SUPPLEMENTARY MATERIAL (BUSSMANN ET AL.)

TABLE S1
Oligonucleotides used in this study

Oligonucleotide	Sequence (5´ to 3´); restriction sites underlined	Application
Oligonucleotides used for construction of plasmids and verification of deletion mutants		
pET2-1324-for	AATAGGATCCGTCCTTTTCGGTGAAAG TGTG	PCR amplification of *rosR* promoter region for construction of pET2-rosR
pET2-1324-rev	AATAGAGCTCGTGTTGTCAAATCACCT ACCCTAG	
narK-RT-1-for	GGTTTCTGCCATCGCTCCCC	PCR amplification of *narK* fragment used for preparation of a calibration curve for qRT-PCR of *narK*
narK-RT-2-rev	GTTCGGGCGGGCGTAGTAGA	
D1-1124	GGTGTTCTAGATGAATTCACC	Primers for construction of pK19mobsacB-ΔrosR for the chromosomal deletion of the *rosR* gene
D2-1124	CCCATCCACTAAACTTAAGTATTCAGT GGAGAGCCATCGTG	
D3-1124	TACTTAAGTTTAGTGGATGGGCTCACC GCAGTGTTGAACTC	
D4-1124	GCGATTTTCCCGGGATGGG	
D1124-fw	GGAAGCGGTCTTGATAACAACC	Primers used for verification of the *rosR* deletion by colony PCR
D1124-rv	GGTGTCGCGCTGCTTCGCC	
D1-cg1322	AATTCTAGAGCATTTTCGGGTTGATTT CC	Primers for construction of pK19mobsacB-Δcg1322 for the chromosomal deletion of the cg1322 gene
D2-cg1322	CCCATCCACTAAACTTAAGTAGACGG CTCCGCAATCAAGG	
D3-cg1322	TACTTAAGTTTAGTGGATGGGGAGGGT CCAGGTGCCAGTC	
D4-cg1322	AATCCCGGGGTTCTTGGCACATGTCTCG	
Dcg1322-fw	GGTTCTACTGCGGTCCCAAT	Primers used for verification of the cg1322 deletion by colony PCR
Dcg1322-rv	CAATTCCCGTTCTACGCAG	
1124-NdeN	GGTAGGTCATATGACAACACC	PCR amplification of *rosR* for construction of pET16b-rosR
1124-XhoC	TAAAAACCCTCGAGAAAACGC	
pET28-10HisTEV-fw	GACCCATGGGCAGCAGCCATCATCATCATCA TCATCATCATCATCATGATTATGATATTCCG	Construction of pET-TEV
pET28-10HisTEV-rv	GACCATATGGCCCTGAAAATACAGGTTCTCG GTGGTCGGAATATCATAATCATGATG	
Oligonucleotides used for qRT-PCR		
narK-RT-3-for	GTGGCTTCTCACACTCGCTG	primers used for qRT-PCR of *narK*
narK-RT-4-rev	CTGAATTATCGAGACGCCGAG	
narG-RT-1-for	CACCGTCGGTTCTGAAGGCC	PCR amplification of *narG* fragment used for preparation of a calibration curve for qRT-PCR of *narG*
narG-RT-2-rev	CGGTGCCTTCAGGAATGCGG	
narG-RT-3-for	GCGCTGACCTCTATGTGTGG	primers used for qRT-PCR of *narG*
narG-RT-4-rev	CAGTGGTGATCATCTGACGTG	

gluS-1-for	CGTCATCCGATTGGGCAGGC	PCR amplification of cg1426 fragment used for preparation of a calibration curve for qRT-PCR of cg1426
gluS-2-rev	CTTCGCCCGCAGGAATTGGG	
gluS-3-for-LC	CGCGGCATTACTGTCCCAGC	primers used for qRT-PCR of cg1426
gluS-4-rev-LC	CTCCGCGGGGTAGAGGTTAG	
2329-1-for	GTCTCCTTCGGCCTCGACAC	PCR amplification of cg2329 fragment used for preparation of a calibration curve for qRT-PCR of cg2329
2329-2-rev	GCCTGCTTGGTCAAGATGTCC	
2329-3-for-LC	CTCGACACCTTCGGCGACAAC	primers used for qRT-PCR of cg2329
2329-4-rev-LC	GTATTCCTCACGGTGGTGCTC	

