Supplemental material to the manuscript:

# Transcription of sialic acid catabolism genes in *Corynebacterium glutamicum* is subject to catabolite repression and control by the transcriptional repressor NanR

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The supplementary data comprise four tables (Table S1, S2, S3, S4) and eleven figures (Fig. S1, Fig. S2, Fig. S3, Fig. S4, Fig. S5, Fig. S6, Fig. S7, Fig. S8, Fig. S9, Fig. S10, Fig. S11).

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**Table S1.** Characteristics of transcriptional regulators of Neu5Ac catabolism in bacteria

| Organism | Name | Regulator Superfamily | Function | Neu5Ac-transporter | Binding motif | Effector/Inducer | References |
|---|---|---|---|---|---|---|---|
| *E. coli* | NanR | GntR/FadR | repressor | secondary | GGTATA | Neu5Ac | (1, 2) |
| *B. breve* | NanR | GntR/FadR | repressor | secondary | ATNAGACATCAGANGTCCCAT | Neu5Ac | (3, 4) |
| *C. glutamicum* | NanR | GntR/FadR | repressor | ABC | AMGYMTGATGTCWKATGTMTA | GlcNAc-6P, ManNAc-6P | (5), this work |
| *V. vulnificius* | NanR | RpiR | repressor | TRAP | GTTTGAAAAAAATCTTCGT | ManNAc-6P | (6) |
| *C. perfringens* | NanR | RpiR | n.d. | secondary | GAAAAATATTTTC | n.d. | (7) |
| *Staph. aureus* | NanR | RpiR | repressor | secondary | n.d. | ManNAc-6P | (8) |
| *H. influenzae* | SiaR | RpiR | repressor | TRAP | n.d. | GlcN-6P (enhances binding) | (9-12) |
| *S. pneumoniae* | NanR | RpiR | activator | ABC + secondary | TCTGAAASTACTTTCARA | n.d. | (13) |

n.d. – not determined

**Table S2.** Oligonucleotides used in this study

| Name | Sequence (5´-3´)[a] | Purpose, restriction site |
|---|---|---|
| PnanR_shift_rev | GTCGCGCACTGCTTCTTGAG | probes PnanR, PnanR-A, PnanR-B, PnanR-C |
| PnanR_shift_fwd | GTGTTAAGTTGAGTGCCGGA | probe PnanR |
| PnanR_shift_A_fwd | GTCGTTAAGTTACTGTGGCG | probe PnanR-A |
| PnanR_shift_B_fwd | GATTTGCAGACTACAGACTA | probe PnanR-B |
| PnanR_shift_C_fwd | CCATTCATTTACACGTAAAAGAC | probe PnanR-C |
| nagA-for | GAGTAGTTACCACCGCAGCT | probes PnagA, PnagA-M4, PnagA-A, PnagA-B |
| nagA-rev | GAGAAAGCCATCAATCACCC | probes PnagA, PnagA-M4 |
| nagA-A-rev | AGGACGTATGATGTCTTATG | probe PnagA-A |
| nagA-B-rev | CGGATTTAGGTTAATTATCTTC | probe PnagA-B |
| siaEFGI_fwd | GCTA*CCTGCAGG*AGGAGATATAACCATGAGCACCACGATTACTC | pEKEx2-*siaEFGI*, *Sbf*I |
| siaEFGI_rev | CTAATGGTGATGATGGTGATGCAAACGAGTTTTGTTCAGCAG | pEKEx2-*siaEFGI* |
| D_nanR_P1 | CGTATG*CTGCAG*TGGTGGTCATTGGGTCGTAG | pK19*mobsacB*Δ*nanR*, *Pst*I |
| D_nanR_P2 | TGTTTAAGTTTAGTGGATGGGGTGTTCACTTCCTAATCTGGAG | pK19*mobsacB*Δ*nanR* |
| D_nanR_P3 | CCCATCCACTAAACTTAAACAGCTCACTACGCGCCGTTTCG | pK19*mobsacB*Δ*nanR* |
| D_nanR_P4 | CTTGA*CCCGGG*AATATCGGTGATGTGGCCATCG | pK19*mobsacB*Δ*nanR*, *Xma*I |
| check_D_nanR_fwd | TGCCGTCTAGAAACTTGCAG | Verification of *nanR* deletion |
| check_D_nanR_rev | AATGAGGCTAGTGCGCATCG | Verification of *nanR* deletion |
| IBA_nanR_fwd | GCTTCA*GGATCC*ACGACAGTCGATATGATCAG | pASK_IBA3_*nanR*, *Bam*HI, probe control |
| IBA_nanR_rev | GCTTA*CTGCAG*GTGCGCATCGAGCATGTTGG | pASK_IBA3_*nanR*, *Pst*I, probe control |
| OE_nanR_fwd | GCTA*CCTGCAGG*ACGACAGTCGATATGATCAG | pEKEx2-*nanR*, *Sbf*I |
| OE_nanR_rev | CTCA*GGATCC*AATGAGGCTAGTGCGCATCG | pEKEx2-*nanR*, *Bam*HI |
| PsiaE_fwd | GCTC*ATGCAT*ACCTGCAAGTTTCTAGACGG | pEPRI-P*siaE*, *NsiI* |
| PsiaE_rev | GCT*GGATCC*GGTTGCTCGGAGGAAATTGC | pEPRI-P*siaE_BamHI* |
| PnanH_fwd | GCC*TCTAGA*TTCCACGCCGGTCTTCCTAC | probe PnanH, pEPRI-P*nanH*, *Xba*I |
| PnanH_rev | CGC*GGATCC*TTATTCGCTGATGCTTGCTGTTG | probePnanH, pEPRI-P*nanH*, *Bam*HI |
| ptsG-probe-fw | CAAACTGACGACGACATC | *ptsG* probe for dot blot analyses |
| ptsG-probe-T7-rv | GGGCCCTAATACGACTCACTATAGGGTGGCAGGAAGTAGAAGAC | *ptsG* probe for dot blot analyses |
| 16S-probe-fw | GAATTCGATGCACCGAGTGGAAGT | 16S RNA gene probe for dot blot analyses |
| 16S-probe-T7-rv | GGGCCCTAATACGACTCACTATAGGGGGTACCGAACCAGTGTGGCACATC | 16S RNA gene probe for dot blot analyses |
| PnanA_fwd | GGTA*GGATCC*GATCACGCCGGTGAAAGTTG | pEPRI-P*nanA*, *Bam*HI |
| PnanA_rev | GGTA*ATGCAT*TAGGACGTATGATGTCTTATG | pEPRI-P*nanA*, *NsiI* |
| PnanR-fwd | GCC*ATGCAT*AGGCCGTGAGTTGAATTGTG | pEPRI-P*nanR*, *NsiI* |
| PnanR_rev | GCG*GGATCC*TTAGTCGCGCACTGCTTCTTGAG | pEPERI-P*nanR*, *Bam*HI |

