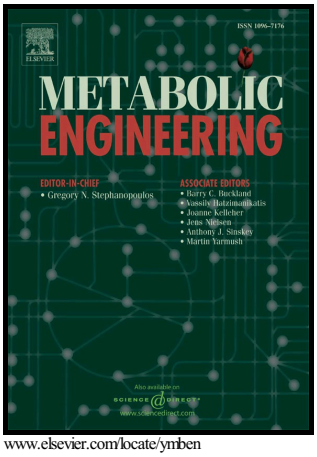

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L-lysine production independent of the oxidative pentose phosphate pathway by *corynebacterium glutamicum* with the *streptococcus mutans gapN* gene

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Figure Legends

Fig. 1. Glycolytic pathways and the pentose phosphate pathways in strain RE2 (right) and its parental Δ*gapB* strain (left). Strain RE2, a suppressor mutant from strain GPN, has a glycolytic pathway that was constructed by replacing its intrinsic NAD-dependent glyceraldehyde 3-phosphate dehydrogenase (GapA) with a non-phosphorylating NADP-dependent glyceraldehyde 3-phosphate dehydrogenase (GapN) from *S. mutans*. The engineering leads to NADPH generation via the glycolytic process but instead bypassing ATP generation. Arrows with solid and dotted lines represent single and multiple enzymatic processes, respectively. Abbreviations are as described in the legend to Fig. S1.

Fig. 2. L-lysine production and growth in strains RE2/pCAK311 and RE2Δ*zwf*/pCAK311 (B and G), strains RE2A^{iol}/pCAK311 and RE2A^{iol}Δ*zwf*/pCAK311 (C-E and H-J), and the control Δ*gapB*/pCAK311 and Δ*gapB*Δ*zwf*/pCAK311 strains (A and F). All strains were cultivated in 30 mL of LFG1 medium containing 5% glucose in a 300-mL baffled Erlenmeyer flask at 30ºC with rotary shaking at 200 rpm. The preceding seed culture conditions for each strain were as follows: BY medium containing 2% glucose (A-C and F-H), BY medium containing 1.5% glucose plus 0.5% *myo*-inositol (D and I), and BY medium containing 2% *myo*-inositol (E and J). White and black symbols represent data for strains with the intact *zwf* gene and the deletants of *zwf*, respectively. *Myo*-inositol presented in (I) and (J) was the carry-over from the seed culture. Values are means of replicated cultures, which showed <5% difference from each other.

Fig. 3. Construction of strain RE2A^{iol}. Strain RE2A^{iol} was constructed from strain RE2 by replacing the genomic *iolT1* open reading frame (ORF) with the endogenous *gapA* ORF. All strains used in this study are also presented.

Fig. 4. Relative transcript levels of *gapA* and *gapN* during L-lysine production. Total RNAs were prepared from cells of the indicated times of L-lysine production shown in Fig. 2. Aliquots of RNAs were reverse transcribed and subjected to qPCR. The transcript levels of *gapN* (black bars) and *gapA* (white bars) were standardized to the constitutive expression level of 16S rRNA, and are presented as relative values to those obtained for the corresponding genes at 8 h in the Δ*gapB*/pCAK311 strain (for *gapA*) (A) and strain RE2/pCAK311 (for *gapN*) (B), respectively. The data for the Δ*gapB*/pCAK311 strain that was precultured on 2% glucose is presented in (A), for strain RE2/pCAK311 that was precultured on 2% glucose is presented in (B), for strain RE2A^{iol}/pCAK311 that was precultured on 2% glucose is presented in (C), for strain RE2A^{iol}/pCAK311 that was precultured on 1.5% glucose plus 0.5% *myo*-inositol is presented in (D), and for strain RE2A^{iol}/pCAK311 that was precultured on 2% *myo*-inositol is presented in (E). Data represent mean value from three independent cultures, and the standard deviation from the mean is indicated as error bars.

Fig. 5. Summary of this work. Growth retardation of strain RE2 was overcome while maintaining its high ability to produce L-lysine using GapA at an effective level in the early exponential phase. Decline in GapA activity from the early to mid-exponential phase is represented with a gradational arrow in strain RE2A^{iol} (rightmost). The best result of strain RE2A^{iol} in terms of production rate was obtained using seed culture on 2% *myo*-inositol. In this condition, the newly engineered strain gave 2.1-fold higher L-lysine titer than the control Δ*gapB*/pCAK311 strain for a comparable period, and yielded a production rate of 2.41 mM/h. In conclusion, well-balanced use of GapA and GapN in strain RE2A^{iol} led to both improved growth and high-level L-lysine production that is independent of the PPP. Abbreviations are as described in the legend to Fig. S1.

