1. Plasmid Construction

All primers are listed in Supplementary Table S1, and all constructed plasmid are listed in Table 1. For marker-free gene deletion, a series of plasmids were constructed. The vector pK18*mobsacB*-△*scrB* was constructed for *scrB* deletion. The upstream and downstream fragments (*scrB*-F and *scrB*-B, forward and backward fragments) were amplified from the *C. glutamicum* genome using primers ScrB-UF/ScrB-UR and ScrB-DF/ScrB-DR respectively. The two fragments were fused and amplified by fusion PCR with primers ScrB-UF/ScrB-DR. The fused fragment (*scrB*-UD) was digested with *Bam*HI and *Xba*I, and then ligated into the same digested sites of pK18mobsacB to create pK18*mobsacB*-△*scrB*. With a similar procedure, pK18mobsacB-△*ptsS*, and pK18*mobsacB*-△*scrB*△*ptsS* were also constructed. The fused fragments pK18mobsacB-△*ptsS* and pK18*mobsacB*-△*scrB*△*ptsS* were amplified with corresponding primers from the *C. glutamicum* 13032 chromosomal DNA. Digested with *Bam*HI-*Xba*I, these two fragments were ligated into the corresponding sites of digested pK18*mobsacB* to yield plasmid pK18mobsacB-△*ptsS* and pK18*mobsacB*-△*scrB*△*ptsS*, respectively.

The shuttle vector pEC-XK99E-*siase*-△*lacIq* was constructed for *siase* expression without addition of isopropyl β-D-1-thiogalactopyranoside (IPTG). The liner fragment of was amplified from vector pEC-XK99E-*siase*2 with the primers pEC-*lacIq*-F/L, then was digested with *Spe*I and ligated itself to yield pEC-XK99E-*siase*2-△*lacIq*.

2. Strains Construction

All constructed strains are listed in Table 1.

The vector pK18*mobsacB*-△*scrB* was integrated into *C. glutamicum* ATCC 13032 chromosome by the first single-crossover recombination and kanamycin-resistant transformants were selected and

verified by PCR. Next, the resulting transformant was cultured in LB liquid medium for 12h, and then the cells were spread on a LB-sucrose agar plate. The *scrB*-deleted strain, denoted as IS1, was selected from the grown colonies by PCR verification using primers ScrB-UF/ScrB-DR. With a similar procedure above, the *ptsS* gene could be knocked out together using pK18*mobsacB*-△*scrB*△*ptsS* verified by primers PtsS-UF/PtsS-DR. The *ptsS* were deleted in *C. glutamicum* ATCC 13032 step by step, generating IS5.

The shuttle vector pEC-XK99E-*siase*0, pEC-XK99E-*siase*1, pEC-XK99E-*siase*2, pEC-XK99E-*siase*3, were introduced into *C. glutamicum* ATCC13032, yielding IS3, IS4, IS6 and IS7, respectively. The transformant IS3 was obtained by introducing the plasmid pEC-XK99E-*siase*0 into IS1. The transformant IS4 was obtained by introducing the plasmid pEC-XK99E-*siase*1 into IS1. The transformant IS6 was obtained by introducing the plasmid pEC-XK99E-*siase*2 into IS5. The transformant IS7 was obtained by introducing the plasmid pEC-XK99E-*siase*3 into IS5. The transformant IS8 was obtained by introducing the plasmid pEC-XK99E-*siase*2-△*lacI* into IS6.

