Supplemental Table 1: Strains and Plasmids

Designation	Genotype or Description	Reference or Source
Burkholderia thailandensis strains		
MJ358	E264 wild type	[1]
AN3	Δ*gbdR1*	This study
AN19	Δ*gbdR2*	This study
AN16	Δ*gbdR1* Δ*gbdR2*	This study
AN126	Δ*souR*	This study
AN202	Δ*gbdR1* Δ*souR*	This study
AN205	Δ*gbdR2* Δ*souR*	This study
AN207	Δ*gbdR1* Δ*gbdR2* Δ*souR*	This study
AN136	*att*Tn7 vector, Zeo^r	This study
AN153	*att*Tn7 vector, Zeo^r	This study
AN128	*att*Tn7 vector, Zeo^r	This study
AN150	*att*Tn7 vector, Zeo^r	This study
AN158	Δ*gbdR1 att*::*gbdR1* comp, Zeo^r	This study
AN173	Δ*gbdR2 att*::*gbdR2* comp, Zeo^r	This study
AN162	Δ*gbdR1* Δ*gbdR2 att*::*gbdR1*, Zeo^r	This study
AN164	Δ*gbdR1* Δ*gbdR2 att*::*gbdR2*, Zeo^r	This study
AN151	*att*::*souR* comp, Zeo^r	This study
AN191	pAN27	This study
AN192	Δ*gbdR1* + pAN27	This study
AN193	Δ*gbdR2* + pAN27	This study
AN194	Δ*gbdR1* Δ*gbdR2* + pAN27	This study
AN192	Δ*souR* + pAN27	This study
AN226	pAN28	This study
AN227	pAN29	This study
AN228	pAN30	This study
AN229	pAN31	This study
Escherichia coli strains		
DH5α		
T7 Express	See manufacturer	NEB
AN133	pAN13 in T7 Express	
AN134	pAN14 in T7 Express	
AN188	pAN19 in T7 Express	

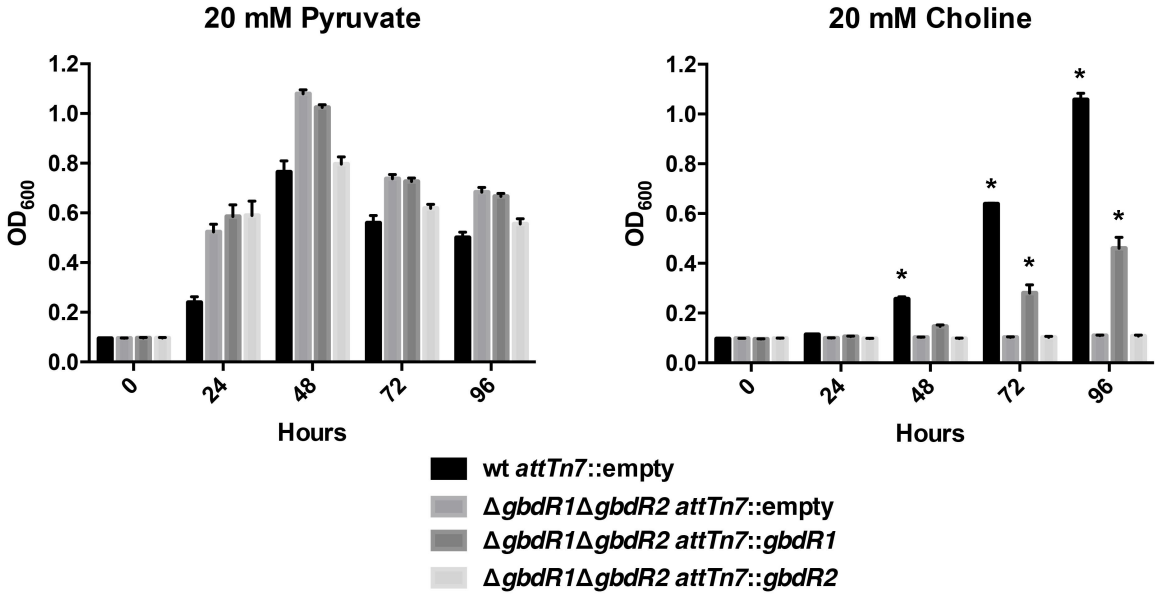
Plasmids		
pMQ132	Yeast recombineering vector, Gm^r	[2]
pAN1	pMQ132 derivative, Tp^r	This study
pAN7	pAN1 derivative, *lacZYA* reporter plasmid, Tp^r	This study
pAN27	pAN7-P_{glyA} -408	This study
pAN28	pAN7-P_{glyA} -340	This study
pAN29	pAN7-P_{glyA} -251	This study
pAN30	pAN7-P_{glyA} -147	This study
pAN31	pAN7-P_{glyA} -85	This study
pMAL-C2X	N-terminal MBP affinity tagging, Amp^r	NEB
pAN13	pMAL-C2X-*gbdR1*	This study
pAN14	pMAL-C2X-*gbdR2*	This study
pAN19	pMAL-C2X-*souR*	This study
pUC18-mini-TN7T-Zeo	*att*Tn7 integration vector, Zeo^r	[3]
pTNS2	carrying the *att*Tn7 transposase, Amp^r	[4]
pAN23	pUC18-mini-TN7T-Zeo-*gbdR1* comp	This study
pAN10	pUC18mini-TN7T-Zeo-*gbdR2* comp	This study
pAN22	pUC18-mini-TN7T-Zeo-*souR* comp	This study