Oligonucleotides for PCR amplification of promoter fragments used for EMSAs

NR-Prom2-for	AGGCCCCACGCCCGTTCCGCCG	PCR amplification of the *narK* promoter
NR-Prom2-rev	CGCCATGCAATTTTCGAGTCCC	
NR-Prom1-for	GACTTATCCAGCTGAGATGCTTC	PCR amplification of parts of *narK* and *narG* as negative control
NR-Prom1-rev	AACCGACGGTGCCTTTTCTTAGG	
NR-Prom2-for	AGGCCCCACGCCCGTTCCGCCG	PCR amplification of *narK* promoter fragment A
nr-2-E-rev	GTTGTGTCACGTGATGCCTC	
NR-Prom2-for	AGGCCCCACGCCCGTTCCGCCG	PCR amplification of *narK* promoter fragment B
nr-2-A-rev	CATTCACCGTCGTGGAGGAG	
nr-2-B-for	CTCCTCCACGACGGTGAATG	PCR amplification of *narK* promoter fragment C
nr-2-B-rev	GTCATCGTTGTGTCTGATCG	
nr-2-C-for	CGATCAGACACAACGATGAC	PCR amplification of *narK* promoter fragment D
nr-2-C-rev	CGTGATTCGGCAAAATTAATTAAACTG	
nr-2-D-for	CAGTTTAATTAATTTTGCCGAATCACG	PCR amplification of *narK* promoter fragment E
nr-2-D-rev	CCCGCATGTTTTTCAACGGG	
nr-2-E-for	CCCGTTGAAAAACATGCGGG	PCR amplification of *narK* promoter fragment F
nr-2-E-rev	GTTGTGTCACGTGATGCCTC	
nr-2-C-for	CGATCAGACACAACGATGAC	PCR amplification of *narK* promoter fragment G
nr2-x1	CGAATTTGTGCTTATATCAACATTCGTG	
nr2-x1	CGAATTTGTGCTTATATCAACATTCGTG	mutation of the *narK* promoter region
nr2-mut1	CGAAAAAGTGCTTATATCAACATTCGTG	
nr2-mut2	CGAATTTCACCTTATATCAACATTCGTG	
nr2-mut3	CGAATTTGTGGAAATATCAACATTCGTG	
nr2-mut4	CGAATTTGTGCTTTATTCAACATTCGTG	
nr2-mut5	CGAATTTGTGCTTATAAGTACATTCGTG	
nr2-mut6	CGAATTTGTGCTTATATCATGTTTCGTG	
nr2-mut7	CGAATTTGTGCTTATATCAACAAACGTG	

cg1324-for	GCCCTTAACGTGTGCATCGC	PCR amplification of *rosR* promoter region
cg1324-rev	GAGTTGTTGCTCTTCAGTGGAG	
cg1324-for	GCCCTTAACGTGTGCATCGC	PCR amplification of RosR binding site a in *rosR* promoter for K_D evaluation
cg1324_Bind1_rev	ATTCACGCCTCCACCTGCGGG	

cg1324-f2-ohne	ATTTCTAAAAAACTTGATGTGGAAAC	PCR amplification of RosR binding site b in *rosR* promoter for K_D evaluation
rev-3	GCTGACCCGTTGCCTCAG	
NCgl0971-for	CAAATGCGTTGAACAGGATTTTCCG	PCR amplification of cg1150 promoter, K_D determination
NCgl0971-rev	GATGATGGCGATCTTGCTC	
glut-S_for	GTCGCGTTGCAGGATGTCGTTG	PCR amplification of cg1426 promoter region
glut-S_rev	GGATCCCGCTGGAACGTCTG	
gluSt- -Mot-fw	CCAAGAGAGGACACCACACAG	PCR amplification of cg1426 promoter without RosR binding site
gluSt+ Mot-fw	GTAATATTGTTGACATATCATCTAAAT TTCC	PCR amplification of cg1426 promoter including RosR binding site
cg1426_Fr2_fw	CCACTTCGTAATATTGTTGACATATCATC	PCR amplification of cg1426 promoter for K_D evaluation
glut-S_rev	GGATCCCGCTGGAACGTCTG	
cg1426_Fr3_fw	CACGTCTGCAATCACCCCGAAC	Further fragmentation of cg1426 promotor
cg1426_Fr4_fw	ACTGCAGCCACGGCAATCG	
cg1426_Fr5_fw	GCACGGTATTCAGCCGCAG	
cg1426_Fr1_rv	CGAAGTGGAATGAAAATCAACGATTG	
NCgl1580-for	GGGAACGGAATACGTGGCAC	PCR amplification of cg1848 promoter
NCgl1580-rev	CTTCGGTGGGGGCTTTGCCG	
NCgl1580-for	GGGAACGGAATACGTGGCAC	PCR amplification of RosR binding site 1 in cg1848 promoter for K_D evaluation
cg1848-Fr1B-rv	CTAGCAGTTTTTAATGGATGCGTC	
cg1848_Fr2D_fw	AACTGCTAGTTTGGTGACATATCAAC	PCR amplification of RosR binding site 2 in cg1848 promoter for K_D evaluation
cg1848-rv-B	AATATAGGCTAAGTGTGTGGTTGG	
coe420-for	CGACGAAGACCTGGTCTCATG	PCR amplification of cg2329 promoter
coe420-rev	CGAAGTGTTTGTGCAGGGG	
Coe420--Mot-fw	CTTTTAATAAGTCTGATCAACAACGTG	PCR amplification of cg2329 promoter without RosR binding site
Coe420 + Mot-fw	CCCCATATTGTTGATATATCTACAAAC	PCR amplification of cg2329 promoter including RosR binding site
coe420-rev	CGAAGTGTTTGTGCAGGGG	PCR amplification of cg2329 promoter for K_D evaluation
cg2329-Fr2-fw-B	CACCGCCAAATGAGAACACC	
NCgl2686-for	CGATCCGTTCGGTTGGCAGAC	PCR amplification of cg3084, promoter, K_D determination
NCgl2686-rev	GTCCAGCCTGACCTGCTCC	
NCgl2826-for	GCGCCTCATCAGCGGTAACC	PCR amplification of cg3237 promoter, K_D-determination
NCgl2826-rev	GCTCGAGAGCGTCGTATGCG	

