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TABLE S1. Primers used in mutant construction

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FIG S1. Lack of accumulation of NAGGN in the Δ*ggn* mutant and trehalose in the Δ*ggn*Δ*tre* mutant.

FIG S2. GbdR activates betaine catabolism in B728a at low osmolarity.

FIG S3. Betaine inhibits the expression of *ggnA* and *treX* in Δ*gbdR*.

FIG S4. Effect of succinate on the expression of the genes *plcA, pchP* and *gbcA*.

Table S1. Primers used in mutant construction

Target genes	Sequence
Δ*gbcAB*	F1: 5'- CCCCTCTTCACCGCCAACCCCAA-3';
(Psyr_4775-Psyr_4776)	R1: 5'- AGCCTACACAATCGCTCAAGACGTATGGGCATCTGCGGGACCTGCAAG-3';
	F2: 5'- AATATCCGGGTAGGCGCAATCACTGAAAACCTATGAGTTCGGCGTGG-3';
	R2: 5'- AGCGAGGTACGCGCCGCCACTTC-3'
Δ*gbdR* (Psyr_4708)	F1: 5'- CCGGAAGCTATGTTGTGTGAAAGC-3';
	R1: 5'- AGCCTACACAATCGCTCAAGACGTTAGGGTCCAACACCGCACAGCA-3';
	F2: 5'- AATATCCGGGTAGGCGCAATCACTCACACTATCTCCTCTCATATGG-3';
	R2: 5'- TGTAGGTCACCGGTACGGAAAT-3'
Δ*tre* (Psyr_2489-	F1: 5'- AAGCGATTCTCTGGTTGCTGTC-3';
Psyr_2491)	R1: 5'- AGCCTACACAATCGCTCAAGACGTCGAGCATCACTCCTCACG-3';
	F2: 5'- AATATCCGGGTAGGCGCAATCACTATCCTCAAACCGAAGAAAGACT-3';
	R2: 5'- TGAACCTGTTCCTGAGCCGAC-3'
Δ*tre*	F1: 5'- GCGACTCGGCTCTCTACAGT-3';
(Psyr_2992-Psyr_3001)	R1: 5'- AGCCTACACAATCGCTCAAGACGTCGGCGCTAATCATCAATTTT-3';
	F2: 5'- AATATCCGGGTAGGCGCAATCACTCTGAGTTCGCTGAATGCCTG-3';
	R2: 5'- TTCCTGCTCGATAGCCTTGT-3'
kan cassette in pKD13	FRT-F: 5'- ATTGTGTAGGCTGGAGCTGCTTC-3'
	FRT-R: 5'- CCATGGTCCATATGAATATCCTCC-3'

TABLE S2. Primers used for quantitative RT-PCR

Primers	**Sequence**
hemD (Psyr_0061)	F: 5'-GCACAGCGTTCGATCATTTTC-3'; R: 5'-TGCTGAACCCACACTGAAC-3'
gbcA (Psyr_4776)	F: 5'-AGAAAATTCCCTACGCCCAC-3'; R: 5'-ACGACCCATCAGTTTCTTGC-3'
dgcA (Psyr_4782)	F: 5'- GCATCAAGGAAGTGGTCAAGG-3'; R: 5'-ATCTTGGCGATCAGGTGC-3'
gbdR (Psyr_4708)	F: 5'-TTGGGAATGTCTGGCGTC-3'; R: 5'-TCATATCCAAAGGTGCGGTG-3'
treX (Psyr_2997)	F: 5'-AAAAGGTTTCGATGCACAGTG-3'; R: 5'-GATAGACAAACCCCTCGCTG-3'
ggnA (Psyr_3747)	F: 5'-TCACCAGTTGCGTATTCAGG-3'; R: 5'-GTTTTGACACTTCACGCGAC-3'
soxA (Psyr_4715)	F: 5'- TTCTCAACCGCATCTACACC-3'; R: 5'-ATCAGGAAGTGGTTGTCAGC-3'