Supplemental Table 2: Primers

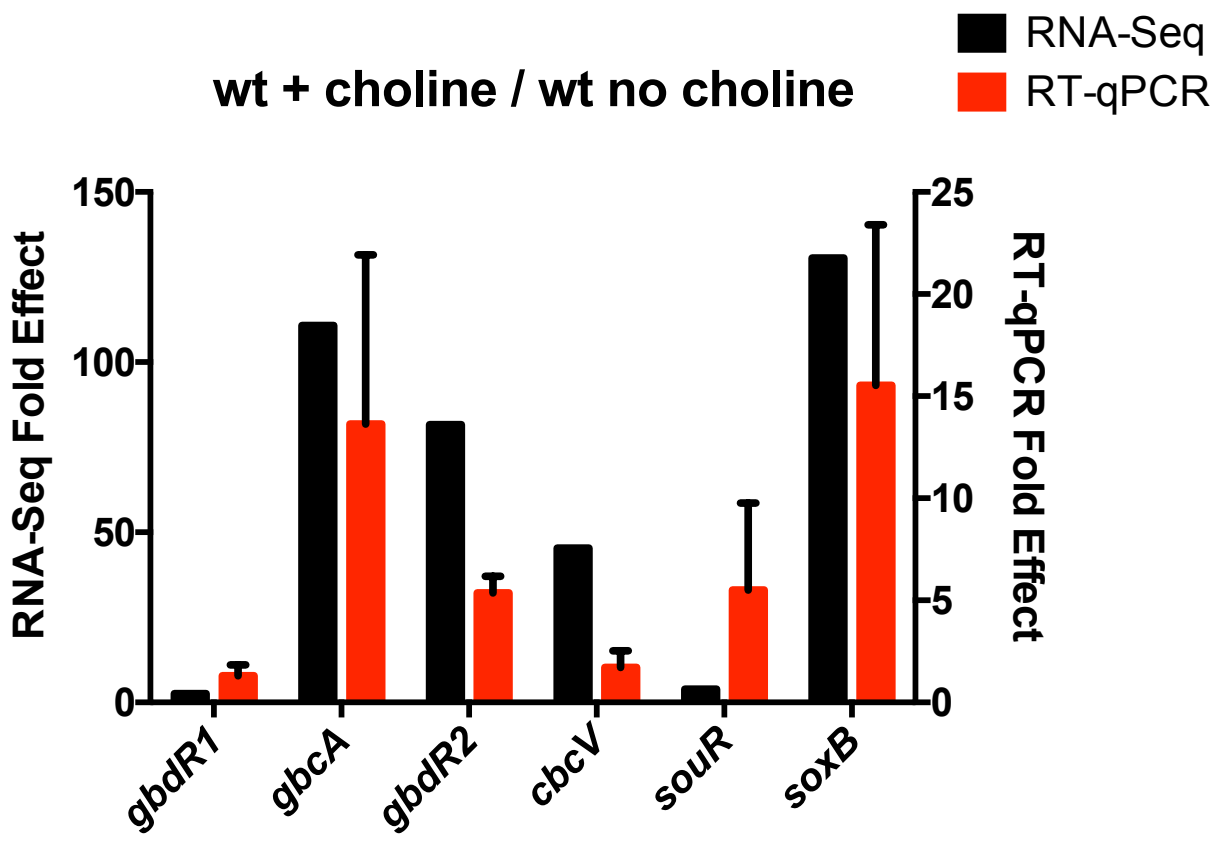
Bt-gbdR1-TpFLP-1F	gcgccccggccgcatcaagatcagatccaaggaacgcttcGAGCTCGAATTAGCTTCAAA	*gbdR1* (*BTH_II1869*) deletion
Bt-gbdR1-TpFLP-1R	ggtgtgcgatttcgggggccggtttcgagttccggtttccGAGCTCGAATTGGGGATCTT	*gbdR1* (*BTH_II1869*) deletion
Bt-gbdR1-2F	CGTAATCGATCAGCATCGTG	*gbdR1* (*BTH_II1869*) deletion
Bt-gbdR1-2R	GAAGCGTTCCTTGGATCTGA	*gbdR1* (*BTH_II1869*) deletion
Bt-gbdR1-3F	GGAAACCGGAACTCGAAAC	*gbdR1* (*BTH_II1869*) deletion
Bt-gbdR1-3R	ACGTCGAGACGCTGAGGAT	*gbdR1* (*BTH_II1869*) deletion
BtgbdR2 proximal F	AAG CCG TGC CGA TGT GGC TG	*gbdR2* (*BTH_II0968*) deletion
BtgbdR2 proximal R	AAG ATC GAC CAG CGG TAA TG	*gbdR2* (*BTH_II0968*) deletion
BtgbdR2 tp F REVISED	ACTACGTCGCGCGCGGACCATTACCGCTGGTCGATCTTgagctcgaattagcttcaaa	*gbdR2* (*BTH_II0968*) deletion
BtgbdR2 tp R REVISED	TACGCCTTGCTGAAATGGCACGGCGAGTGAAAGCCGCAGAgagctcgaattggggatctt	*gbdR2* (*BTH_II0968*) deletion
BtgbdR2 post F REVISED	TCTGCGGCTTTCACTCGCCG	*gbdR2* (*BTH_II0968*) deletion
BtgbdR2 post R REVISED	CGCCGCCGCTCAGATAGTCG	*gbdR2* (*BTH_II0968*) deletion