Oligonucleotides used for site-directed mutagenesis of RosR

c1-for	GCGCCTGCGAGACATGTCCCAAGAAC TAGATTGGGAC	Primers for site-directed mutagenesis of RosR-Cys64 to Ser using pET16b-rosR as template
c1-rev	GTCCCAATCTAGTTCTTGGGACATGTC TCGCAGGCGC	
c2-for	CTTAGTGGCCAAGGTTAAATCCGCAG GTGACGCACGAGG	Primers for site-directed mutagenesis of RosR-Cys92 to Ser using pET16b-rosR as template
c2-rev	CCTCGTGCGTCACCTGCGGATTTAAC CTTGGCCACTAAG	
c3-for	GTTGAACTCCAACACCTCCATTGAGA TCAACAACCAAC	Primers for site-directed mutagenesis of RosR-Cys151 to Ser using pET16b-rosR as template
c3-rev	GTTGGTTGTTGATCTCAATGGAGGTGT TGGAGTTCAAC	
Oligonucleotides used for primer extension and 5'-RACE experiments		
IRD800-cg1322-1	CTTGATTTCGGTGTGTGCAGGGTC	Oligonucleotides for primer extension of cg1322
IRD800-cg1322-2	CCTTGGTAACCATTGCGTGGCGAGC	
IRD800-cg1426-1	CGTCTGCTGATGCATTTTGTGGGGC	Oligonucleotides for primer extension of cg1426
IRD800-cg1426-2	CATCGATGTAGTTGGTATCGCGAACGAA	
IRD800-cg3084-1	CCGCGAGTCCAGCCTGACCTGCTCC	Oligonucleotides for primer extension of cg3084
IRD800-cg3084-2	GTGAAACCGCGGCGGGAAAGTTCATG	
cg1322-A	GTGGTCAGGTTGTGGTTTTC	Oligonucleotides for primer extension of cg1322
cg1322-B	GTGTACTCGGTGAATTCACC	
cg1426-A	GTCGCGTTGCAGGATGTCGT	Oligonucleotides for primer extension of cg1426
cg1426-B	GAACGTCTGCGACGATGCG	
cg3084-A	CGATCCGTTCGGTTGGCAGA	Oligonucleotides for primer extension of cg3084
cg3084-B	GAGGACGAGAAAATCTTTTCC	
cg1324-sp1	GAAAGAGTAACTAGTACTGC	Primers for 5´ RACE of *rosR*
cg1324-sp2	ATTCTGAAGTGGTCAGGTTG	
cg2329-sp1	CTCCGATGCCGATGATATCC	Primers for 5´ RACE of cg2329
cg2329-sp2	CCCCGACTTTGTCTGCCATC	
cg1150-sp1	CGTCGAGGACGGGGAAGTTG	Primers for 5´ RACE of cg1150
cg1150-sp2	AATCGGCGATATCAACGAGC	
cg1848-sp1	GTGCTCGCCGGTGGCAAAGAC	Primers for 5´ RACE of cg1848
cg1848-sp2	CATCGAGGCCTACTTCTTCAG	

