Supplemental Material

Construction of a prophage-free *Corynebacterium glutamicum* - a platform strain for basic research and industrial biotechnology

Recombinant DNA work

Construction of deletion plasmids. The up- and downstream regions (~500 bp) of the respective gene cluster (here exemplified for CGP1) were amplified using the oligonucleotide pairs CGP1-D1/CGP1-D2 and CGP1-D3/CGP1-D4, respectively. The resulting PCR products served as templates for overlap extension PCR using the oligonucleotide pair CGP1-D1/CGP1-D4. The resulting DNA fragment was digested with EcoRI and BamHI and cloned into pK19*mobsacB* cut with the same enzymes. The other deletion plasmids were constructed analogously.

Construction of pACYC184-*cglM-Lac*. The following PCR products were amplified: a 500 bp fragment of the *cglM*-gene using the primer pair cglMRR-fw-XbaI/cglMRR-rv and *C. glutamicum* chromosomal DNA as template. *LacI* was amplified with the primer pair Prom_LacI/Prom_RBS-rv and pAN6 as template. *LacZ* was amplified using the primers lacZ-fw/lacZ-rv-XbaI and the plasmid pK18-lacZ as template. All three fragments were purified and used for an overlap-extension-PCR with the primer pair lacZ-rv-XbaI/cglMRR-fw-XbaI. The resulting fragment was gel-purified, digested with XbaI and cloned into pACYC184. Positive clones could easily be identified as blue colonies on agar plates containing 200 µM IPTG and 100 µM 5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-gal).

Construction of pMBX1-eYFP. pEKEx3 was cut with NdeI and the overhangs were filled using Klenow polymerase (NEB) to remove unnecessary parts of the plasmid and the NdeI sites at unwanted positions. The RBS was inserted *via* Phosphorothioate-based ligase-independent gene cloning (PLICing) (1) using the primer pair pMBX1-RBS-fw/pMBX1-RBS-rv and the resulting plasmid was named pMBX1. Finally, *eyfp* was cloned by PLICing using the primer pairs pMBX1-clone-fw/ pMBX1-clone-rv and YFP-PLIC-fw/YFP-PLIC-rv with pEKEx2-eYFP as template for *eyfp*.

Construction of pAN6-crimson. The Crimson coding sequence was amplified using the primer pair crimson-NdeI-fw/crimson-EcoRI-rv and the plasmid pE2-crimson as template. The PCR-product was cut with NdeI/EcoRI and ligated into pAN6 cut with the same enzymes.

Construction of pK18*mobsacB*-int1. The intergenic regions were amplified using the primer pairs pK18-int-1/pK18-int-2 as well as pK18-int-3/pK18-int-4 and *C. glutamicum* chromosomal DNA as template. The resulting fragments were fused with the pK18*mobsacB* plasmid cut with EcoRI/NheI using the In-Fusion® cloning kit (Clontech, Saint-Germain-en-Laye, France).

Construction of chromosomal fluorescence markers. A DNA fragment encoding eYFP or crimson under the control of the P_{tac} promoter was amplified using the primer pairs Ptac-eYFP-MfeI-fw/Ptac-eYFP-XhoI-rv and Ptac-eYFP-MfeI-fw/Ptac-crimson-XhoI-rv with pMBX1-eYFP or pAN6-crimson as template. The resulting products were cut with MfeI/XhoI and ligated into pK18*mobsacB*-int1 cut with the same enzymes. All plasmids were sequenced to exclude unwanted mutations.