BtII0994proxF	CGGCAATCTACGATGAGGCT	*souR* (*BTH_II0994*) deletion
BtII0994proxR	CGATAGGGGGAAGAACACCG	*souR* (*BTH_II0994*) deletion
BtII0994postF	GGTCAGCGTGAAGTTAGGCA	*souR* (*BTH_II0994*) deletion
BtII0994postR	CCGATGTCGTCCTTCTCGTC	*souR* (*BTH_II0994*) deletion
BtII0994tpF	GCCGCCGGCCGCATCGGCGTCGGTGTTCTTCCCCCTATCGgagctcgaattagcttcaaa	*souR* (*BTH_II0994*) deletion
BtII0994tpR	GCGCTTCGGGATCGTGCTGCTGCCTAACTTCACGCTGACCgagctcgaattggggatctt	*souR* (*BTH_II0994*) deletion
gbdR1compFHindIII	CCTAAGCTTGAGCGACATCCCGAGCACCG	*gbdR1* complement
gbdR1compRKpnI	CTTGGTACCCGGGGGCCGGTTTCGAGTTC	*gbdR1* complement
KpnIgbdR2compF	CTT GGTACC CTGACGTTTTCCGCCTTCG	*gbdR2* complement
EcoRIgbdR2compR	CTT GAATTC GGGATCGGTTCGTGGCTG	*gbdR2* complement
KpnI II0994compF	CTT GGTACC CCTTGCGTAACGATGCGTGGT	*souR* complement (use MBP-SouR reverse primer)
Tp pMQ132F	TACGCCGTGGGTCGATGTTTGATGTTATGGAGCAGCAACGgatcccctgattccctttgt	Generation of pAN1
Tp pMQ132R	ACTCCGCGGCCGGGAAGCCGATCTCGGCTTGAACGAATTGagcgcttttgaagctgatgt	Generation of pAN1
BtPFusionYHRp	CTACTGCCGCCAGGCAAATTCTGTTTTATCAGACCGCTT	Generation of pAN7 step 1