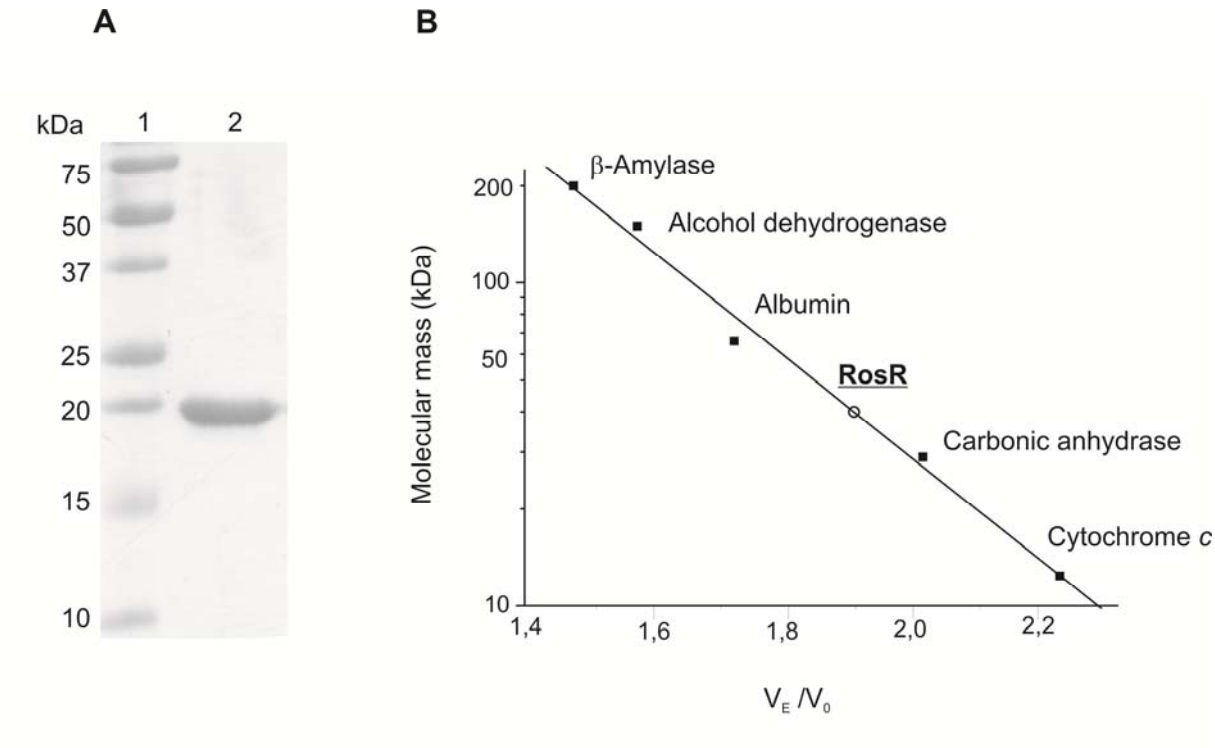


Fig. S1. Purification of RosR and determination of its native mass. (A) Coomassie-stained SDS-polyacrylamide gel showing RosR-His (lane 2) after purification by Ni^{2+}-NTA affinity chromatography from *E. coli* BL21(DE3)/pLysS carrying the expression plasmid pET16b-RosR. Lane 1 contains protein molecular mass standards. (B) Result of size exclusion chromatography of purified RosR-His using a HiLoad™ 16/60 Superdex™ 200 column (Amersham Biosciences). The column was equilibrated with 20 mM HEPES buffer pH 7.5 containing 500 mM NaCl and elution was performed with the same buffer at 1 ml/min. Calibration was performed with cytochrome *c* (12.4 kDa), carbonic anhydrase (29 kDa), albumin (66 kDa), alcohol dehydrogenase (150 kDa), and β-amylase (200 kDa). V_E, elution volume of the protein, V_0, void volume of the column.

