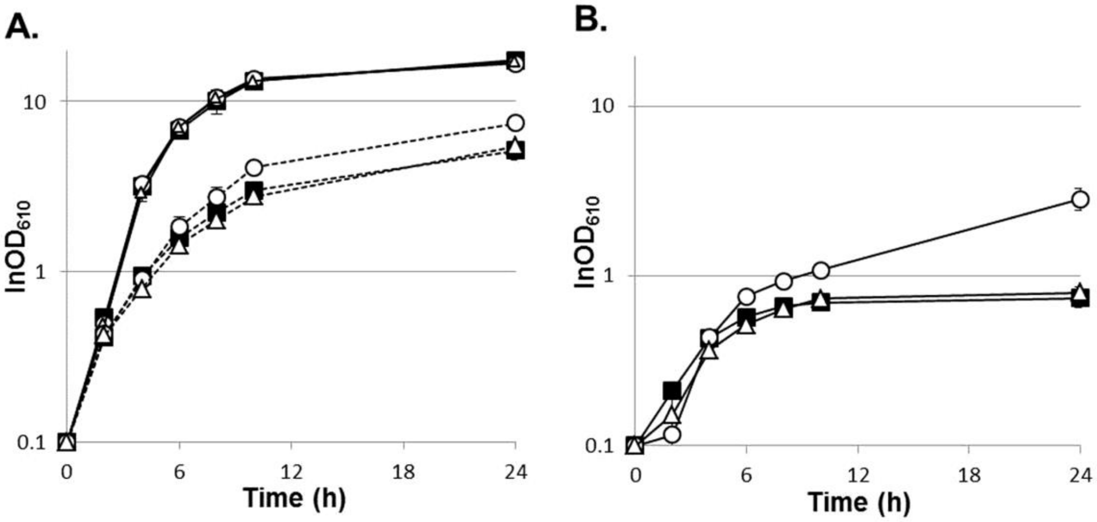
Supplementary Figure



Supplementary Figure 1. Plasmid-based overexpression of *pfk* and *pyk* restores the GLY3-like stress tolerance to GLY3dfy.

Growth curves of different strains with standard deviations (n = 3). GLY3 with the empty vector pCRB12i (closed square), GLY3dfy with pCRB12i (open circle), and GLY3dfy with pCRE558, the construct for overexpression of *pfk* and *pyk* (open triangle), grown in nutrient-rich medium supplemented with 50 μg/ml kanamycin and 10 μM IPTG. **A.** Growth at 33 ℃ (solid lines) and 40 ℃ (dashed lines). **B.** Growth in the presence of 1.75 % (v/v) isobutanol.

Supplementary Tables

Supplementary Table 1. mRNA ratio of the genes in the regions 1 and 2

Gene (locus_tag)	Region	mRNA ratio						
		trm2/GLY3		tam44/GLY3		tam45/GLY3		GLY3
		33℃	41℃	33℃	41℃	33℃	41℃	41/33℃
CgR_2322	region 1[*1]	0.03	0.02	0.01	0.02	0.02	0.02	0.57
CgR_2323		0.15	0.09	0.1	0.1	0.08	0.06	1.01
CgR_2324		0.05	0.01	0.03	0.02	0.04	0.02	1.60
CgR_2325		0.09	0.02	0.04	0.01	0.06	0.04	2.69
CgR_2326		0.01	0.08	0.02	0.21	0.01	0.11	0.09
CgR_2327		0.001	0.003	0.005	0.001	0.002	0.002	0.38
CgR_2328		0.19	0.19	0.22	0.17	0.19	0.09	0.45
CgR_2329		0.21	0.32	0.29	0.42	0.20	0.30	0.35
CgR_0733	region 2[*1]	0.05	0.12	0.03	0.03	0.04	0.03	0.38
CgR_0734		0.56	0.62	0.74	0.56	0.52	0.37	0.52
CgR_6038		0.06	0.05	0.10	0.07	0.08	0.07	0.35
CgR_6039		0.23	0.34	0.25	0.36	0.18	0.23	0.35
CgR_6040		0.02	0.02	0.02	0.05	0.02	0.02	0.59
CgR_6041		0.02	0.05	0.02	0.03	0.01	0.03	0.25
CgR_6042		0.02	0.03	0.01	0.04	0.01	0.04	0.26
CgR_6043		0.03	0.03	0.04	0.04	0.04	0.05	0.36
CgR_6044		0.32	0.45	0.51	0.75	0.34	0.44	0.23
CgR_6045		0.18	0.17	0.25	0.21	0.16	0.12	0.22
CgR_6046		0.09	0.16	0.11	0.19	0.10	0.15	0.38
CgR_6047		0.02	0.02	0.04	0.04	0.01	0.03	0.56
CgR_6048		0.32	0.47	0.42	0.46	0.31	0.28	0.37