1F	C GAATTC CTGCCCG	
BtPFusionYHRp1R	GCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGG CAG GAATTC GAAGCGG	Generation of pAN7 step 1
BtPFusionYHRp2F	CTACTGCCGCCAGGCAAATTCTGTTTTATCAGACCGCTT C ccaagcttgcatgcctgcag	Generation of pAN7 step 2
BtPFusionYHRp2R	GCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGG CAG gaattCGCAGCGTATCAGGC	Generation of pAN7 step 2
glyAtogbdR1interF	CCTGCATGCAAGGCGTCTCGTCACTGGAT	Generation of pAN27
glyAtogbdR1interR	CTTGCATGCGGGGAAGCGTTCCTTGGATCT	Generation of pAN27
SphI P.glyA1LacZ1	CTT GCATGC CGCAACTAGCGTGCCATACG	Generation of pAN28, use with glyAtogbdR1interR
SphI P.glyA1LacZ2	CTT GCATGC CTGCGCAGCGCGAGTG	Generation of pAN29, use with glyAtogbdR1interR
SphI P.glyA1LacZ3	CTT GCATGC GAAAGCGTCTGAATTCATCAATCGG	Generation of pAN30, use with glyAtogbdR1interR
SphI P.glyA1LacZ4	CTT GCATGC CCGCTGCGGCGGAAC	Generation of pAN31, use with glyAtogbdR1interR
gbdR1EMSA2F	GGGGTTGGCGTTCGACATAA	EMSA Probe 2
gbdR1EMSA2R	CGGAAAAGGGAAAGAAAGCGT	EMSA Probe 2
BIOgbdR1EMSA2F	BIO-GGGGTTGGCGTTCGACATAA	EMSA Probe 2
gbdR1EMSA3R	GCTAAACCCTAGATCGGCGG	EMSA Probe 1
gbdR2EMSA1F	CAGCGTATGCAGGAACGGG	EMSA Probe 1
BIOgbdR1EMSA3F	BIO-TGTCGTTCGGATTTTTAGCCG	EMSA Probe 1
SarOxiEMSA1F	CGGTCATGTCTGCAAAATCGT	EMSA Probe 3
SarOxiEMSA1R	GCCGATAGAACAAAAACGGCA	EMSA Probe 3
BioSarOxiEMSA1F	BIO-CGGTCATGTCTGCAAAATCGT	EMSA Probe 3
pMALC2XgbdR1F	CCTGGATCCTCGCCCGACCGCACCGCG	MBP-GbdR1
RSFgbdR1R	CTT AAG CTT TCA GCG CGC CGA CAC GCG	MBP-GbdR1
MALgbdR2F	CCG GGATCC GTG ACG TCC GCC GCC GCT	MBP-GbdR2
6hisgbdR2R	CTTAAGCTTTCAGCGCGCCGACACGCG	MBP-GbdR2
BamHI II0994 pMAL F	CCG GGATCC GTGCGTTTCGGCGATGGTTC	MBP-SouR
HindIII II0994 pMAL R	CCT AAGCTT TCAGCGAGCGCCCCTC	MBP-SouR and *souR* complementation
BtRplURTF	CAGTACAAGGTTGCCGTTGG	*rplU* qRT-PCR set
BtRplURTR	CCGTGCTTTTGGTAGTGCTTC	*rplU* qRT-PCR set
II1861RTF	GCAGCAGATACGGCTCCATC	*gbcA* qRT-PCR set
II1861RTR	GCAACGAGGAAAAAGGGTCG	*gbcA* qRT-PCR set
II0996RTF	CTCGAACTACCTGTGGGACG	*soxB* qRT-PCR set