[a] Restriction sites in the oligonucleotides are shown in italics and T7 polymerase stat sites are underlined

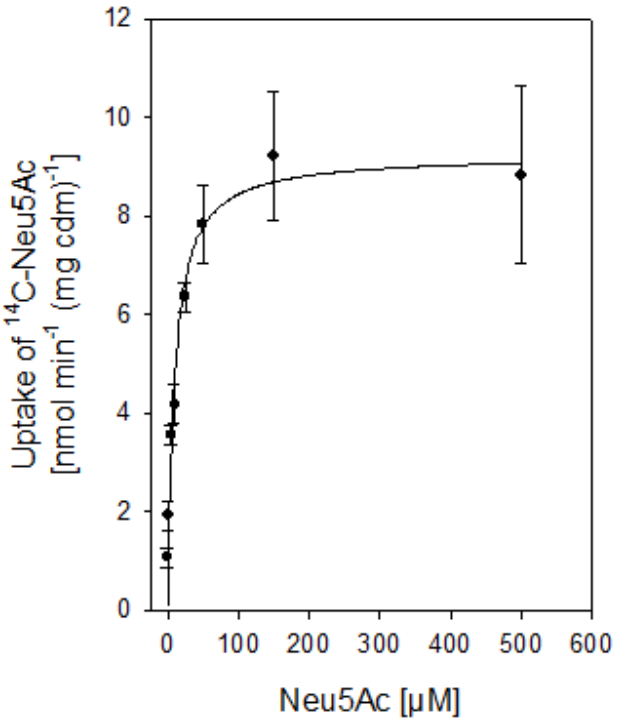



**Fig. S1:** Neu5Ac uptake of *C. glutamicum* WT cultivated in minimal medium with 0.2 % (w/v) Neu5Ac, different concentrations (0.5 - 500 µM) of [$^{14}$C]-Neu5Ac were tested. Data represent mean values of three independent measurements from 2 independent cultivations and were fitted according to the Michaelis-Menten equation.

