Supplementary Data

Table S1. Number of conserved bases in the *lux* box homologues located upstream of *gtaR* and *orfg1*.

Promoter	***lux* box homologue location**	**No. of bases conserved based on *lux* box sequence proposed by Fuqua (Fuqua et al., 1994)**	**No. of bases conserved based *lux* box consensus sequence proposed by Horng (Horng et al., 2002)**
gtaR	5'-most (in *rplQ* gene)	10 of 20	4 of 8
gtaR	3'-most	13 of 20	7 of 8
orfg1	only one	10 of 20	8 of 8

Table S2. Primers used in this study

Primer Name	**Sequence 5'-3'**	**Construct(s)**	**Restriction Enzyme**
GTA5	GATGCGGCTGCAGACCGATCC	p601-g65	*Pst*I
GTA2.6	GAACCGGATCCATCGCCAGGG	p601-g65	*Bam*HI
LHR2.2	GTGATCTGTCGGATCCTCGGGATAGG	p601-P2R, p601-P25, p601-P23	*Bam*HI
LHR2	ACACTTGTTGGGATCCGTGTGTATG	p601-P1R	*Bam*HI
KOR1F	CGATGAAGGTCGACACTGACGGTT	p601-P2R, p601-	*Pst*I

		P1R	
LHR5	AGACCACCCTGCAGAAAGCCA	p601-P25	*Pst*I
LHR3	TTTCGAAGCTGCAGACGAATAAGC	p601-P23	*Pst*I
PR28n	CTGTCTAAAAACATATGTCCATACACAC	pET28R	*Nde*I
PR28c	CGCTGCGGGATCCTACAG	pET28R	*Bam*HI
gtaR_comp_up	GTATCGGTACCAGCAAAACGACCTTAGGA	pIND4R	*Kpn*I
gtaR_comp_down	GTACAGGATCCGAAAGTGTGGTGGTCTGC AT	pIND4R	*Bam*HI
Primer Name	**Sequence 5'-3'**	**Intergenic Region**	**Restriction Enzyme**
rplQ-gtaR_up	CGACGTTTCGGCGAAGGGCG	*rplq-gtaR*	n/a
rplQ-gtaR_down	CCGCAGACACTTGTTGAT	*rplq-gtaR*	n/a
gtaR-gtaI_up	GCTGCGAAGCTTGGCATCA	*gtaR-gtaI*	n/a
gtaR-gtaI_down	AATGATGAAACTTTGCCGCC	*gtaR-gtaI*	n/a
gtaI-rcc00330_up	GCTGGTATCGCCGCTGC	*gtaI*-rcc00330	n/a
gtaI-rcc00330_down	CCTCGGACAGATGCTGCG	*gtaI*-rcc00330	n/a
Primer Name	**Sequence 5'-3'**	**EMSA Fragment(s)**	**Restriction Enzyme**
LHR2.2	GTGATCTGTCGGATCCTCGGGATAGG	DR9, DR7, DR5, DR3, DR1, P23	*Bam*HI
LHR3	TTTCGAAGCTGCAGACGAATAAGC	P23, DRR	*Pst*I

LHR3	TTTCGAAGCTGCAGACGAATAAGC	345 bp	*Pst*I
KOR1F	CGATGAAGGTCGACACTGACGGTT	800 bp	*Pst*I
LF9	AGGCAATTCCCGGTTGATCTT	DR9	n/a
LF7	TGGCACCTGTCTAAAAAGACATGTCCA	DR7	n/a
LF5	GTCTAAAAAGACATGTCCATACACACCG	DR5	n/a
LF3	ACATGTCCATACACACCGAAATCAA	DR3	n/a
LF1	CCGAAATCAACAAGTGTCTGCG	DR1	n/a
LHRrf	GAAAAGATCAACCGGGAATTG	DRR	n/a
3RPL	GCTTATTCGTCTGCAGCTTCGAAA	R23	n/a
M200R	GCGCAACGCATTTAATG	M1	n/a
Lux-upstream-F	AGACCCATGCGTCACG	*gtaR* 681 bp	n/a
Lux-downstream-R	CCGATGAAATAGCCATCC	*gtaR* 681 bp	n/a

Supplementary material figure legends.