Table S1. Primers used in this study

Primer name	Sequence (5'-3') *
△*scrB* construction	
ScrB-UF	GCGGATCCCTGGGCCAATGGCGATGAAT (*Bam*HI)
ScrB-UR	AGGAAAGTAGTGTGTGGGGCTATCATAAAAAAGGGTCTTTTG T
ScrB-DF	ACAAAAGACCCTTTTTTATGATAGCCCCACACACTACTTTCCT
ScrB-UR	TCTAGAGCGCAGGGGTATGACGCTT (*Xba*I)
△*ptsS* construction	
PtsS-UF	GGATCCGACGGTAACCCACCGCAGCTTCACT(*Bam*HI)
PtsS-UR	CCCGGTTAAGGAGAAATTCAAGTTGAAACCTTGAGTGTTC
PtsS-DF	GAACACTCAAGGTTTCAACTTGAATTTCTCCTTAACCGGG
PtsS-DR	TCTAGAATTTCACACCCCATTACCGCGAT(*Xba*I)
△l*acq* construction	
Lacq-F	CGACTAGTGAGCGCAACGCAATTAATGTGAGTT (Spe*I*)
Lacq-R	ACTAGT ATTCACCACCCTGAATTGACTCTC (Spe*I*)
siase0	
F0-	CGGAATTCCGCAACGAATATACAAAAGTCCGCTGATTTTCCC AT (*EcoR*I)
R0-	GGTCGACGTTCAGCTTATAGATCCCGGCTTGCCACGGAGC(*Sal* I)
siase1	
F1	CGGAATTCATGTTTCTTAATGGATTTAAG(*EcoR*I)
R1	GGTCGACGTTCAGCTTATAGATCCCGGCTTGCCACGGAGC(*Sal* I)
siase2	
F2	CGATATCTGAGCTGTTGACAATTAATCAT (*EcoR*V)
R2	TCTAGACAAAAAAGGGCATCCGTCAGGAT (*Xba*I)
siase3	
F3	CGATATCTGAGCTGTTGACAATTAATCAT (*EcoR*V)
R3	TCTAGACAAAAAAGGGCATCCGTCAGGAT (*Xba*I)

*Restriction sites are underlined, and the restriction enzymes are indicated in parentheses.

Table S2. Comparison of the costs from different substrates for isomaltulose production by different engineered food-grade strains and purity. The cost of feedstocks for producing one ton of isomaltulose was calculated based on the current market prices and the reported conversion rate of sucrose.

Strains	Substrate cost (yuan/ton isomaltulose)	Isomaltulose Yield (g/g)	Isomaltulose purity (%)	References
Lactococcus lactis	Sucrose; 12,500	0.72	< 90	30
Saccharomyces cerevisiae	Sucrose; > 20,000	0.07	< 10	49
Yarrowia lipolytica	Sucrose; 8,646	0.96	97.8	44
Bacillus subtilis	PCM; 3,800	0.92	< 92.4	16
Yarrowia lipolytica	PCM; 3,600	0.96	97.4	13
Yarrowia lipolytica	PBM; 2,700	0.94	85.8	14
Corynebacterium glutamicum	UCM; 1,646	0.97	98%	In this study
Corynebacterium glutamicum	UBM; 1,916	0.97	98%	In this study

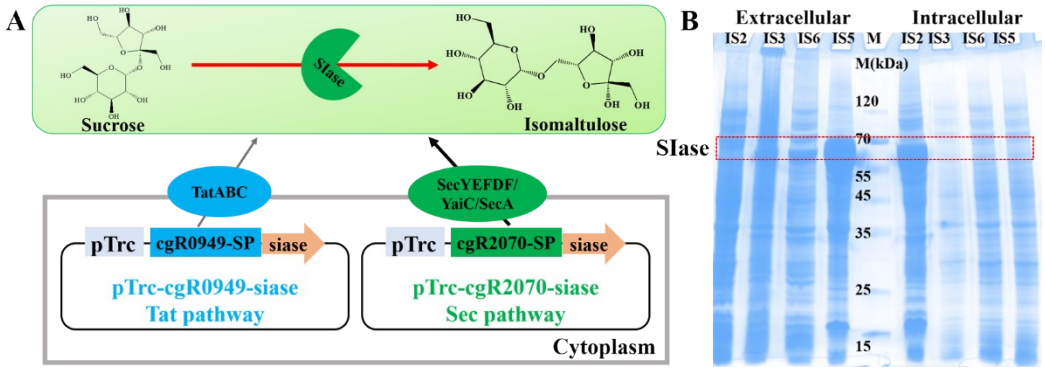

Figure S1. Secretory expression of sucrose isomerase in *C. gluctamicum*. (A) Siagram of extracellular isomaltulose conversion. SIase, sucrose isomerase; SecYEGDF/YajC/SecA, proteins involving SEC secretion pathway; TatABC, proteins involving TAT secretion pathway. (B) SDS-PAGE of the expression of sucrose isomerase. IS2 strain, *C.glutamicum* carrying pEC-XK99E-*siase* 0 vector without native signal peptide; IS3, *C.glutamicum* carrying pEC-XK99E-*siase* 1 vector with native signal peptide; IS5, *C.glutamicum* carrying pEC-XK99E-*siase*2 vector with cgR-2070 signal peptide through Sec pathway; IS6, *C.glutamicum* carrying pEC-XK99E-*siase*3 vector with cgR-

0949 signal peptide through Tat pathway. Samples were collected after cultured in shake flasks for 48 h at 30°C, 200 rpm and pH 7.0.