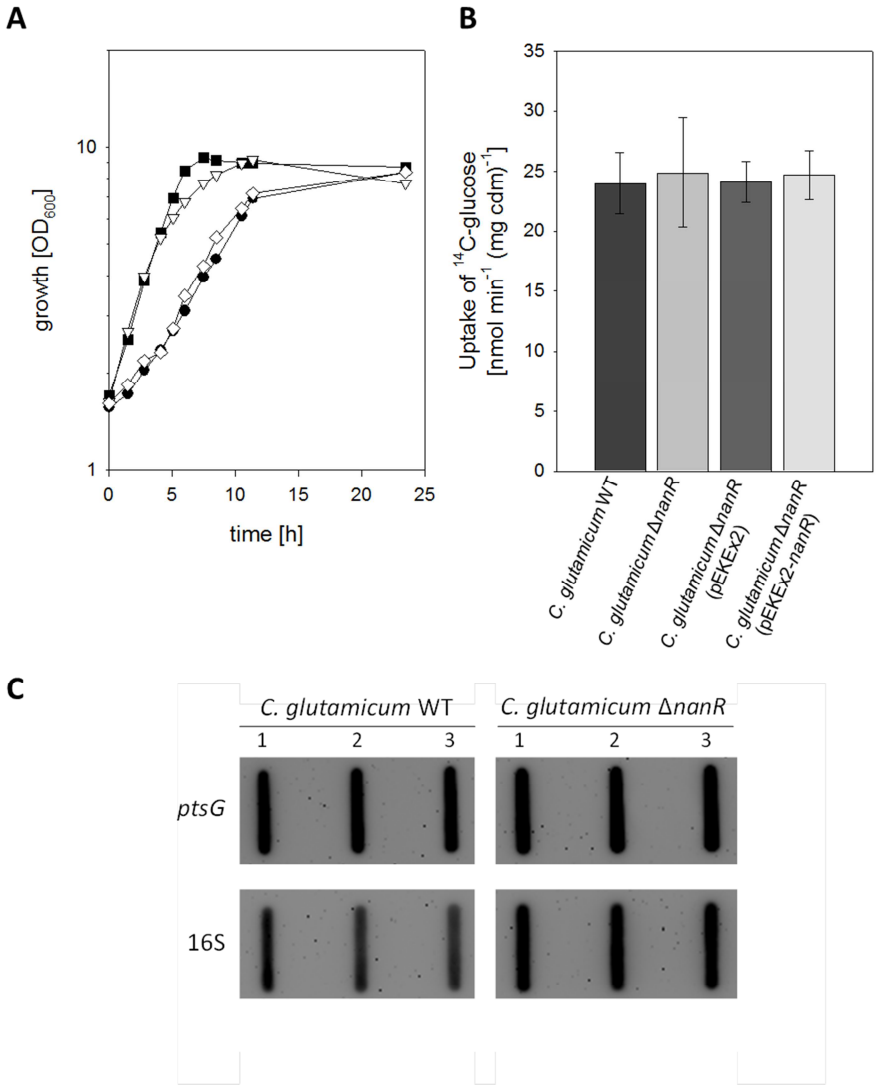



**Fig. S2:** Growth of *C. glutamicum* WT [black squares], *C. glutamicum* Δ*nanR* [black circles], *C. glutamicum* Δ*nanR* (pEKEx2) [white diamonds], and *C. glutamicum* Δ*nanR* (pEKEx2-*nanR*) [white triangles] in minimal medium with 1 % (w/v) glucose (A). Three independent cultivations were performed; data from one representative experiment are shown, results of each of the cultivations were comparable. Analyses of [$^{14}$C]glucose uptake in *C. glutamicum* WT, *C. glutamicum* Δ*nanR, C. glutamicum* Δ*nanR* (pEKEx2), and *C. glutamicum* Δ*nanR* (pEKEx2-*nanR*) (B) after cultivation in TY complex medium. The glucose uptake data represent mean values and standard deviations of three independent measurements from three independent cultivations. Analyses of *ptsG* transcription (C) in *C. glutamicum* WT and *C. glutamicum* ΔnanR. For each strain samples from three independent cultivations in minimal medium with 1 % (w/v) glucose were analyzed. The *ptsG* and 16S RNA levels were monitored in the RNA hybridization experiments with DIG-labeled antisense RNA probes.

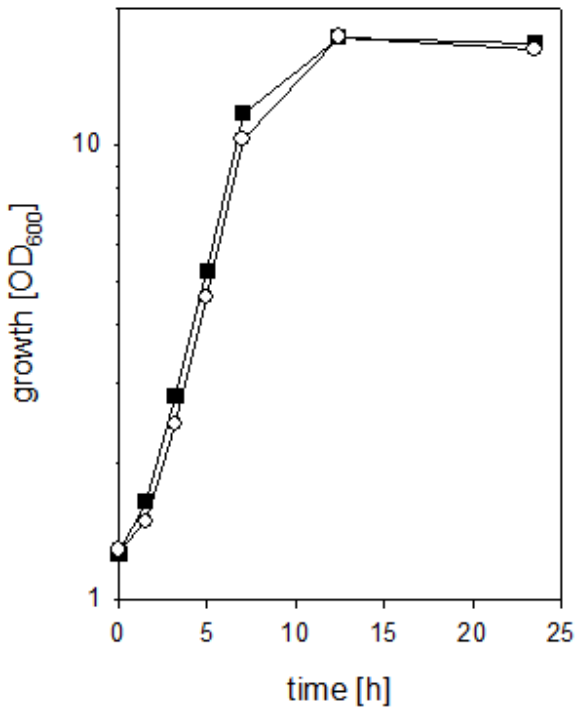



**Fig. S3:** Growth of *C. glutamicum* WT [black squares] and *C. glutamicum* Δ*nanR* [white diamonds] in minimal medium with 1 % (w/v) fructose. At least three independent cultivations were performed; data from one representative experiment are shown, results of each of the cultivations were comparable.

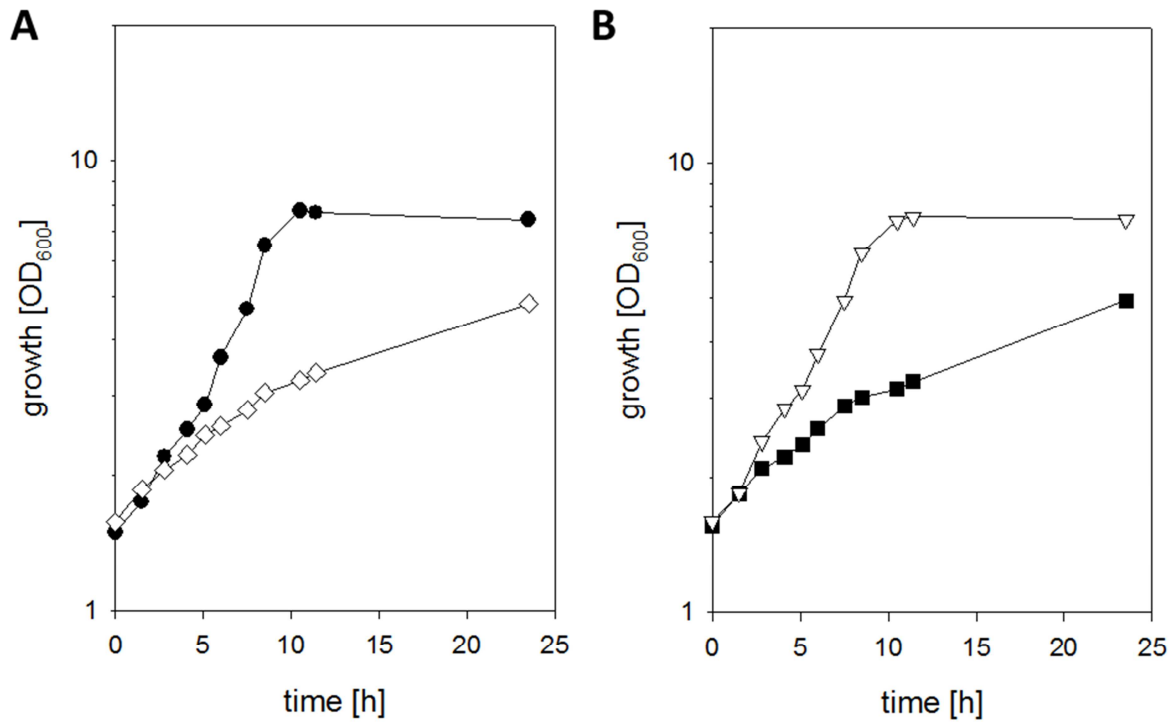



**Fig. S4.** Growth in minimal medium with 0.5 % (w/v) glucosamine as sole source of carbon and energy of *C. glutamicum* WT [black circles] and *C. glutamicum* Δ*nanR* [white diamonds] (in panel A) as well as *C. glutamicum* Δ*nanR* (pEKEx2-*nanR*) [white triangles] and *C. glutamicum* Δ*nanR* (pEKEx2) [black squares] (in panel B). Three independent cultivations were performed; data from one representative experiment are shown, results of each of the cultivations were comparable.