Table S1: Oligonucleotides used in this study

Oligonucleotide	Sequence (5' → 3') and properties[a]
CGP1-D1	CCGGAATTCCACCGCAAGTGGAGTCTTTTTC
CGP1-D2	**CCCATCCACTAAACTTAAACA**CCAGAAACTTGGGGGTAGTTTC
CGP1-D3	**TGTTTAAGTTTAGTGGATGGG**GCAGTTTGAGTGACATCACCACC
CGP1-D4	GCGCGGATCCGCATCACATTTTAGAACGCCACC
CGP1-Dfw	CAGTTCCGCCGGAAATTCCTG
CGP1-Drv	GTGTCATTGACCAACTGGAAGG
CGP2-D1	CCGGAATTCCAAAAGCCTCTTCCCCACGCT
CGP2-D2	**CCCATCCACTAAACTTAAACA**GTCTAATGGACGGTGAAGTATC
CGP2-D3	**TGTTTAAGTTTAGTGGATGGG**GAACCTACGCCAATTCCACGA
CGP2-D4	CGCGGATCCGTGGAACGCACCGACGGCGAA
CGP2-Dfw	CACCACTTCGCAGGCTTTGAG
CGP2-Drv	CGTAGTCGTCCTCGGATTCAAC
CGP3-D1	CCGGAATTCCTGAGCTAAACGCGCGGGAC
CGP3-D2	**CCCATCCACTAAACTTAAACA**CACCCAGCTTCTATATGGAAGA
CGP3-D3	**TGTTTAAGTTTAGTGGATGGG**CTCCTCGATTAACGTCAGCCAA
CGP3-D4	CGCGGATCCCGGTTTCATTAAGAATCATGTCCA
CGP3-Dfw	CTTTAGGTACGTGGTAAACATACC
CGP3-Drv	CCTTCTCCAGTGATGACTAAATC
cglMRR-D1	CCGGAATTCATCTCAAGCGACCGTTCAAAAGC
cglMRR-D2	**CCCATCCACTAAACTTAAACA**ACAATAGTGGGTTTTGTACTCATG
cglMRR-D3	**TGTTTAAGTTTAGTGGATGGG**CATACAAACACCGCTGTTGATTAC
cglMRR-D4	CGCGGATCCTAAGCTCGCTGACATGCGGTTG
cglMRR_downstr_rv	TGAAGAAGCAGAGGTCAATGATG
cglMRR_upstr_fw	ATCGTGATCACCCAGTGGAGC
prophage-1611-for	GCAGAGTTCGCACGAGTGTTGAGCG
prophage-1816-rev	CACGTACTTTGCACGGATTCGTCGG
D_cg2040_for	CTGCTCTGATAACCATATTGAAGAA

D_cg2040_rev	AGGCCGTCGATTTACTAAAAACC
lacZ-fw	ATGACCATGATTACGGATTCACTG
lacZ-rv-XbaI	GCGCTCTAGAATTATTTTTGACACCAGACCAACTGG
Prom_lacI	**TTGGAGGCCAAGATCGAG**TCAAGCCTTCGTCACTGGTCC
Prom_RBS-rv	**TGAATCCGTAATCATGGT**CATATGTATATCTCCTTCTGCAGG
cglMRR-fw-XbaI	GCGCTCTAGAGTAGGAGCTGGGTGCTTGAAATC
cglMRR-rv	CTCGATCTTGGCCTCCAAGAGG
pMBX1-RBS-fw	G*A*G*A*T*A*T*A*C*A*T*A*TGGTCGACTCTAGAGGATCCC
pMBX1-RBS-rv	T*A*T*G*T*A*T*A*T*C*T*C*CTTCTGCAGGCATGCAAGCTTGG
pMBX1-clone-fw	G*G*T*A*C*C*G*A*G*C*T*C*GAATTCACTGGC
pMBX1-clone-rv	C*A*T*A*T*G*T*A*T*A*T*C*TCCTTCTGCAGGCATG
YFP-PLIC-fw	G*A*T*A*T*A*C*A*T*A*T*G* GTGAGCAAGGGCGAGGAGCTG
YFP-PLIC-rv	G*A*G*C*T*C*G*G*T*A*C*C* TTATCTAGACTTGTACAGCTCGTCC
crimson-NdeI-fw	ATACATATGGATAGCACTGAGAACGTCATCAAG
crimson-EcoRI-rv	GGCGAATTCCTACTGGAACAGGTGGTG
pK18-int-1	**GCAGCGTGAAGCTAG**ATCTGCGGCAGTCACTTTGATTTAC
pK18-int-2	**TGATCCAATTGACACGAGCAAGAGGAA**TTCGCGTATGGCAATGACAGTTTG
pK18-int-3	**GTGTCAATTGGATCAGCTCGAGCGAA**CTCATGGACACAATTTAAACTTCTTAAATAG
pK18-int-4	**CCATGATTACGAATT**GGCTCCGTTGTTCACGTCTAC
Ptac_eYFP_MfeI_fw	GCGCCAATTGTCAAGCCTTCGTCACTGGTCC
Ptac_eYFP_XhoI_rv	GCGCCTCGAGTTATCTAGACTTGTACAGCTCGTC
Ptac_Crimson_XhoI_rv	GCGCCTCGAGCTACTGGAACAGGTGGTGGC
ddh-LC-for	ACGTGCTGTTCCTGTGCATGG
ddh-LC-rev	GCTCGGCTAAGACTGCCGCT
qPCR-pEKEx-5	AATGTCGGGCAATCAGGTGCG
qPCR-pEKEx-6	TACCTGGAATGCTGTTTTCCCGG

[a]In some cases oligonucleotides were designed to introduce recognition sites for restriction endonucleases (recognition sites underlined). Complementary sequences used for overlap extension PCR or In-Fusion® cloning are written in bold letters. Asterisks (*) mark the locations of phosphorothioate bonds.

