Metabolic engineering of coryneform bacteria for the enhanced supply of acetyl-CoA and its application to the fermentative production of useful compounds

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Research aims

Corynebacterium glutamicum is a biotin auxotrophic Gram-positive soil bacterium and has been used for the industrial production of amino acids, particularly glutamate and lysine. Recent progress in metabolic engineering strategies has enabled the development of *C. glutamicum* strains capable of producing a variety of useful compounds, including isobutanol, itaconate, and cadaverine. However, little progress has been made in the production of compounds derived from acetyl coenzyme A (acetyl-CoA), an essential intermediate of the central metabolism of *C. glutamicum*. The present study aimed to identify factors involved in increasing the pool of acetyl-CoA (using genetic engineering to modulate the expression of genes related to the supply and consumption of acetyl-CoA) and to apply this knowledge to the production of acetyl-CoA-derived compounds.

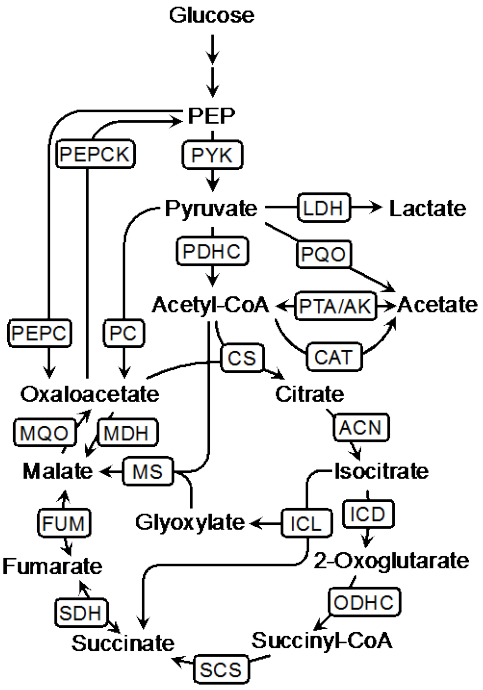

Fig. 1 Central metabolism of *C. glutamicum*

Methods

Using *C. glutamicum* ATCC 13032 as a parental strain, mutant strains with in-frame deletions of *ramA*, *ldhA*, *pqo*, *cat*, and *pta-ackA* were generated via a two-step homologous recombination procedure. All *C. glutamicum* strains were grown in minimal medium supplemented with 222 mM glucose to analyze their fermentation profiles.

Results

1. Effects of *ramA* deletion on the glucose metabolism of *C. glutamicum*

One effective way to enhance the supply of acetyl-CoA is to decrease the carbon flow into the tricarboxylic acid (TCA) cycle. RamA, a global transcriptional regulator, directly regulates many genes involved in the central metabolism of *C. glutamicum* (**Fig. 1**), particularly those involved in the TCA cycle[1]. Compared to the wild type, when the Δ*ramA* strain was grown in a minimal medium supplemented with glucose, the expression of the genes encoding CS, ACN, ODHC, SCS, SDH, FUM, and MQO was down-regulated. Notably, the Δ*ramA* strain showed approximately 90% less CS activity than the wild type[2]. Hence, the effects of *ramA* deletion on glucose metabolism were investigated further. Compared to the wild type, the Δ*ramA* strain showed an increased glucose consumption rate, decreased growth, a higher formation of lactate and acetate, and lower accumulation of 2-oxoglutarate and succinate (**Table 1**). These results indicate a reduction of the aerobic metabolism (such as the TCA cycle) and an increase of fermentation in the Δ*ramA* strain. However, the specific activity of PDHC, the enzyme producing the acetyl-CoA, in the Δ*ramA* strain was significantly lower than in the wild type (4.40 mU·(mg of protein)$^{-1}$ vs. 13.5 mU·(mg of protein)$^{-1}$). Unfortunately, this finding is not in line with the aims of this study. Therefore, new approaches need to be considered, such as (i) investigating the new factors that can attenuate the TCA cycle, and (ii) improving PDHC activity in the Δ*ramA* strain.

Table 1: Fermentation profiles of the *C. glutamicum* strains analyzed. The data are mean values and standard deviation from three independent experiments. For growth and metabolites, the values represent the maxima.