Figure S1. The *gtaR* gene, surrounding orfs, and the sequence of the *ctrA* 5' regulatory region. **A**, Representation of *gtaR* and flanking genes. Annotations are according to Genbank accession # CP001312. From left to right: *rpoA* is annotated as a DNA-directed RNA polymerase subunit alpha; *rplQ* is annotated as the 50S ribosomal protein L17; *gtaR* encodes the GtaR response regulator; *gtaI* encodes a homoserine lactone synthase; and rcc00327 is a predicted protein of unknown function. **B**, DNA sequence of the *gtaR* 5' region, showing key features. Bent arrows

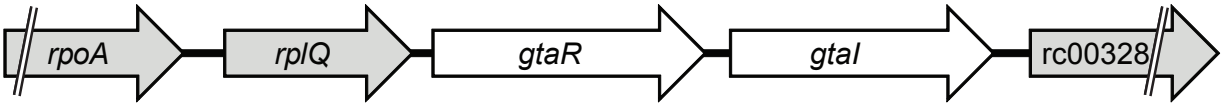
show the *rplQ* and *gtaR* start codons. Green boxes indicate the –35 and –10 sites predicted by the Softberry program BPROM (http://linux1.softberry.com/berry.phtml). Red boxes are the stop codons for *rpoA* and *rplQ*. The black horizontal bars indicate predicted *lux* boxes. An alternative predicted *gtaR* start codon is indicated by a yellow box. The vertical arrow labeled "2.2" indicates the 3' juncture of all *gtaR::lacZ* fusions except p601-P1R, which has a 3' juncture indicated by the vertical arrow labeled "2.1". The vertical arrows labeled with odd numbers and "R" indicate the 5' end of *gtaR* promoter region deletions. Each arrow is identified with a label that corresponds to the plasmid name (*i.e.*, the arrow labeled "5" corresponds to plasmid p601-P25). Numbers on the right indicate the number of bases before or after the *ctrA* start codon.

Figure S2. The GtaR-6xHis protein does not bind to the RcGTA promoter region. **A**, Representation of RcGTA *orfg1* and 5' sequences. The black boxes represent predicted -10 and -35 sites and the empty box is a predicted *lux* box. The bracket indicates the 650 bp DNA fragment used for EMSAs. **B**, EMSA using the GtaR-6xHis protein and the 650 bp DNA fragment. O indicates the origin of eletrophoresis.

Figure S3. Comparison of RcGTA gene expression in the *R. capsulatus gtaRI* mutant in the absence and presence of exogenous acyl-HSLs. **A**, western blots of cells probed with RcGTA capsid protein antiserum; **B**, frequency of RcGTA-mediated gene transfer of *gtaRI* knockout (*ΔgtaRI*) with no addition of acyl-HSL and the addition of C12-, C16- and C18-HSL (C12, C16 and C18). Error bars represent the standard deviation of the mean between samples (n=3), except that * indicates the range between samples (n=2).

Figure S4. An unrooted phylogenetic tree of LuxR homologs in gram-negative bacteria. The branch containing GtaR is encircled with a dashed line, and GtaR is marked with two asterisks. The two additional *R. capsulatus* ORFs with homology to LuxR-type transcriptional regulators are marked with a single asterisk. Members of the previously identified EsaR clade are enclosed within a solid line. Names of α-proteobacteria are in green, β-proteobacteria in blue, and γ-proteobacteria in red. Support values were based on 1000 replicates and the scale bar represents the number of substitutions per site.

A



B

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CGGAATCGGC CTCGAAGACC GATGCCGACG ACGGGCTCGA GTTCAACCCG CTGCTGCTGA -900
                                                          R
AGAAAGTGGA CGAGCTGGAA CTGTCGGTCC ..(N190).. TCTGGCCAAG CGCTTCGAAG -840
         rpoA
ACCAGTTCTG AAAACGTCCG GGGGGTGGGT GACCGCCCCC CGAAAATGCC CGGACTTCCG -780

GGGAACCCTG GCCATGATGC CCCAACGAAG CCCCCCGCAC GCACGGGGCG CAACACAAAG -720
                                rplQ
CAAAACGACC TTAGGAGAGA CCCATGCGTC ACGCCCGCGG CTACCGCCGT CTGAACCGTA -660

CCCACGAACA CCGCAAGGCG CTGTTCGCCA ACATGTGCGG CTCGCTCATC GAACACGAAC -600

AGATCAAGAC CACCCTGCCG AAAGCCAAGG AACTGCGTCC GATCATCGAA AAGCTGATCA -540
                    5                                        -35
CGCTGGCCAA GCGCGGCGAC CTGCATGCCC GTTGTCAGGC GGCGGCGATG CTTGAAGCAG -480
                  -10
GACAAGGACG TGGCCAAGCT TTTCGACGTG CTCGGGCCCC GCTACAAGGA CCGTCAGGGC -420