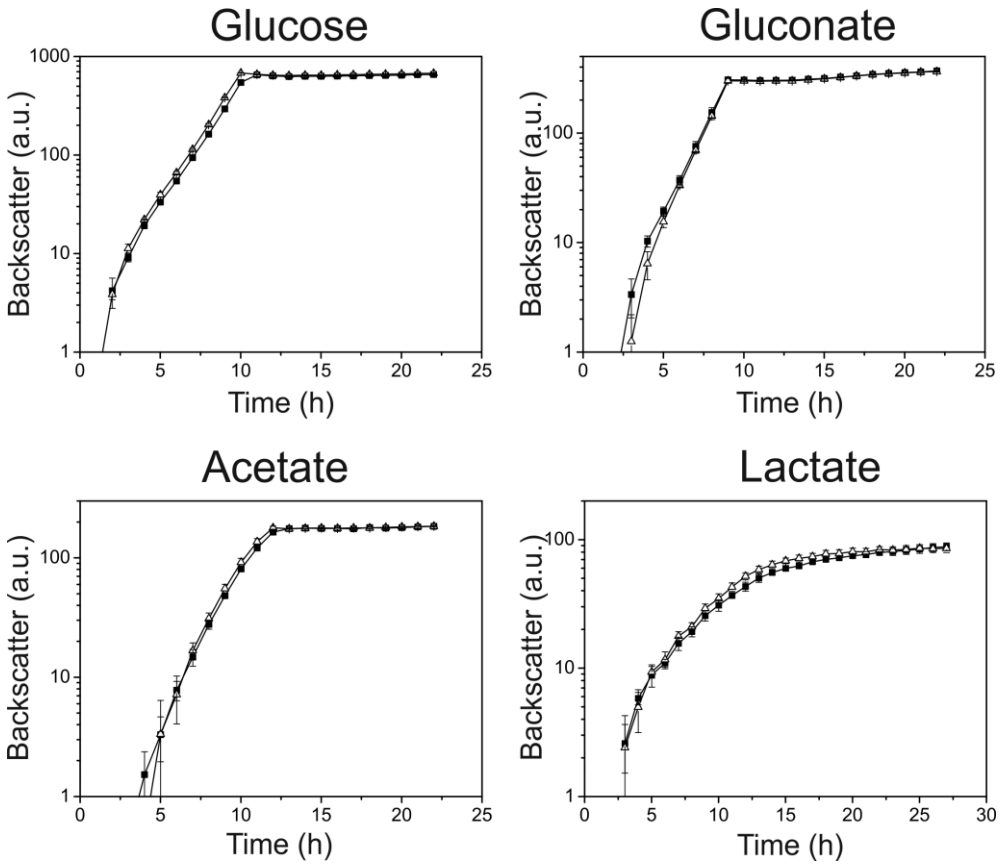

Fig. S1: Growth of *C. glutamicum* MB001 (Δ) and ATCC 13032 (■) in CGXII minimal medium with different carbon sources. The strains were precultivated in BHI medium and washed once with PBS before inoculation in CGXII minimal medium to an initial OD_{600} of 1. Each graph shows the average values and standard deviations resulting from four biological replicates. Glucose, 2 % (w v^{-1}); sodium gluconate, 100 mM; sodium acetate, 2 % (w v^{-1}); sodium lactate, 100 mM. The cells were grown in 48well Flowerplates using the Biolector microcultivation system (see Material and Methods).