**Table S3.** Gene expression differences between *C. glutamicum* WT and *C. glutamicum* Δ*nanR* during exponential growth in LB medium. This table lists genes that showed statistically significant ($P < 0.05$) expression changes by at least a factor of four. For operons, all genes are listed.

| Gene[a] | Annotation[b] | mRNA[c] (ΔnanR/WT) |
|---|---|---|
| cg0809 | septumformationproteinMaf-likeprotein | 0.14 |
| cg0810 | conservedhypotheticalprotein | 0.23 |
| cg0811 | AccD2, acetylCoAcarboxylase□-subunit | 0.16 |
| cg0814 | BirA, biotinproteinligase | 0.19 |
| cg0815 | putative membrane protein | 0.19 |
| cg0828 | putativedihydrofolatereductase | 0.16 |
| cg0829 | hypothetical protein | 0.13 |
| cg0830 | putative membrane protein | 0.12 |
| cg1139 | Allophanate hydrolase subunit 2 | 0.15 |
| cg1140 | Allophanate hydrolase subunit 1 | 0.16 |
| cg1141 | putative LamB-family lactam utilization protein | 0.19 |
| cg1142 | Na+/proline, Na+/panthothenatesymporter | 0.31 |
| cg3226 | putative L-lactate permease | 0.16 |
| | | |
| cg0043 | ABC transporter ATP-bindingprotein | 19.7 |
| cg0395 | hypotheticalprotein | 4.9 |
| cg0759 | PrpD2, methylcitratedehydratase | 14.9 |
| cg0760 | PrpB2, methylisocitratelyase | 4.0 |
| cg0762 | PrpC2, methylcitratesynthase | 8.5 |
| cg0796 | PrpD1, methylcitratedehydratase | 1.4 |
| cg0797 | PrpB1, methylisocitratelyase | 4.0 |
| cg0798 | PrpC1, methylcitratesynthase | 1.5 |
| cg1295 | putativealpha/beta superfamilyhydrolase/acyltransferase | 6.1 |
| cg1420 | GatB, aspartyl/glutamyl-tRNAamidotransferasesubunit B | 9.9 |
| cg1590 | putativesecretedmagnesiumchelatasesubunit | 6.5 |
| cg1612 | putativeacetyltransferase | 6.1 |
| cg2430 | hypotheticalprotein | 4.0 |
| cg2479 | hypotheticalprotein | 14.0 |
| cg2623 | PcaI, acyl-CoA:3-ketoacid-coenzyme A transferasesubunit | 4.3 |
| cg2746 | putativetranscriptionalregulator | 6.5 |
| cg2917 | conservedhypotheticalprotein | 6.1 |
| cg2927 | ScrB, sucrose 6-phosphate hydrolase | 2.0 |
| cg2928 | NagB, glucosamine-6-phosphate deaminase | 10.6 |
| cg2929 | NagA1, N-acetylglucosamine-6-phosphate deacetylase | 337.8 |
| cg2931 | NanA, N-acetylneuraminatelyase (aldolase) | 147.0 |
| cg2932 | NanK, N-acetylmannosaminekinase | 168.9 |
| cg2933 | NanE, N-acetylmannosamine-6-phosphate 2-epimerase | 97.0 |
| cg2937 | dipeptide/oligopeptideABC transport system, substrate-binding lipoprotein | 9.9 |
| cg2938 | dipeptide/oligopeptideABC transport system, permease subunit | 78.8 |
| cg2939 | dipeptide/oligopeptideABC transport system, ATPase subunit | 25.2 |
| cg2940 | dipeptide/oligopeptideABC transport system, ATPase subunit | 274.4 |
| cg2942 | putative AsnC-family transcriptional regulator | 7.5 |
| cg3185 | conservedhypotheticalprotein | 4.6 |
| cg3252 | putatitve membrane proteintranslocasesubunit | 4.0 |
| cg3407 | putative membrane protein | 12.1 |
| cg3419 | hypothetical protein | 4.3 |

[a]Genes showing significantly ($P < 0.05$) altered mRNA levels by at least a factor of four are listed based on three independent DNA microarray hybridizations. For operons, all genes are listed. [b]Gene identifiers and annotations are given according to BX927147. [c]The mRNA levels were derived from a three independent cultivations.

**Table S4**. NagA and NagB specific activity in *C. glutamicum* strains during exponential growth in LB medium.

| **Strain** | **NagA [U/mg protein]** | **NagB [U/mg protein]** |
|---|---|---|
| *C. glutamicum* WT | 0.012 ± 0.001 | 0.014 ± 0.008 |
| *C. glutamicum ΔnanR* | 0.453 ± 0.119 | 0.251 ± 0.078 |