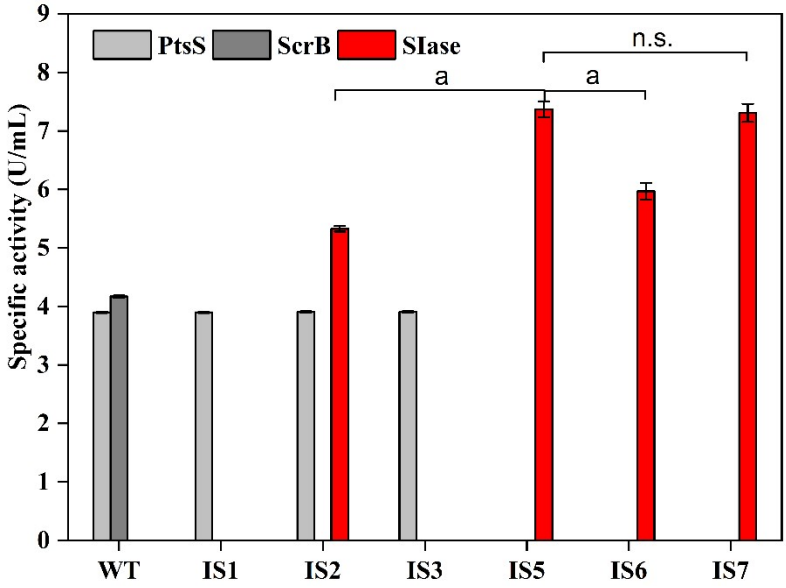

Figure S2. The activity of PtsS, ScrB and SIase in different strains. The strains grown on the CGXII medium at 30°C, 180 rpm for 48 h. The amount of enzyme producing 1umol/min of production was defined as one unit of enzyme activity (U). The symbol (a) was the difference ($p < 0.05$) in SIase activity between two groups; n.s. meant no statistic difference.

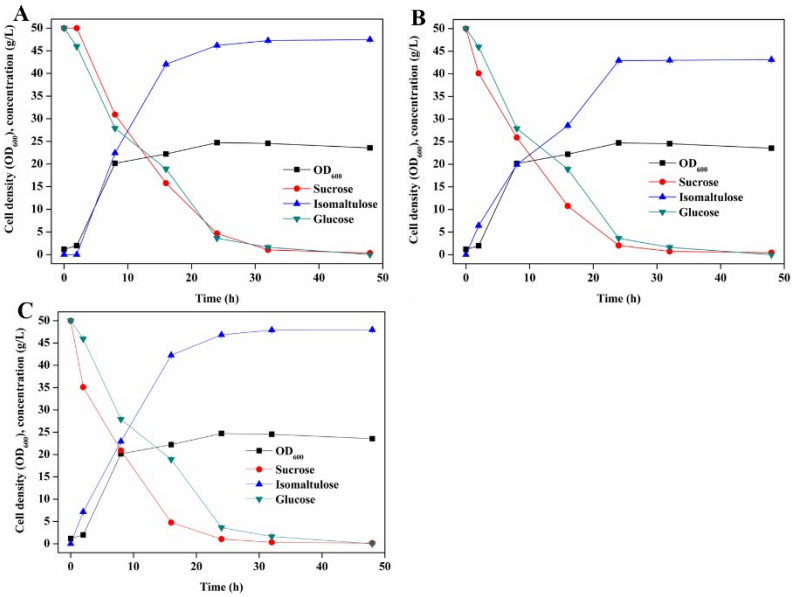

Figure S3. Metabolic profiles of the strains IS5, IS6 and IS7. The strains were cultured in CGXIIY medium with 50 g/L glucose and 50 g/L sucrose at 30°C and 180 rpm in 300 mL flasks with a final culture volume of 60 mL. A: The fermentation characteristics of IS5; B: the fermentation characteristics of IS6; C: the fermentation characteristics of IS7.