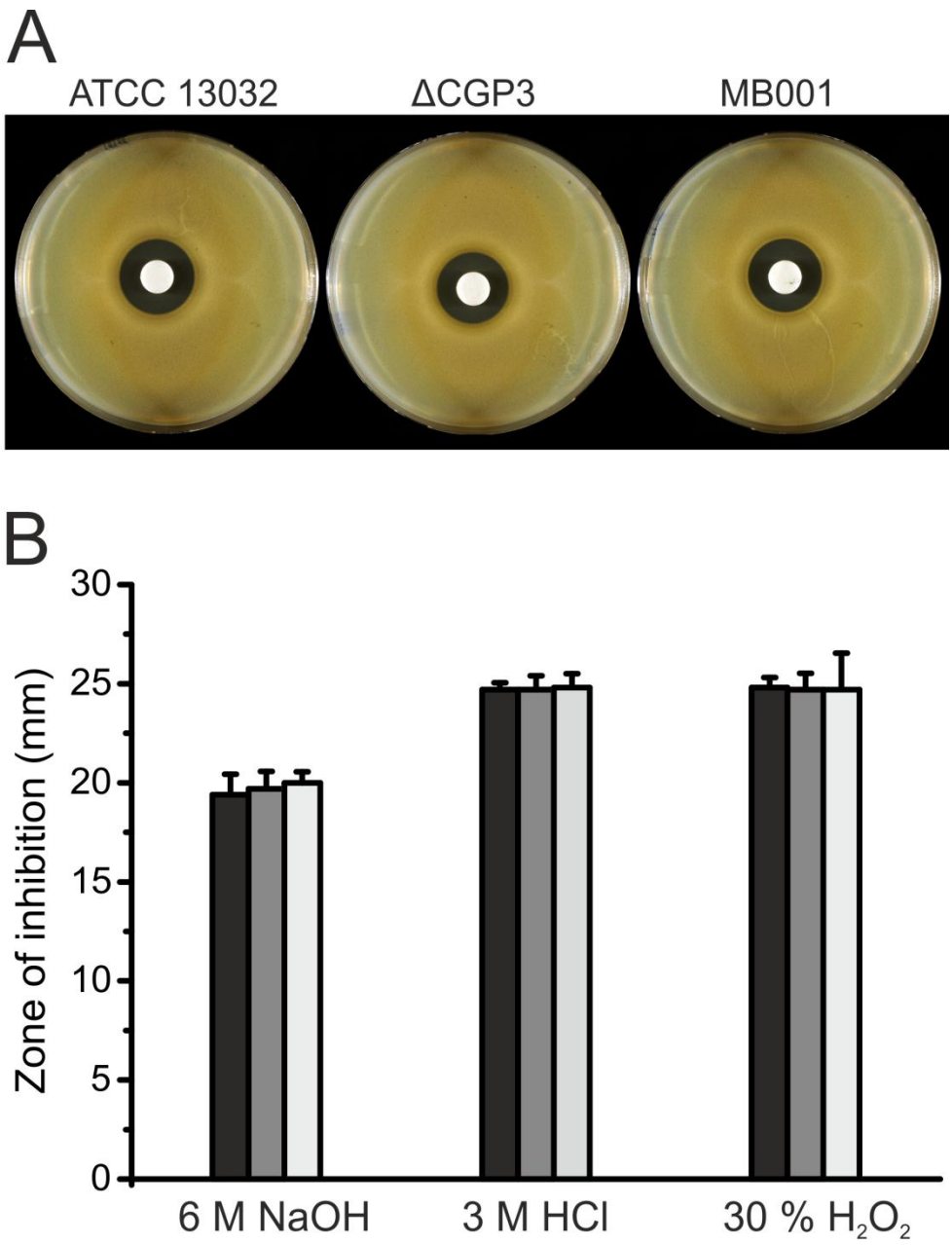

Fig. S2: Comparison of the stress resistance of *C. glutamicum* ATCC 13032, DCGP3 and MB001 using agar diffusion tests. A, Comparison of the growth of ATCC 13032, ATCC 13032 ΔCGP3 and MB001 in the presence of 6 M HCl. B, Zone of inhibition in the presence of different stress inducing agents. Shown is the average value and SD of at least three biological replicates.

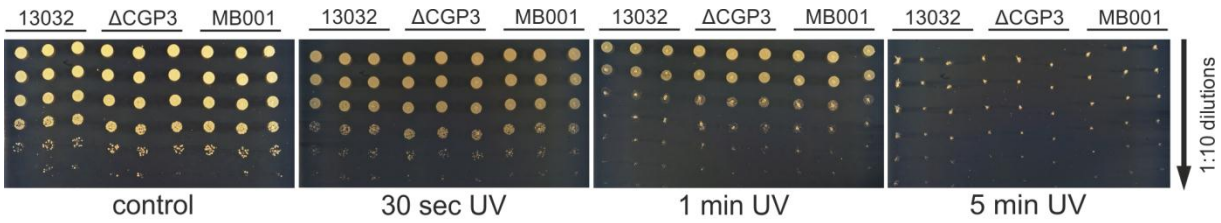

Fig. S3: Growth of *C. glutamicum* ATCC 13032, DCGP3 and MB001 after UV radiation. A dilution series of *C. glutamicum* cells from the exponential growth phase was prepared and 3 µl of each were spotted onto CGXII minimal medium agar plates containing 4 % (w v^{-1}) glucose. After exposure to UV light, the agar plates were incubated for 2 days at 30 °C and photographed.

References

1. **Blanusa M, Schenk A, Sadeghi H, Marienhagen J, Schwaneberg U.** 2010. Phosphorothioate-based ligase-independent gene cloning (PLICing): An enzyme-free and sequence-independent cloning method. Anal. Biochem. **406:**141-146.