GGCTACACCC GCGTCCTGAA AGCCGGTTTC CGCTATGGCG ACATGGCGCC GATGGCCTTC -360

ATCGAATTCG TCGAGCGCGA CGTTTCGGCG AAGGGCGCGG CTGACAAGGC CCGTGAAGCG -300
                        rplQ
GCTTTCGAAG CGGCCGACGA ATAAGCTTTC CGCAAGGGAA GCCGGAAGCC CGCCCCTCGG -240
                 3  -35                      -10
GGCGGGTTTT CGTTTTTGCG GGCGGCGCAA CCGCAGGGCG AGACTTGGCG GCAATCTGCC -180
                                                          -35
CGTAATGGCG CCGATTTCAG CAAAAACCAC CCCGCCGGAG GCAATTCCCG GTTGATCTTT -120
                    -10
TCCGGTGGCA CCTGTCTAAA AAGACATGTC CATACACACC GAAATCAACA AGTGTCTGCG -60


GGAAATCGGT CGCGTCGCGA CGGATGGCTA TTTCATCGGC CTGCATATCC GTTTCGCCGC -10
                                                  2.1
          gtaR
CCCGATCATG CAATTCCAGA CCTATCCCGA GGCGTGGACA GATCACTACA CCCGGCAGGC 50
                                        2.2
```

Fig S1

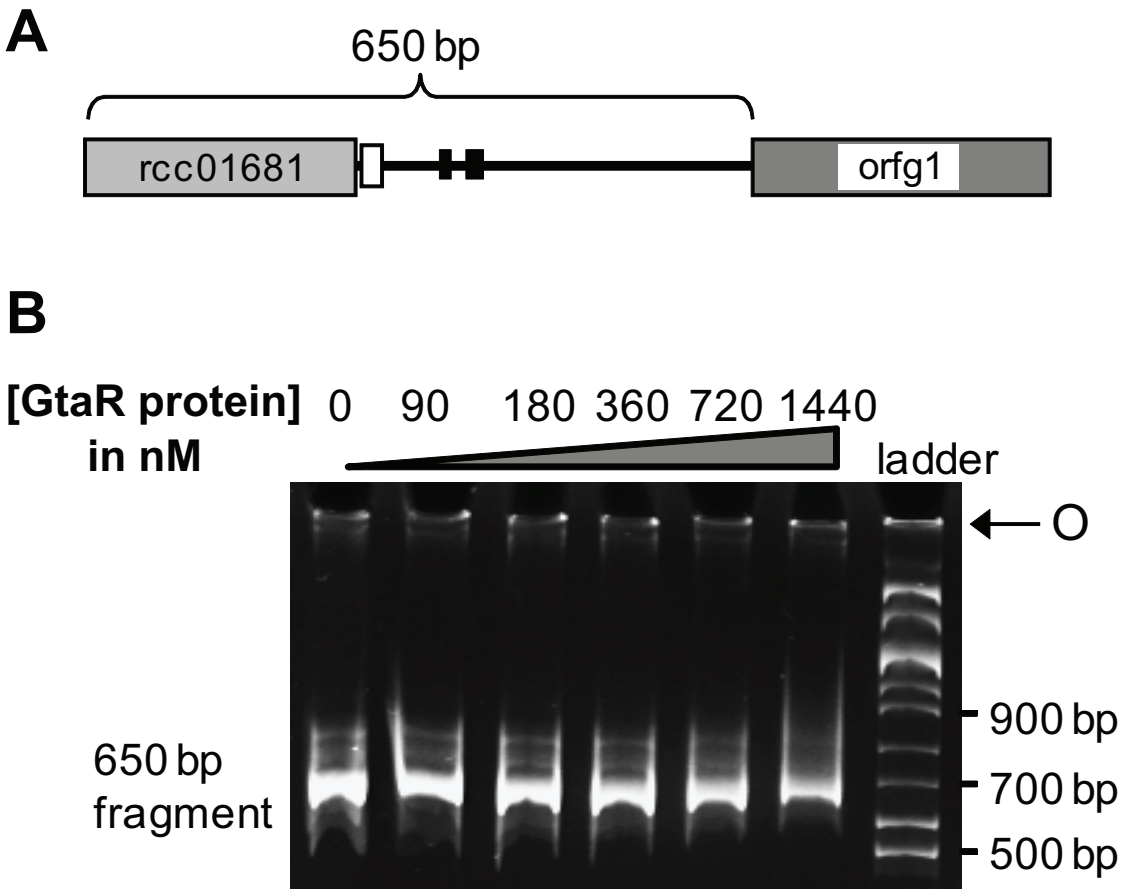
A
650 bp
rcc01681
orfg1
B
[GtaR protein]
in nM
0
90
180
360
720
1440
ladder
O
650 bp
fragment
900 bp
700 bp
500 bp

Fig S2

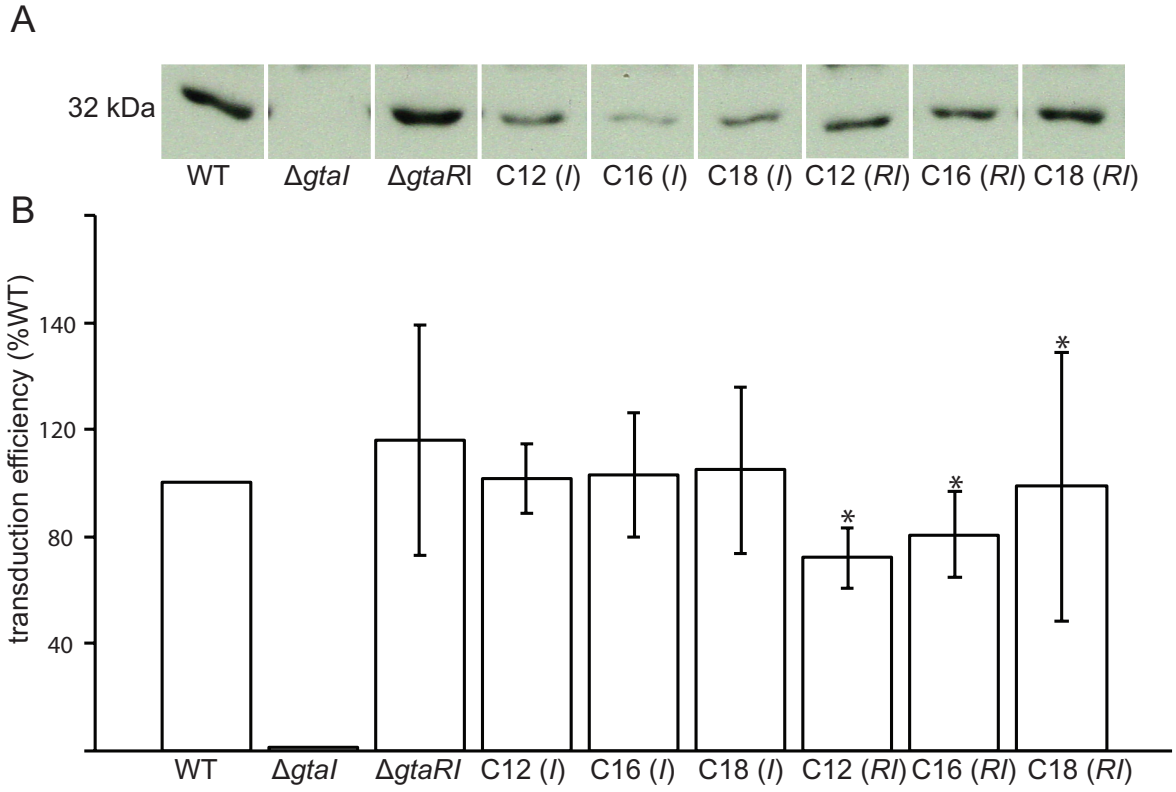
A
32 kDa
WT
ΔgtaI
ΔgtaRI
C12 (I)
C16 (I)
C18 (I)
C12 (RI)
C16 (RI)
C18 (RI)
B
transduction efficiency (%WT)
140
120
80
40
*
*
*
WT
ΔgtaI
ΔgtaRI
C12 (I)
C16 (I)
C18 (I)
C12 (RI)
C16 (RI)
C18 (RI)

Fig S3

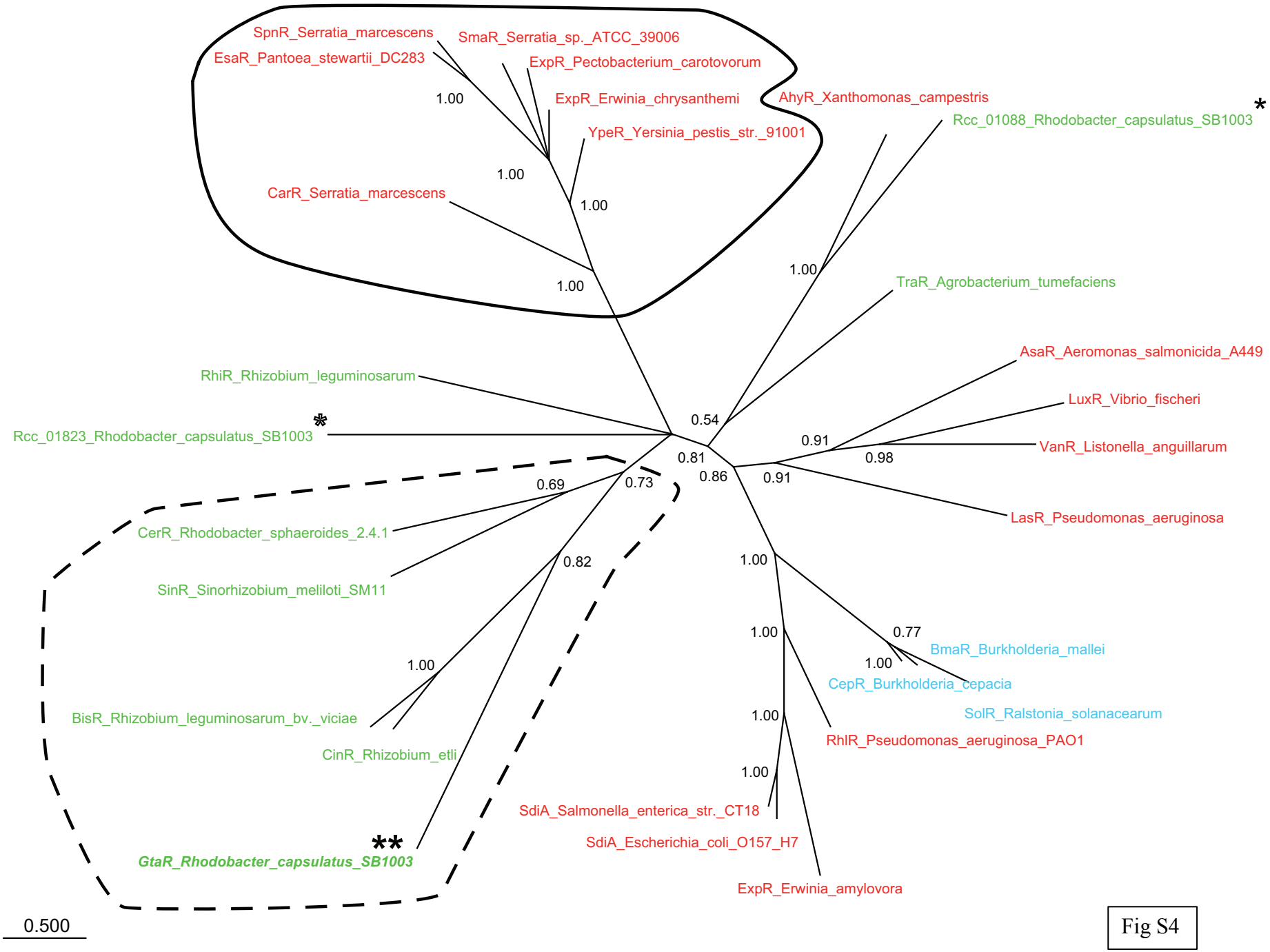
SpnR_Serratia_marcescens
SmaR_Serratia_sp._ATCC_39006
EsaR_Pantoea_stewartii_DC283
ExpR_Pectobacterium_carotovorum
ExpR_Erwinia_chrysanthemi
YpeR_Yersinia_pestis_str._91001
CarR_Serratia_marcescens
AhyR_Xanthomonas_campestris
Rcc_01088_Rhodobacter_capsulatus_SB1003
TraR_Agrobacterium_tumefaciens
AsaR_Aeromonas_salmonicida_A449
LuxR_Vibrio_fischeri
VanR_Listonella_anguillarum
LasR_Pseudomonas_aeruginosa
RhiR_Rhizobium_leguminosarum
Rcc_01823_Rhodobacter_capsulatus_SB1003
CerR_Rhodobacter_sphaeroides_2.4.1
SinR_Sinorhizobium_meliloti_SM11
BisR_Rhizobium_leguminosarum_bv._viciae
CinR_Rhizobium_etli
GtaR_Rhodobacter_capsulatus_SB1003
BmaR_Burkholderia_mallei
CepR_Burkholderia_cepacia
SolR_Ralstonia_solanacearum
RhlR_Pseudomonas_aeruginosa_PAO1
SdiA_Salmonella_enterica_str._CT18
SdiA_Escherichia_coli_O157_H7
ExpR_Erwinia_amylovora
1.00
0.54
0.91
0.98
0.81
0.86
0.73
0.69
0.82
0.77
0.500

Fig S4