[*1] For genomic organization of each region, see Figure 2.

Supplementary Table 2. Genomic deletions common to the three evolved strains.

Position (nt)	Gene		Protein	Deletion
	Locus_tag	Name		
region1: 2555774-2560443	CgR_1327	*pfk* (transgene)	6-phosphofructokinase	Entire ORF
	CgR_2811	*pyk* (transgene)	Pyruvate kinase	Entire ORF
	CgR_2322		Putative transposase	Entire ORF
	CgR_2323		Putative transposase	Entire ORF
	CgR_2324		Putative transposase	Entire ORF
	CgR_2325		Putative transcriptional regulator	Entire ORF
	CgR_2326		Putative major facilitator transporter	Entire ORF
	CgR_2327		Hypothetical protein	1-363 nt of ORF
region2: 793044-799509	CgR_0733		Hypothetical protein	Entire ORF
	CgR_0734		Hypothetical protein	54-126 nt of ORF
	CgR_6038		Hypothetical protein	Entire ORF
	CgR_6039		Hypothetical protein	Entire ORF
	CgR_6040		Hypothetical protein	Entire ORF
	CgR_6041		Hypothetical protein	Entire ORF
	CgR_6042		Hypothetical protein	Entire ORF
	CgR_6043		Hypothetical protein	Entire ORF
	CgR_6044		Hypothetical protein	Entire ORF
	CgR_6045		Hypothetical protein	Entire ORF
	CgR_6046		Hypothetical protein	Entire ORF
	CgR_6047		Hypothetical protein	Entire ORF

Supplementary Table 3. Point mutations identified in the evolved strains.

Presence/Absence (p/a)			Number of mutations identified			
trm2	tam44	tam45	Intragenic		Intergenic	Sum
			Non-synonymous	Synonymous		
p	p	p	14	7	5	26
p	p	a	26	17	6	49
p	a	p	0	0	0	0
a	p	p	0	0	0	0
p	a	a	30	18	11	59
a	p	a	37	15	3	55
a	a	p	68	29	9	106
Total			175	86	34	295

Supplementary Table 4. Intergenic mutations common to the evolved strains.

Position (nt)	Mutation	Upstream			Downstream			Thermotolerance*[2]	
		Position*[1]	Gene (locus_tag)	Protein	Position*[1]	Gene (locus_tag)	Protein	Introduction	Reversion
1123184	T>C	+24 nt	CgR_1015	Thiol-disulfide isomerase	-56 nt	CgR_1016	Hypothetical protein	+/-	+/-
1509663	G>A	+572 nt	CgR_1377	Hypothetical protein	-385 nt	CgR_1378	Hypothetical protein	+/-	+/-
1553806	G>A	+194 nt	CgR_1414	Hypothetical protein	-65 nt	CgR_1415	Putative trypsin-like protease	+/-	+/-
1616056	A>G	+80 nt	CgR_1462	Arginine repressor	-88 nt	CgR_1463	Argininosuccinate synthase	+/-	+/-
3284814	T>C	+131 nt	CgR_2959	Proline-betaine transporter	-119 nt	CgR_2960	Putative membraneprotease	+/-	+/-

*[1] Position of the start or the end of an ORF is shown relative to the position of a mutation.

*[2] Mutations were introduced to GLY3 (introduction), or replaced by the wild-type genes in the evolved strains (reversion). +/-: unchanged; +:increased; -:decreased