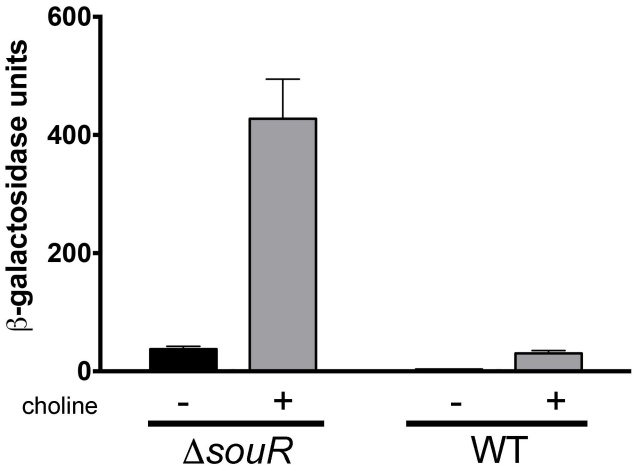
II0966RTR	AAGTTGATCGTCGGCTCGAT	*soxB* qRT-PCR set
II0994RTF	CGTTGCCTTTCTGCATCTCG	*souR* qRT-PCR set
II0994RTR	TGCGTGAGCTGGTTCCATTA	*souR* qRT-PCR set
BtgbdR1 RT F	AGTGCTCAGGATGGCAAACT	*gbdR1* qRT-PCR set
BtgbdR1 RT R	CAGATTTTCCCAATGGATCG	*gbdR1* qRT-PCR set
gbdR2 set3 RTF	CAGGTGTCCGAGCAGTTCAT	*gbdR2* qRT-PCR set
gbdR2 set3 RTR	CGGCGACACGTTCAGATAGA	*gbdR2* qRT-PCR set



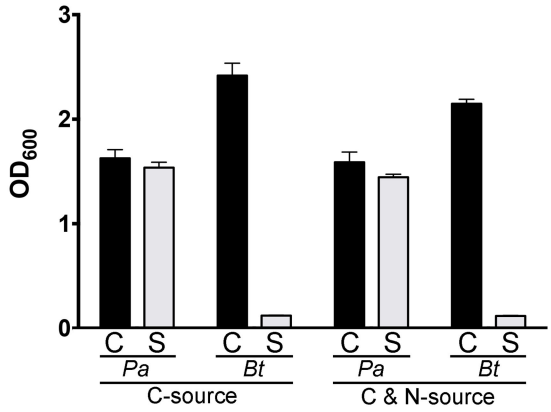
Supplementary Figure 1. Complementation of Δ*gbdR1*Δ*gbdR2* with *gbdR1* alone is able to restore the ability of *B. thailandensis* to utilize choline as a carbon source, but *gbdR2* alone is unable to complement the metabolic deficiency. Data shown are averaged from three experiments each with three biological replicates and error bars represent SEM. Data analyzed by two-way ANOVA with Dunnett's post-test using the double deletion mutant as the comparator making comparisons within each time point. The asterisks indicate $p < 0.01$.



Supplementary Figure 2. Validation of RNA-Seq experiments by qRT-PCR supports the findings of the RNA-Seq experiment. RNA-Seq values were normalized within DEseq based on library size while the RT-qPCR values were normalized to the *rplU* transcript levels. Both data sets are expressed here as fold change in expression scaled to their own respective y-axes.



Supplementary Figure 3. pAN27 is still responsive to choline in ΔsouR, and β-galactosidase activity seems to be induced to even higher levels as compared to wild type. This suggests that SouR is not required for the induction of *glyA* promoter. Data shown are averaged from three experiments each with three biological replicates and error bars represent SEM.



Supplementary Figure 4. *B. thailandensis* can utilize choline as either a carbon or carbon and nitrogen source. *B. thailandensis* is unable to utilize sarcosine as a carbon or carbon and nitrogen source, unlike *P. aeruginosa*. Strains were grown in MOPS minimal media or MOPS minimal media without NH_4, with either 40 mM or choline (C) or sarcosine (S) for 72 hours at 37 ^{o}C. Data shown are averaged from three experiments each with three biological replicates and error bars represent SEM.

Supplemental References

1. Brett, P.J., D. DeShazer, and D.E. Woods, *Burkholderia thailandensis sp. nov., a Burkholderia pseudomallei-like species.* Int J Syst Bacteriol, 1998. **48 Pt 1**: p. 317-20.
2. Shanks, R.M., et al., *New yeast recombineering tools for bacteria.* Plasmid, 2009. **62**(2): p. 88-97.
3. Choi, K.H. and H.P. Schweizer, *mini-Tn7 insertion in bacteria with single attTn7 sites: example Pseudomonas aeruginosa.* Nat Protoc, 2006. **1**(1): p. 153-61.
4. Choi, K.H., et al., *A Tn7-based broad-range bacterial cloning and expression system.* Nat Methods, 2005. **2**(6): p. 443-8.