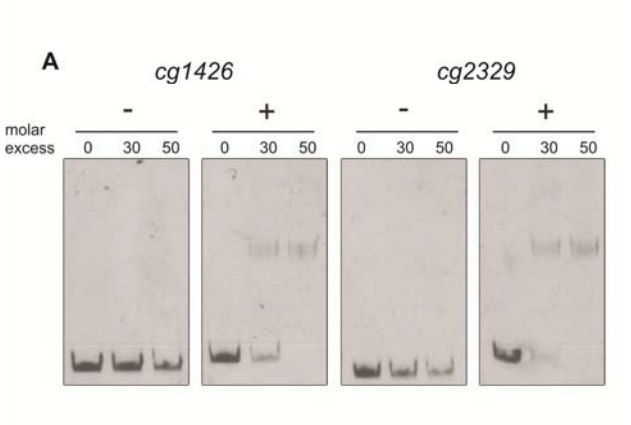
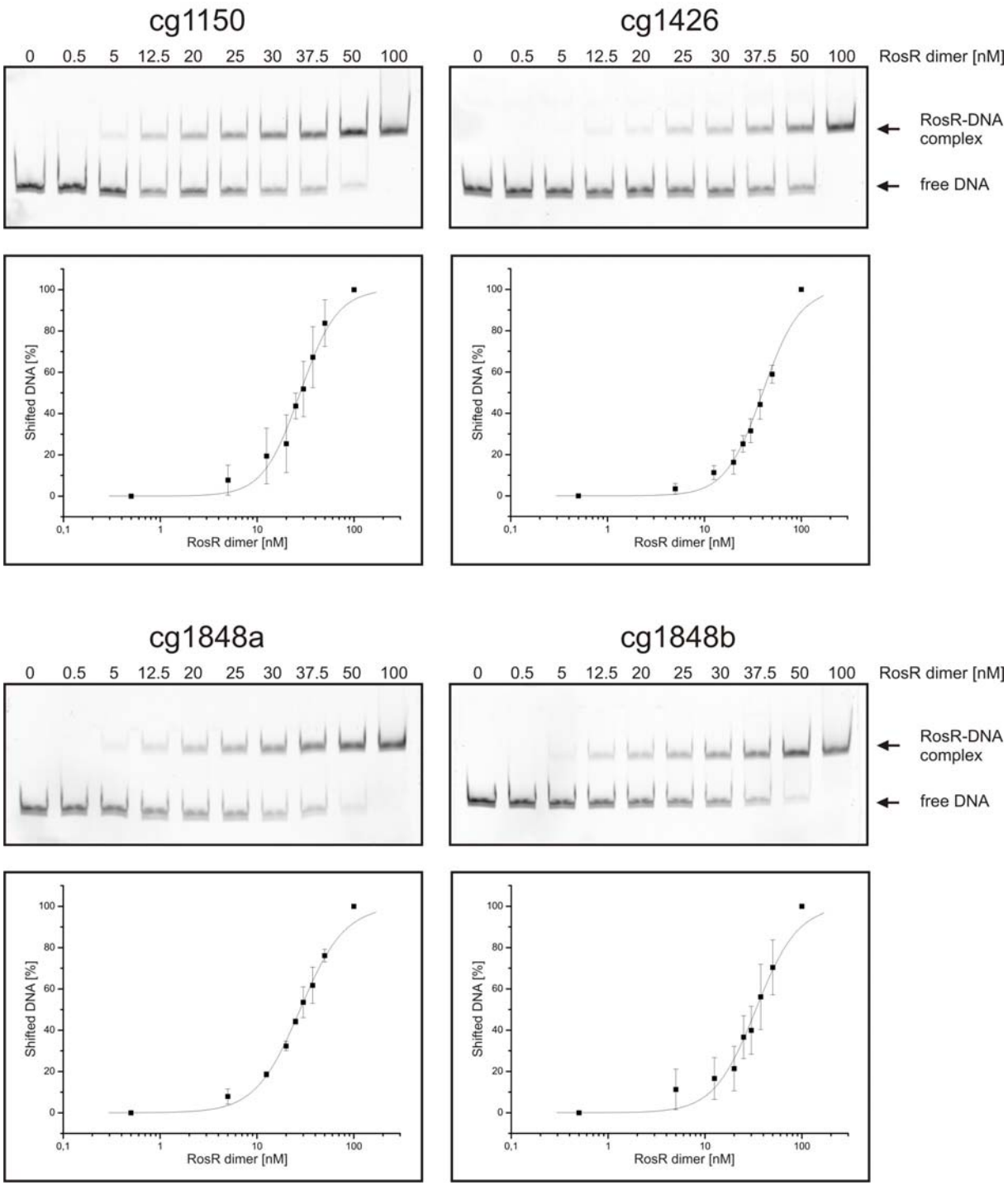


Fig. S2. EMSAs with purified RosR protein and the promoter regions of cg2329 and cg1426. DNA fragments (15 nM) of the cg2329 and cg1426 promoter regions containing (+) or missing (-) the predicted RosR binding site were incubated either without or with 30- or 50-fold molar excess of purified RosR-His dimer (225 nM or 375 nM, respectively).


cg1150
cg1426
0 0.5 5 12.5 20 25 30 37.5 50 100
RosR dimer [nM]
RosR-DNA complex
free DNA
Shifted DNA [%]
RosR dimer [nM]
cg1848a
cg1848b