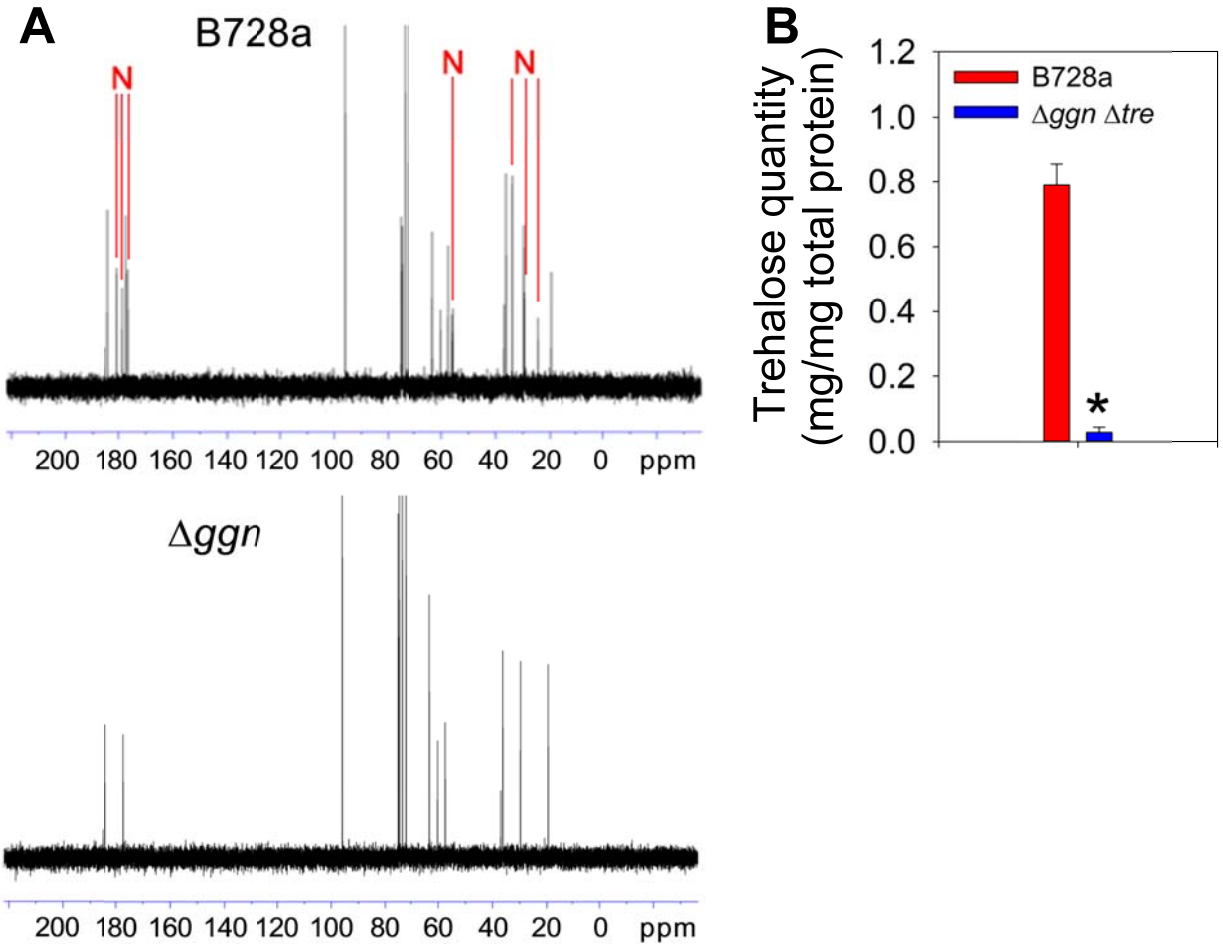

FIG S1. Lack of accumulation of NAGGN in the Δ*ggn* mutant and trehalose in the Δ*ggn*Δ*tre* mutant. (A) ^{13}C-NMR analysis of compatible solutes in log-phase cells that of B728a and Δ*ggn* that had been grown in MinA-pyruvate with 0.6 M NaCl for 24 h. Resonances (peaks) from the dipeptide NAGGN (N) are indicated. (B) Quantification the trehalose accumulated in log-phase cells of B728a and Δ*ggn*Δ*tre* that had been similarly grown in MinA-pyruvate with 0.6 M NaCl for 24 h. Trehalose was quantified using the trehalose assay kit (Megazyme, Ireland), and was normalized to total protein as measured using the Pierce BCA protein assay kit (Thermo Fisher Scientific Inc., Rockford, IL). Asterisks indicate differences between B728a and Δ*ggn*Δ*tre* cells (P <0.05). Values are means ± SEM (n = 3).