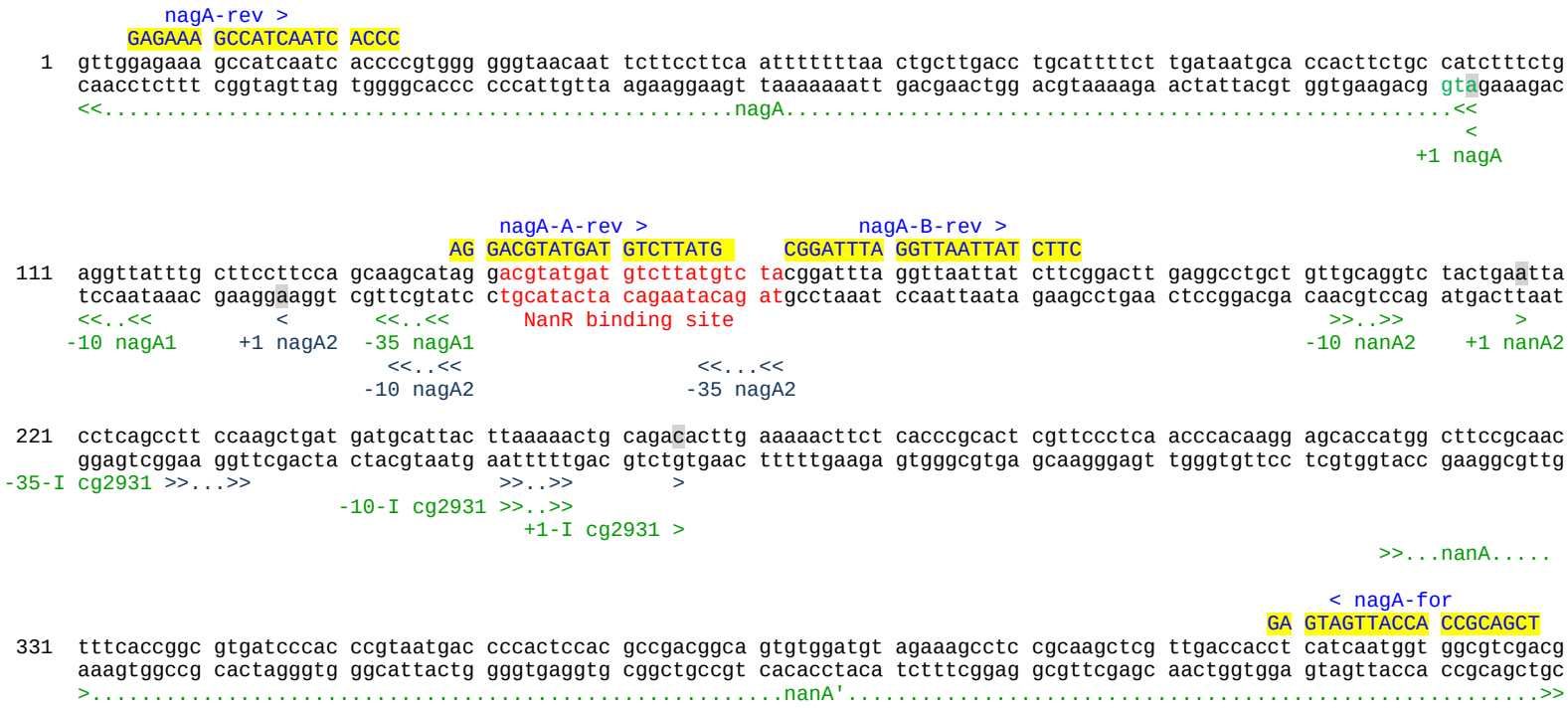



**Fig S5.** Genomic locus of the intergenic region of the *C. glutamicum nagA* and *nanA* genes, localization of primers used for EMSA experiments, the NanR binding site, transcriptional start sites as well as -10 and -35 regions. The transcriptional start sites, -10 regions, and -35 regions are taken from the publication by Pfeifer-Sancar et al., 2013 (14).