Parameter	Wild type	Δ*ramA*	Δ4	Δ4 Δ*ramA*
Specific growth rate (h^{-1})[a]	0.470 ± 0.009	0.443 ± 0.005[c]	0.494 ± 0.011[c]	0.425 ± 0.009[d]
Growth (OD_{600})	27.4 ± 0.7	23.0 ± 0.2[c]	27.7 ± 0.5	20.7 ± 0.4[d]
Glucose consumption rate[b]	0.957 ± 0.045	1.27 ± 0.04[c]	0.913 ± 0.016[c]	1.22 ± 0.01[d]
Pyruvate (mM)	46.9 ± 3.8	44.2 ± 4.8	89.3 ± 4.0[c]	161 ± 4[d]
Lactate (mM)	43.8 ± 15.5	103 ± 10[c]	4.31 ± 0.26[c]	5.87 ± 0.12[d]
Acetate (mM)	76.9 ± 10.5	109 ± 4[c]	13.5 ± 0.4[c]	12.7 ± 0.6
Succinate (mM)	18.5 ± 0.3	13.8 ± 0.2[c]	26.6 ± 2.5[c]	18.9 ± 0.7[d]
2-Oxoglutarate (mM)	5.26 ± 2.75	0.687 ± 0.203[c]	4.01 ± 0.03	2.97 ± 0.18[d]

[a] The specific growth rates were calculated from data obtained during the logarithmic growth phase (between 3 h and 6 h).

[b] The glucose consumption rates were calculated from data obtained during the early stationary phase (between 6 h and 15 h) and were expressed as consumed glucose (mM)·(average

$OD_{600})^{-1} \cdot h^{-1}$.

[c] *Statistically* significant differences (*t*-test *P*-values < 0.05), when compared to the wild-type strain.

[d] *Statistically* significant differences (*t*-test *P*-values <0.05), when compared to the Δ4 strain.

2. Construction of pyruvate-producing mutants

In the context of the second approach proposed above (improving PDHC activity in the Δ*ramA* strain), using as host a strain that can accumulate high amount of pyruvate, a precursor of acetyl-CoA, would be favorable. To date, a prototrophic pyruvate-producing strain of *C. glutamicum* (i.e., a strain without inactivation of PDHC) has not been reported[3]. The experiments described in section **1.** have shown that the deletion of *ramA* led to a fermentative phenotype. Therefore, a mutant strain lacking the enzymes involved in all known lactate- and acetate-producing pathways (i.e., LDH, PQO, CAT, and PTA/AK; see **Fig. 1**) was constructed, referred to as Δ4. The effects of *ramA* deletion on glucose metabolism were then evaluated in this mutant (**Table 1**). The Δ4 strain produced about two times more pyruvate than the wild-type and Δ*ramA* strains. Moreover, the pyruvate titer further increased in the Δ4 Δ*ramA* strain and was almost two times higher than that in the Δ4 strain. Furthermore, compared to the Δ4 strain, the Δ4 Δ*ramA* strain showed an increased glucose consumption rate, decreased growth, and lower accumulation of 2-oxoglutarate and succinate. These results indicate that the TCA cycle is attenuated in the Δ4 Δ*ramA* strain, as is the case in the Δ*ramA* strain[5].

Conclusions

This study aimed to (i) identify factors that can increase the pool of acetyl-CoA and (ii) apply this knowledge to the production of acetyl-CoA-derived compounds in *C. glutamicum*. At this stage of the project, I have made very encouraging progress towards the first aim. I have shown that *ramA* deletion can effectively attenuate the TCA cycle, and I have generated a *C. glutamicum* strain that can produce high levels of pyruvate, a precursor of acetyl-CoA. RamA has been reported to positively regulate the expression of the *aceF* gene, which encodes the E2 subunit of PDHC (formerly known as *sucB* and assumed to encode the E2 subunit of ODHC)[1,4], likely explaining the decreased PDHC activity observed in the Δ*ramA* strain and providing a clue for prospective work. Furthermore, the amino acid sequence of AceF differs between *C. glutamicum* strains, and data suggest that these differences affect PDHC activity. In the near future, I would like to further explore these lines of investigation and achieve the objectives of this research project.

References

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