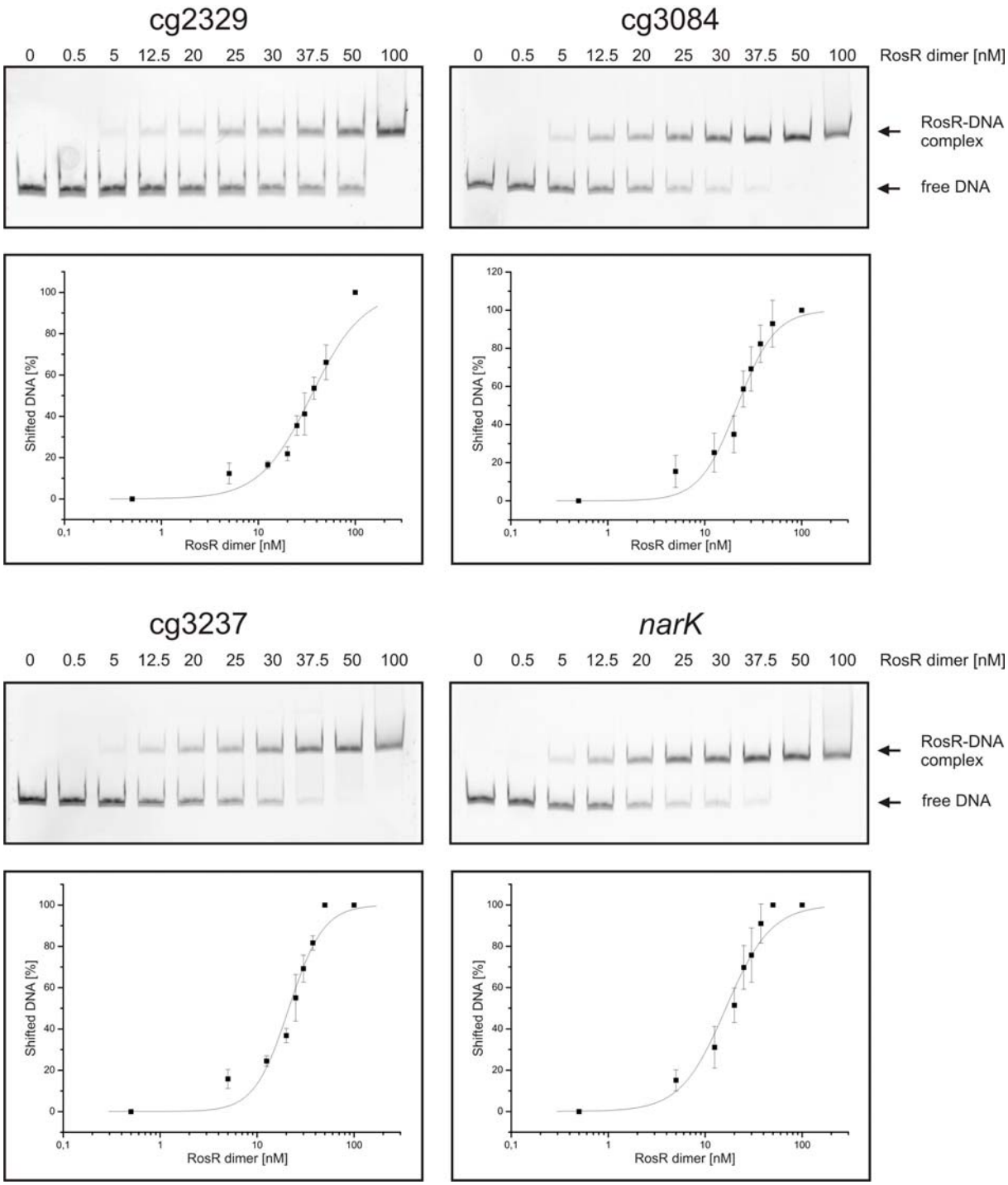


Fig. S3. Determination of the apparent K_D values for RosR-binding sites of all currently known RosR target genes except those for *rosR* itself, which are depicted in Fig. 5. DNA fragments (80 ng), each carrying a single RosR binding site, were incubated with increasing RosR concentrations, resolved on a 10% native polyacrylamide gel and stained with GelRed™. At least three independent gels were performed for each binding site. The bands were quantified using ImageQuant Software (GE Healthcare) and the percentage of shifted DNA was calculated. These values were plotted against the RosR concentration in $\log_{10}$ scale and a sigmoidal fit was performed. The turning point of the curve was defined as apparent K_D value.