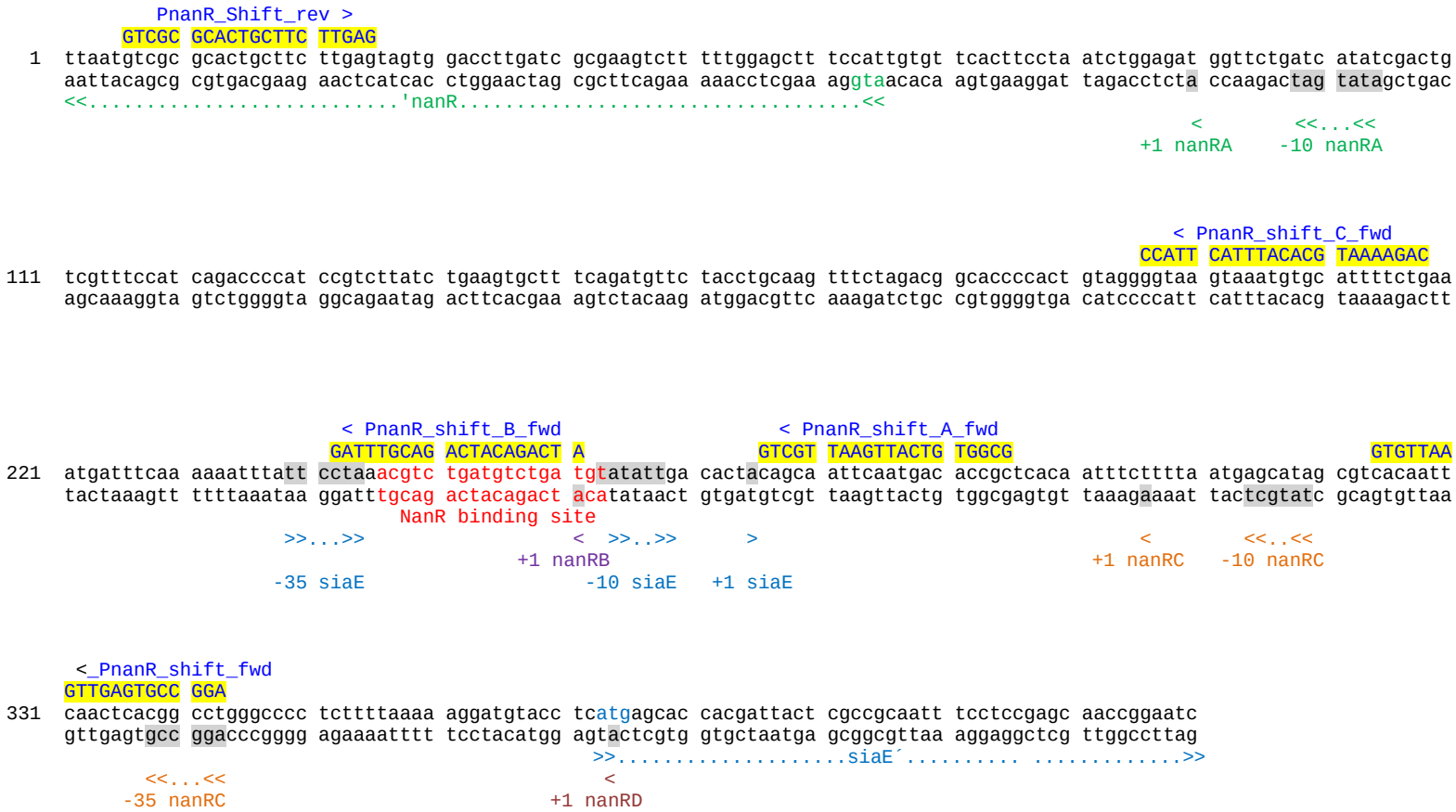



**Fig S6.** Genomic locus of the intergenic region of the *C. glutamicum nanR* and *siaE* genes, localization of primers used for EMSA experiments, the NanR binding site, transcriptional start sites as well as -10 and -35 regions. The transcriptional start sites, -10 regions, and -35 regions are taken from the publication by Pfeifer-Sancar et al., 2013 (14).

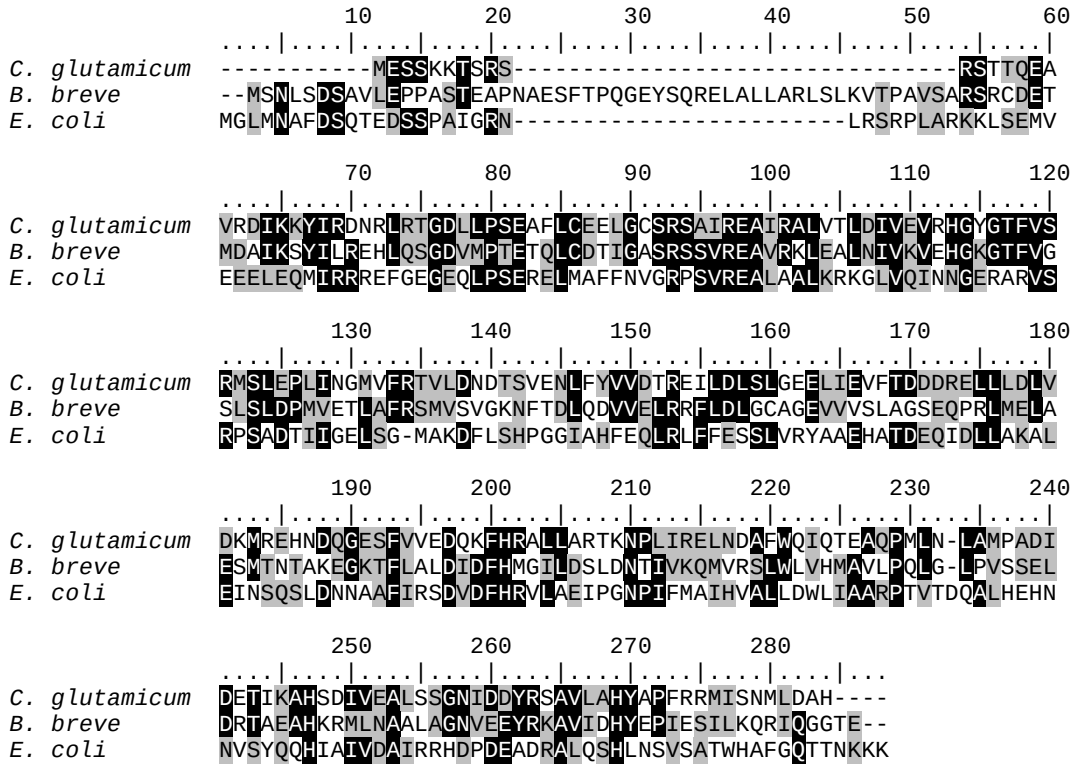



**Fig. S7:** Alignment of NanR sequences of *C. glutamicum*, *E. coli*, and *B. breve,* black shading indicates identical amino acids; grey shading indicates similar amino acids.

