

Supplemental Table 1. Summary of significantly differentially expressed genes. RNA-sequencing data for significantly differentially expressed genes ( $P_{adj} < 0.5$ ) is shown. OD<sub>600</sub> of 0.2, OD<sub>600</sub> of 0.5, and OD<sub>600</sub> of 1.0 correspond to late lag phase, exponential phase, and early stationary phase, respectively. Genes that are expressed significantly higher in the  $\Delta ltdR$  mutant strain compared to WT are labelled in black. Genes that are expressed significantly higher in WT compared to the  $\Delta ltdR$  mutant strain are labelled in red.

Supplemental Table 2. Results of RT-qPCR to confirm select RNA-sequencing hits. Fold changes comparing WT expression to the  $\Delta ltdR$  mutant strain expression of GBSCOH1\_RS05780 (MtaR), GBS COH1\_RS06260 (hemolysin III), GBSCOH1\_RS06255 (hemolysin III regulator), GBSCOH1\_RS07960 (3-hydroxybutyryl-CoA dehydrogenase), GBSCOH1\_RS07790 (MetQ), GBSCOH1\_RS08590 (hypothetical protein), GBSCOH1\_RS09575 (5-methyltetrahydropteroyltriglutamate homocysteine S-methyltransferase), GBSCOH1\_RS09570 (bifunctional homocysteine S-methyltransferase/methylenetetrahydrofolate reductase), and GBSCOH1\_RS00325 (N-acetylmannosamine-6-phosphate 2-epimerase) are shown. Statistically significant fold change values ( $P < 0.05$ ) are indicated in bold.

Supplemental Table 3. Primer sequences used for RT-qPCR to validate GBS RNA-sequencing hits.

Supplemental Figure 1. RT-qPCR to compare gene expression between WT, the  $\Delta ltdR$  mutant, and the complemented strains.