into the EMP under aerobic conditions by replacing the promoter of the glucose-6-phosphate isomerase (*pgi*) gene with the lactate dehydrogenase (*ldhA*) promoter. The constructed strain was grown in minimal medium to produce 1,5-diaminopentane under aerobic conditions. For succinate production, the strain was first grown in nutrient rich medium, and then the cells were washed with minimal medium, followed by preparation of 6% of cell suspension to produce succinate under anaerobic conditions. The products were analyzed by high-performance liquid chromatography (HPLC).

Results

Under aerobic conditions, four moles of NADPH are required to synthesize one mole of 1,5-diaminopentane. Since the PPP is the primary source of NADPH production, ideally, all the glucose carbons should be metabolized via this pathway. To that end, we constructed a promoter exchange strain by replacing the native promoter of the *pgi* gene with the promoter of the *ldhA* gene, which is induced under oxygen-deprived conditions. Hence, under aerobic conditions, *pgi* expression is shut off and glucose is metabolized only via the PPP (Fig. 1). In contrast, under anaerobic conditions, *pgi* expression is induced, channeling the glucose flux into the EMP to produce succinate through pyruvate (Fig. 1).

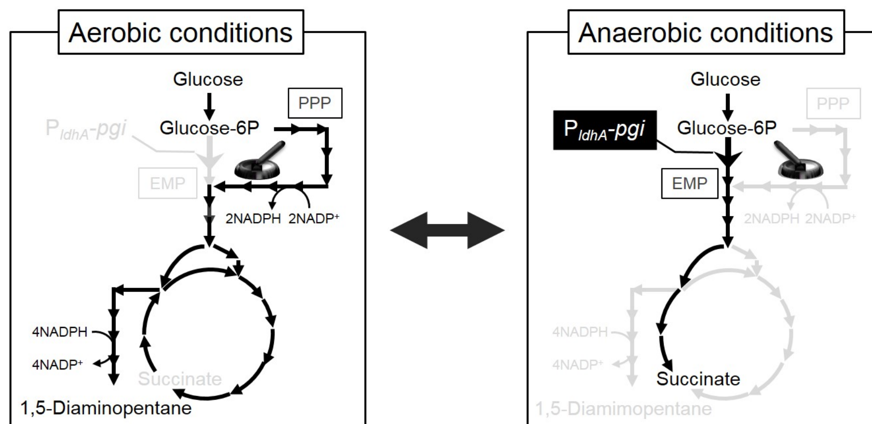

Fig. 1 Schematic of the oxygen level controlled bidirectional metabolic switch between the Embden-Meyerhof and Pentose Phosphate pathways.

Compared to the parental strain, the promoter exchange strain produced 1,5-diaminopentane with a 4.1-fold and 2.3-fold higher yield (g per g of consumed glucose) and productivity (g/L·h), respectively (Fig. 2). However, compared to the parental strain, the succinate productivity was decreased by 27% in the promoter exchange strain (Fig. 3). To resolve this unexpected decrease in succinate productivity, we introduced in the genome of the promoter exchange strain, an additional copy of the *pgi* gene under the control of the *ldhA* promoter. In the resulting second copy strain, the succinate productivity and yield were comparable to

those in the parental strain (Fig. 2). Moreover, although the second copy of the *pgi* cassette decreased the 1,5-diaminopentane yield by 27%, this strain exhibited an increased growth rate, resulting in a 10% increase in the 1,5-diaminopentane productivity compared to that in the promoter exchange strain (Fig. 2).

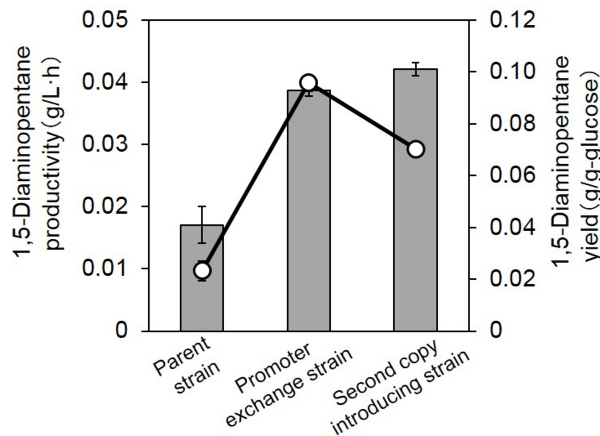

Fig. 2 1,5-Diaminopentane productivity (▪) and yield (-o-) under aerobic conditions.

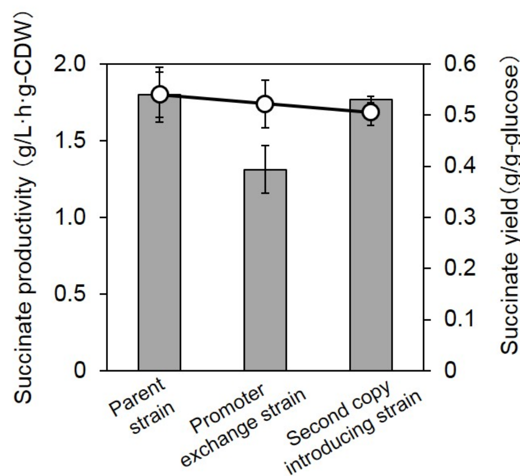

Fig. 3 Succinate productivity (▪) and yield (-o-) under anaerobic conditions.

Conclusion

Using oxygen as a metabolic switch, two polyamide monomers were produced by a single *C. glutamicum* strain through switching the carbon flow between the central metabolic pathways. This strategy could be applied to produce other useful bio-chemicals and fuels.

References

1) Kind S. *et al*. (2014) From zero to hero – Production of bio-based nylon from renewable resources using engineered *Corynebacterium glutamicum*. *Metab. Eng.* **25:** 113–123.
2) Okino S., Noburyu R., Suda M., Jojima T., Inui M., Yukawa H. (2008) An efficient succinic acid production process in a metabolically engineered *Corynebacterium glutamicum* strain. *Appl. Microbiol. Biotechnol.* **81:** 459–464.