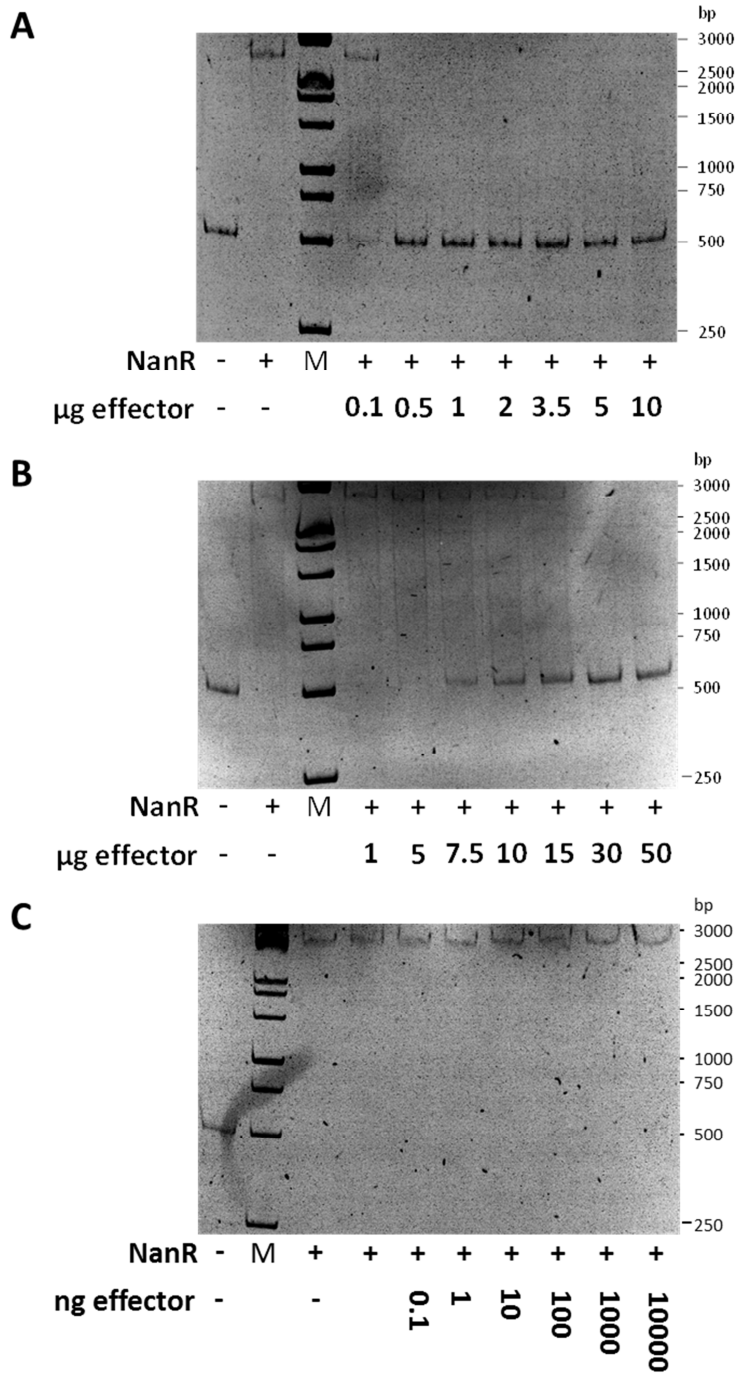



**Fig. S8.** Representative EMSA experiments using 0.3 µg NanR with 10 ng of the probe PnagA and (0 – 10 µg) GlcNAc-6P [A], (0 – 50 µg) ManNAc-6P [B] or [0– 10 µg] Neu5Ac.

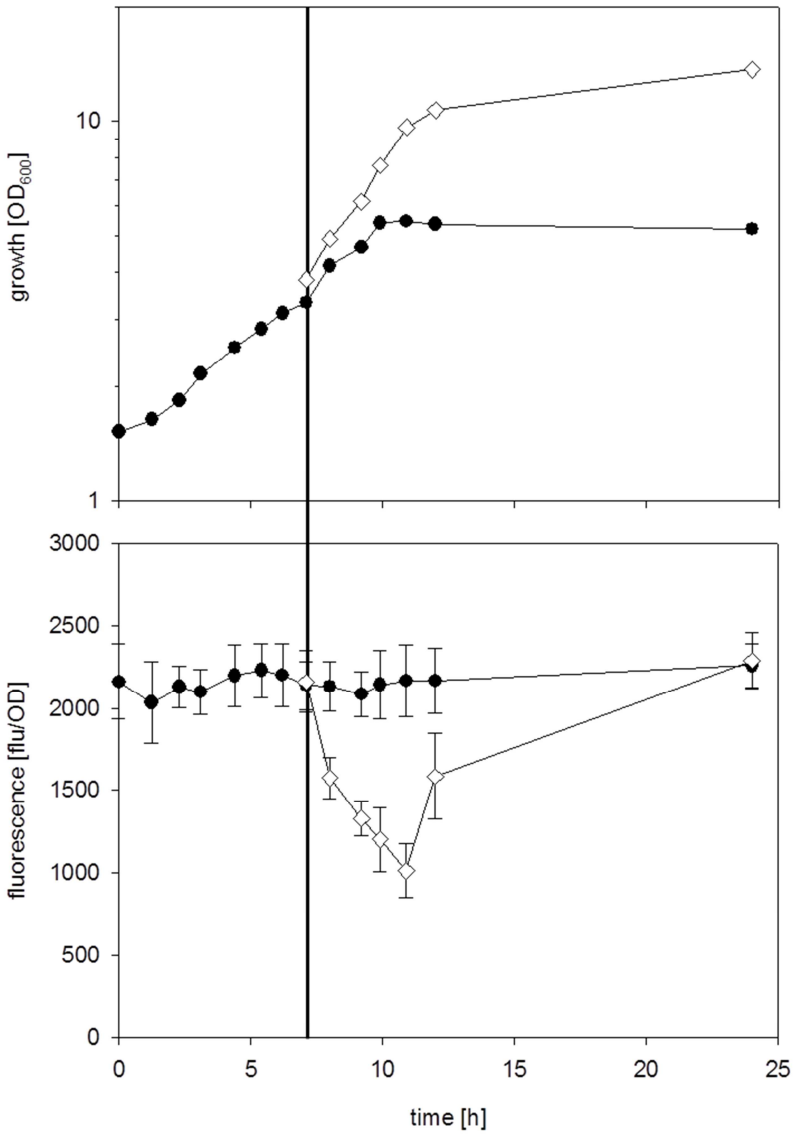



**Fig. S9.** Growth of *C. glutamicum* WT (pEPRI_PRnagAB_WT) and relative fluorescence of the GFP reporter in minimal medium with initially 0.3 % (w/v) Neu5Ac as substrate, after 6 h of cultivation the culture was split into two separate cultures (indicated by the line), of these two cultures one contained additionally 0.3 % (w/v) glucose (white diamonds), whereas no additional carbon source was added to the second culture (black circles). Cells were pre-cultivated in minimal medium with 0.3 % (w/v) Neu5Ac. Three independent cultivations were performed; growth data from one representative experiment are shown, results of each of the cultivations were comparable. GFP fluorescence data represent mean values and standard deviations of two independent measurements each from three independent cultivations.