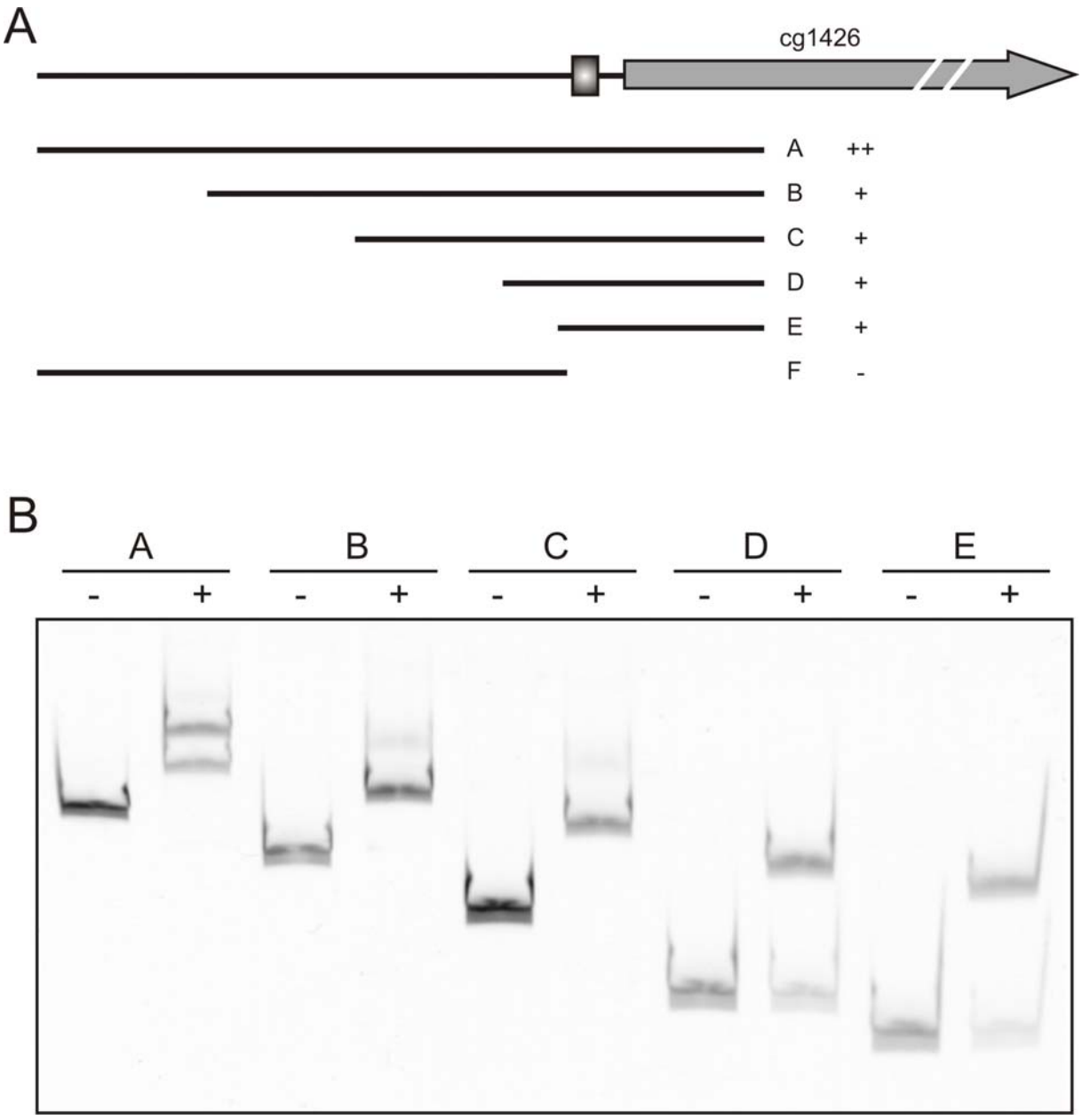


Fig. S4. EMSAs with different cg1426 promoter fragments. A, Scheme of the cg1426 promoter region showing the position of the confirmed RosR binding site (box) and of fragments A-F used for EMSAs. "++" indicates formation of two RosR-DNA complexes, "+" formation of a single RosR-DNA complex and "-" no formation of a RosR-DNA complex. B, EMSA with fragments A-E. 80 ng of each DNA fragment were incubated either without RosR (-) or with 50 nM of RosR dimer (+) for 20 min at room temperature. Then the samples were separated by native polyacrylamide gel electrophoresis (10%) and the DNA was stained with GelRed™.

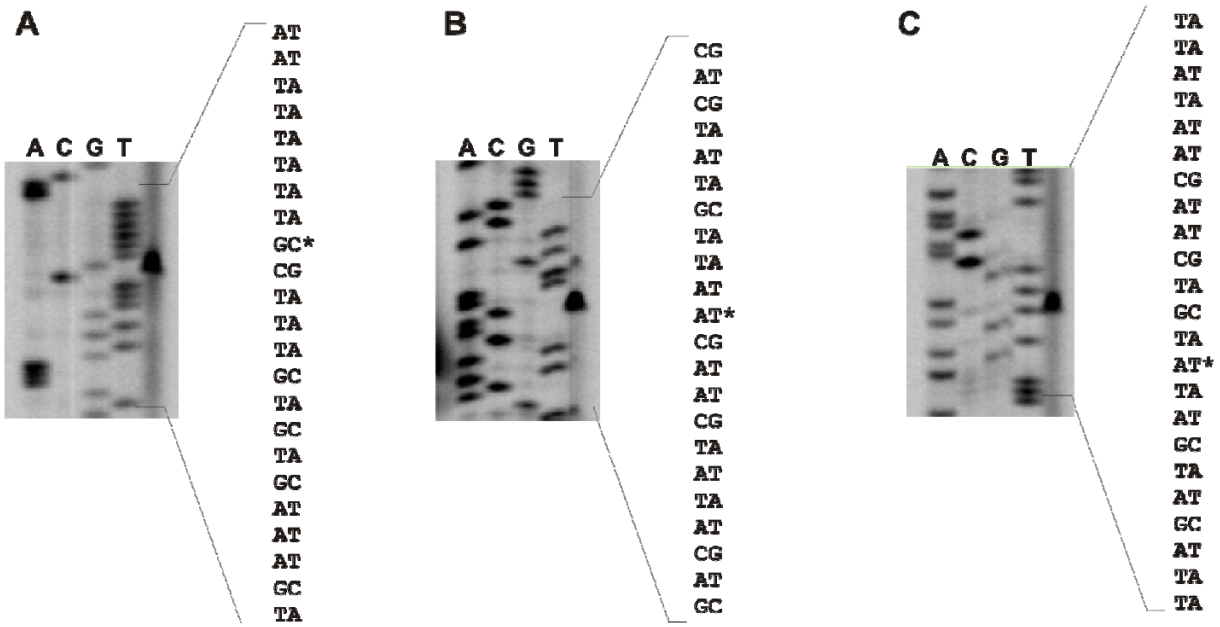


Fig. S5. Determination of the transcriptional start sites of cg1322 (A), cg3084 (B) and cg1426 (C) by primer extension analysis using oligonucleotides cg1322A, cg3084A and cg1426A and total RNA isolated from *C. glutamicum* Δ*rosR* cells grown in glucose minimal medium. The residues marked with the star represent the identified transcription start sites.