Table 1 Specific activities of GapA and GapN in the engineered *C. glutamicum* strains.

Strain	Carbon source in seed culture	Enzyme activity (mU/mg)							
		Early exponential phase				Mid-exponential phase			
		GapA		GapN		GapA		GapN	
		NAD^+	$NADP^+$	NAD^+	$NADP^+$	NAD^+	$NADP^+$	NAD^+	$NADP^+$
Δ*gapB*/pCAK311	Glucose	1645.1 ± 64.6	–	ND	ND	1224.6 ± 79.7	–	ND	ND
RE2/pCAK311	Glucose	ND	ND	18.7 ± 6.3	845.7 ± 6.9	ND	ND	11.1 ± 3.8	310.5 ± 57.0
$RE2A^{iol}$/pCAK311	Glucose	–	ND	32.7 ± 19.6	724.8 ± 66.5	–	ND	6.7 ± 2.3	344.1 ± 45.1
	Glucose + *Myo*-inositol	181.8 ± 12.9	ND	ND	462.3 ± 38.2	–	ND	ND	317.5 ± 32.8
	Myo-inositol	571.9 ± 21.1	ND	ND	460.8 ± 19.8	58.2 ± 7.4	ND	ND	331.2 ± 112.1

Data represent mean values and standard deviations of three independent cultures. –, not detected; ND, not determined.

Highlights

- Growth of the *C. glutamicum* strain with *S. mutans gapN* was improved by growth phase-specific induction of endogenous *gapA*.
- The engineered GapN strain achieved a 1.8- to 2.1-fold higher L-lysine production rate than the control GapA strain.

- The efficient production was demonstrated to be independent of the oxidative pentose phosphate pathway.
- Intracellular NADPH/$NADP^+$ ratio of the GapN strain during L-lysine production was significantly higher than that of the GapA strain.