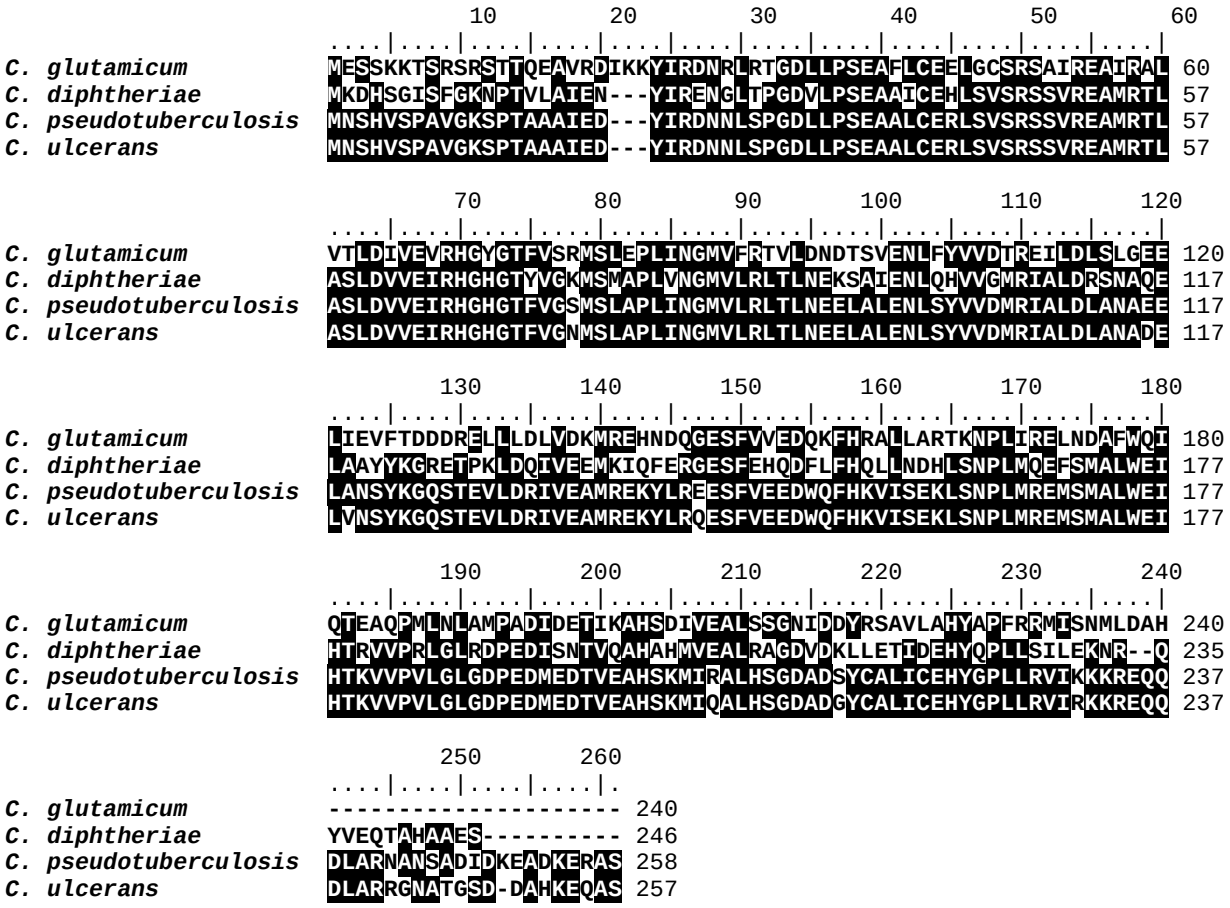
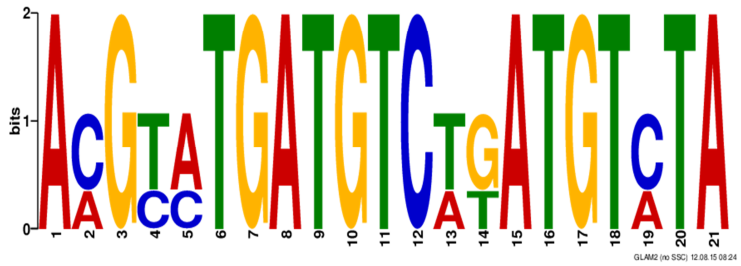



**Fig. S10:** Alignment of (putative) NanR sequences of *C. glutamicum*, *C. diphtheria*, *C. pseudotuberculosis*, and *C. ulcerans*, black shading indicates identical amino acids, grey shading indicates similar amino acids.

**A**




**B**

| | |
|---|---|
| *C. glutamicum nagA-nanA* | ACG TAT GAT GTC TTA TGT CTA |
| *C. glutamicum M4 nagA-nanA* | ACG TAT GAC GTC TTA TGT CTA |
| *C. glutamicum nanR-siaE* | ACG TCT GAT GTC TGA TGT ATA |
| *C. glutamicum nanH* | AAG CAT GAT GTC AGA TGT CTA |

**C**

| | |
|---|---|
| *C. diphtheriae nanR-siaE* | ACG TCT GAT GTC AGG GGT GAA |
| *C. ulcerans nanR-siaE* | ACG TCA GAC GTC TGA TGT ATT |
| *C. pseudotuberculosis nanR-siaE* | ACG TCA GAC GTC TGA TGT ATT |

**Fig. S11:** Sequence logo for the consensus motif of the NanR binding site in *C. glutamicum* generated using Glam2 (15) (A). Sequences of NanR binding site in promoter regions in *C. glutamicum* (B), the nucleotide exchange in the NanR binding site within the *nagA-nanA* intergenic region is indicated in yellow. Sequences of putative NanR binding sites within the intergenic regions of *nanR-siaE* in *C. diphteriae*, *C. ulcerans*, and *C. pseudotuberculosis* identified using FIMO (16) (C).

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