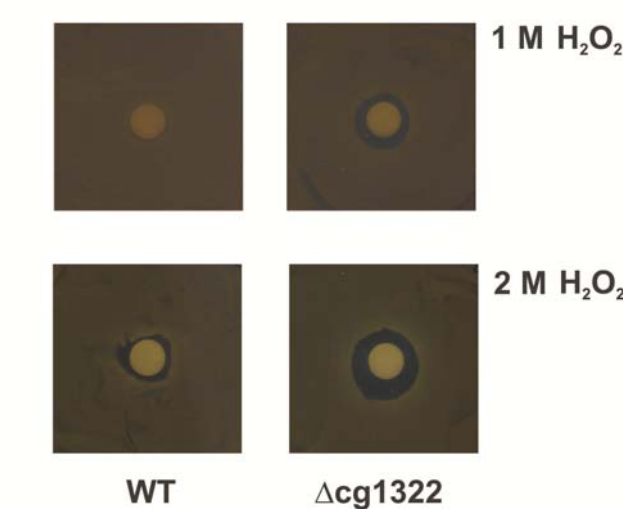


Fig. S6. Agar plate assays showing the increased sensitivity of the Δcg1322 mutant to hydrogen peroxide. A paper disc impregnated with 70 µl of either 1 M or 2 M H_2O_2 was placed on BHI plates containing either Δcg1322 or wild-type cells. The plates were incubated at 30°C for 24 h. Experiments were performed in triplicate starting with independent precultures.

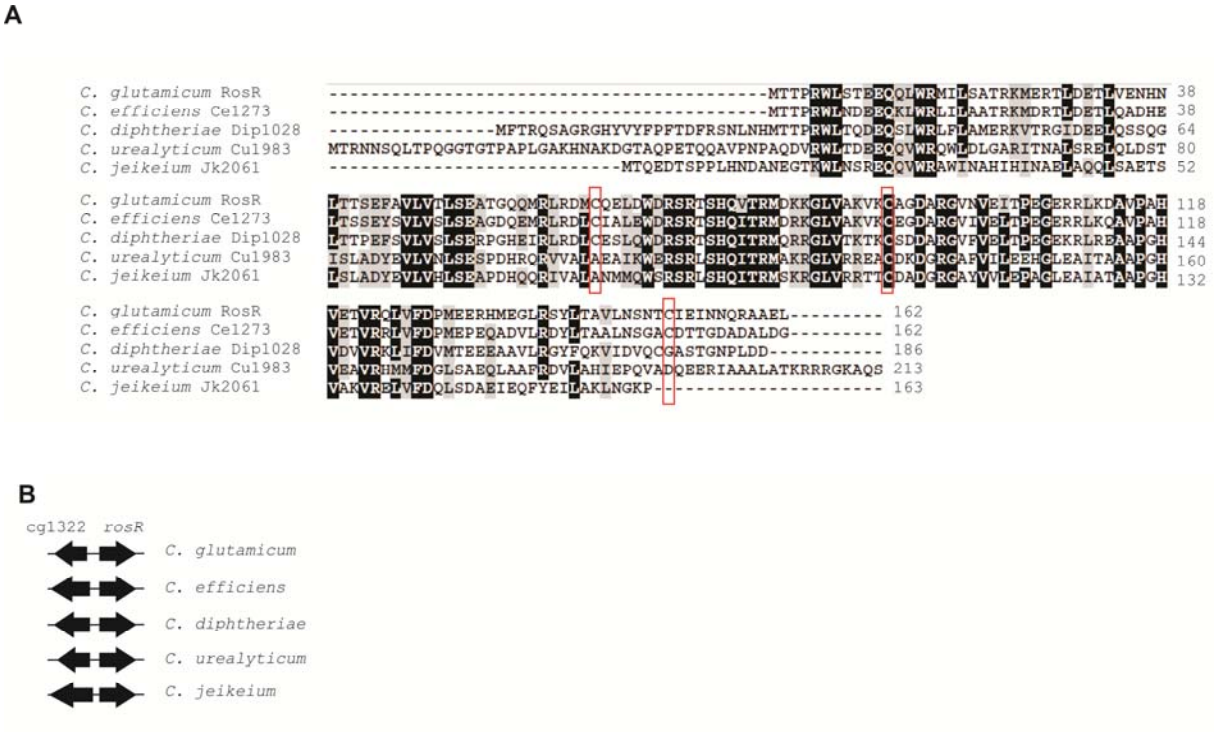


Fig. S7. (A) Amino acid sequence alignment of RosR homologs (cysteine residues marked by red boxes) and (B) genomic organisation of *rosR* and cg1322 homologs in different corynebacteria.