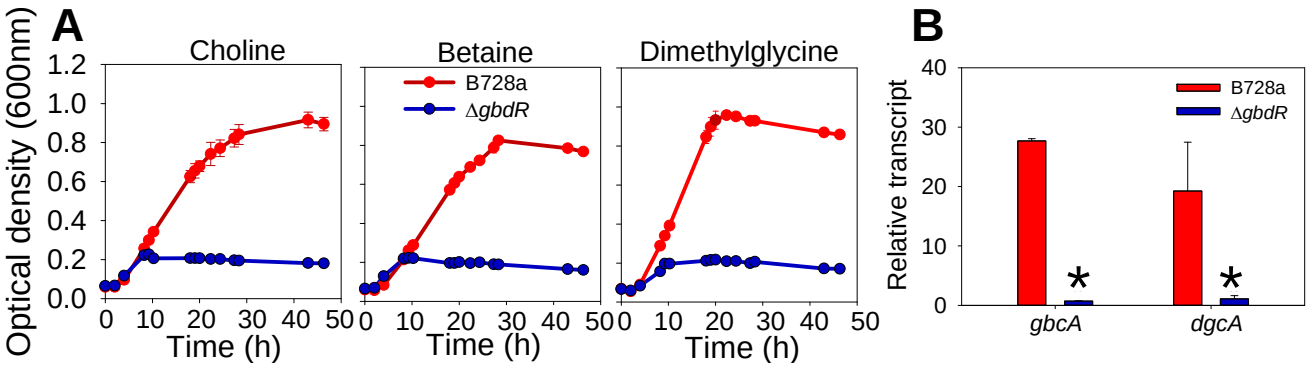

FIG S2. GbdR activates betaine catabolism in B728a at low osmolarity. (A) Growth of B728a and Δ*gbdR* in MinA medium with 20 mM choline, betaine or dimethylglycine as sole carbon source. Growth was monitored in microtiter plates based on optical density. Values are means ± SEM (n = 3). Initial growth of Δ*gbdR* was likely due to the catabolism of the small amount of citrate in the MinA medium. (B) qRT-PCR analysis of *gbcA* and *dgcA* in B728a and Δ*gbdR* at 30 min after washed, log-phase cells were suspended in MinA medium with 20 mM betaine as a carbon source. Values are expressed relative to transcript ıevels at 30 min after washed, log-phase cells were suspended in MinA medium with 20 mM pyruvate as a carbon source. Asterisks indicate differences between B728a and Δ*gbdR* cells (P <0.05). Values are means ± SEM (n = 3).

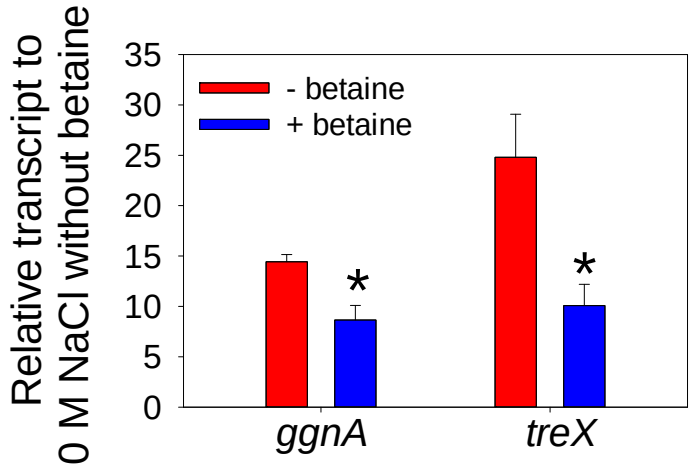

FIG S3. Betaine inhibits the expression of *ggnA* and *treX* in Δ*gbdR*. qRT-PCR analysis of *ggnA,* and *treX* in Δ*gbdR* at 30 h after amending cells with 0.6 M NaCl in the presence or absence of betaine. Values are expressed relative to transcript levels at 0 M NaCl without betaine. Values are means ± SEM (n = 3).

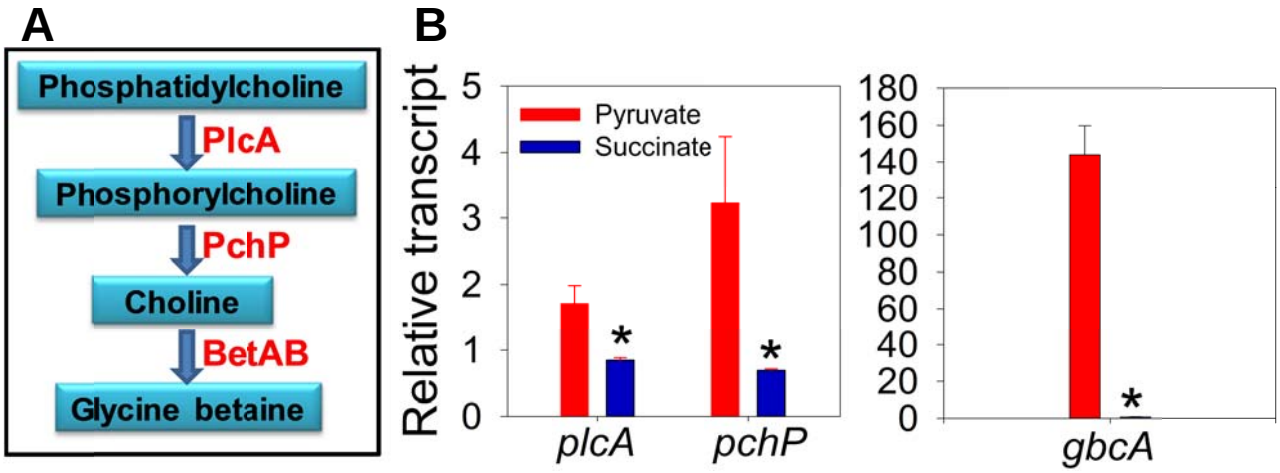

FIG S4. Effect of succinate on the expression of the genes *plcA, pchP* and *gbcA,* which are involved in the catabolism of the quaternary ammonium compounds phosphatidylcholine, phosphorylcholine and betaine, respectively. (A) Phosphatidylcholine degradation pathway and the enzymes that are involved. (B) qRT-PCR analysis of *plcA, pchP* and *gbcA* in B728a at 40 min after suspension of washed, log-phase cells in MinA medium with 10 mM pyruvate or succinate and betaine. Values are expressed relative to transcript levels in cells in MinA medium with pyruvate but not betaine. Asterisks indicate differences between pyruvate- and succinate-grown cells (P <0.05). Values are means ± SEM (n = 3).