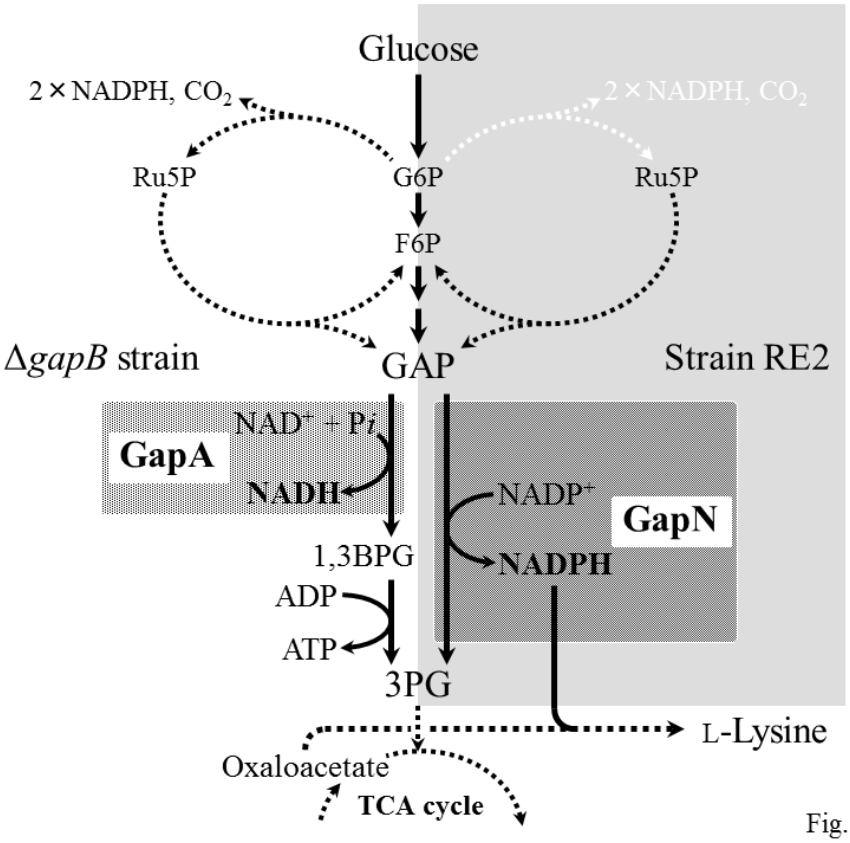

Fig. 1. Takeno

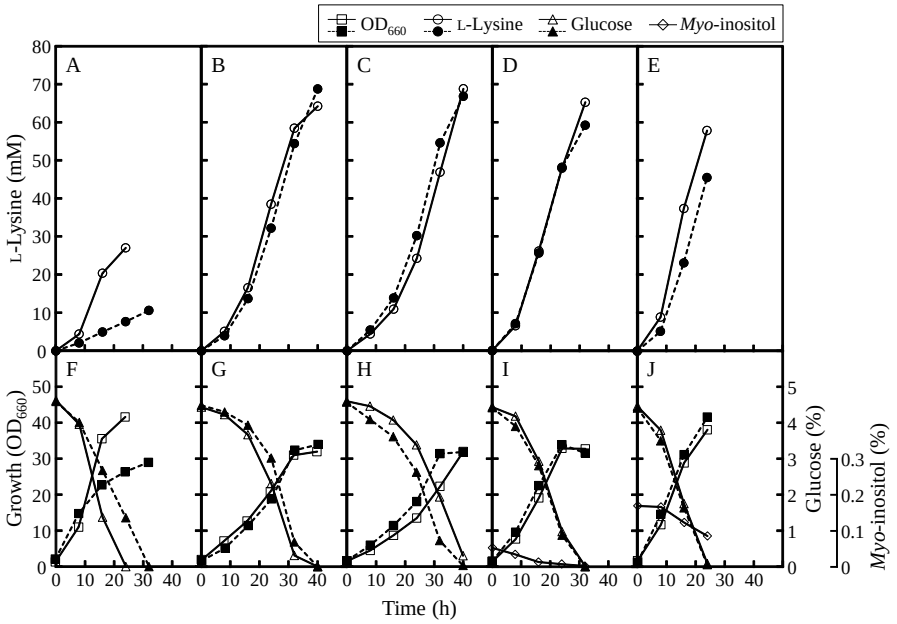

Fig. 2. Takeno

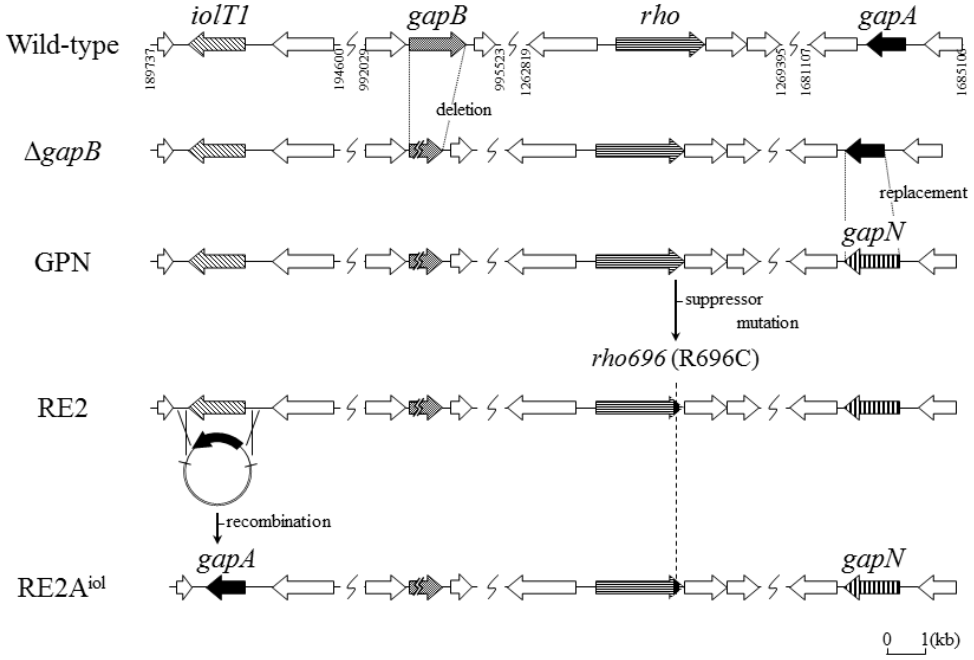

Fig. 3. Takeno

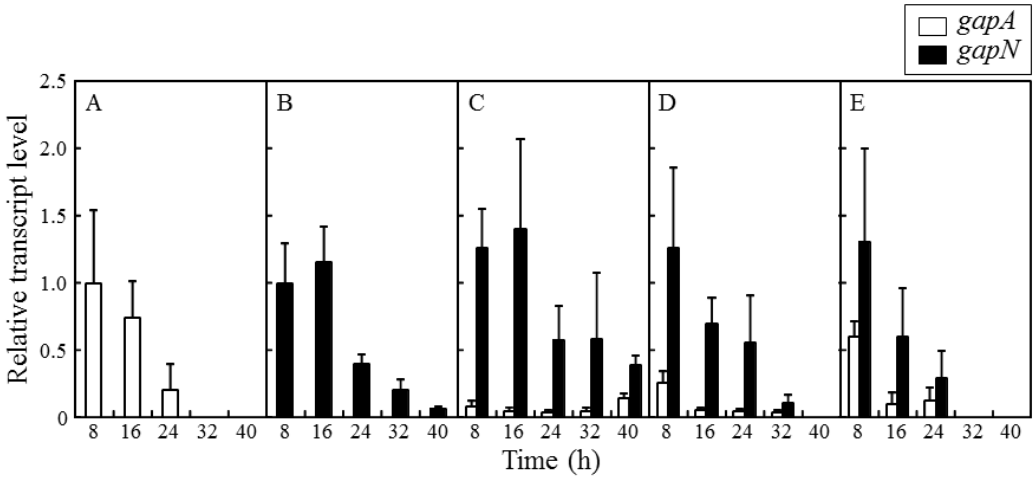
gapA
gapN
A
B
C
D
E
Relative transcript level
2.5
2.0
1.5
1.0
0.5
0
8 16 24 32 40 8 16 24 32 40 8 16 24 32 40 8 16 24 32 40 8 16 24 32 40
Time (h)

Fig. 4. Takeno

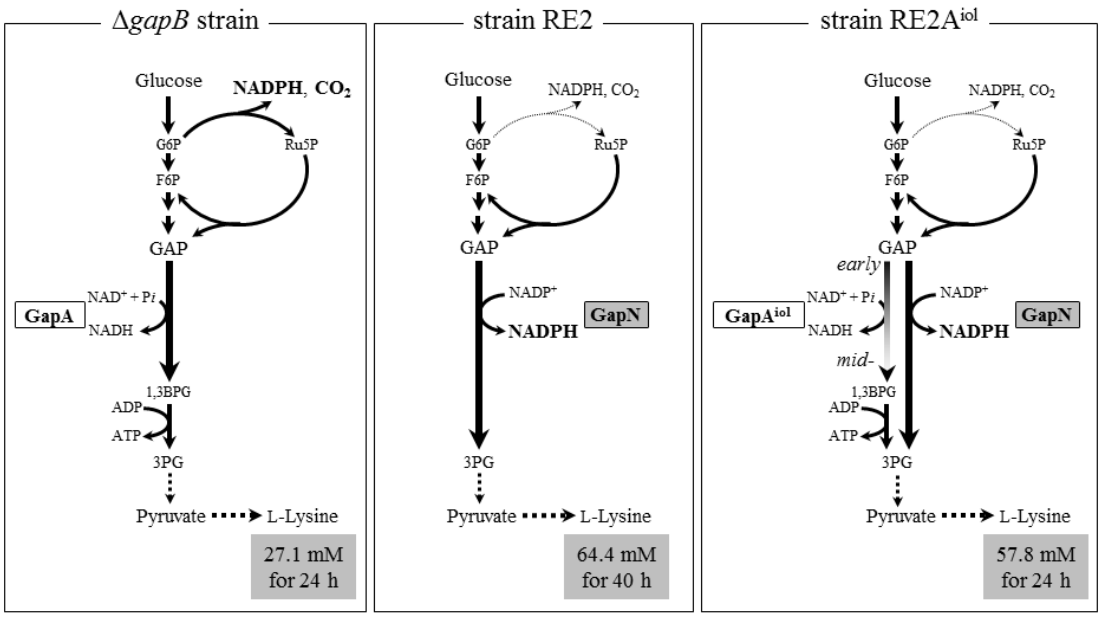
Δ*gapB* strain
Glucose
NADPH, CO2
G6P
Ru5P
F6P
GAP
GapA
NAD+ + Pi
NADH
1,3BPG
ADP
ATP
3PG
Pyruvate
L-Lysine
27.1 mM
for 24 h
strain RE2
Glucose
NADPH, CO2
G6P
Ru5P
F6P
GAP
NADP+
NADPH
GapN
3PG
Pyruvate
L-Lysine
64.4 mM
for 40 h
strain RE2A^iol
Glucose
NADPH, CO2
G6P
Ru5P
F6P
GAP
early
GapA^iol
NAD+ + Pi
NADH
NADP+
NADPH
GapN
mid-
1,3BPG
ADP
ATP
3PG
Pyruvate
L-Lysine
57.8 mM
for 24 h

Fig. 5. Takeno