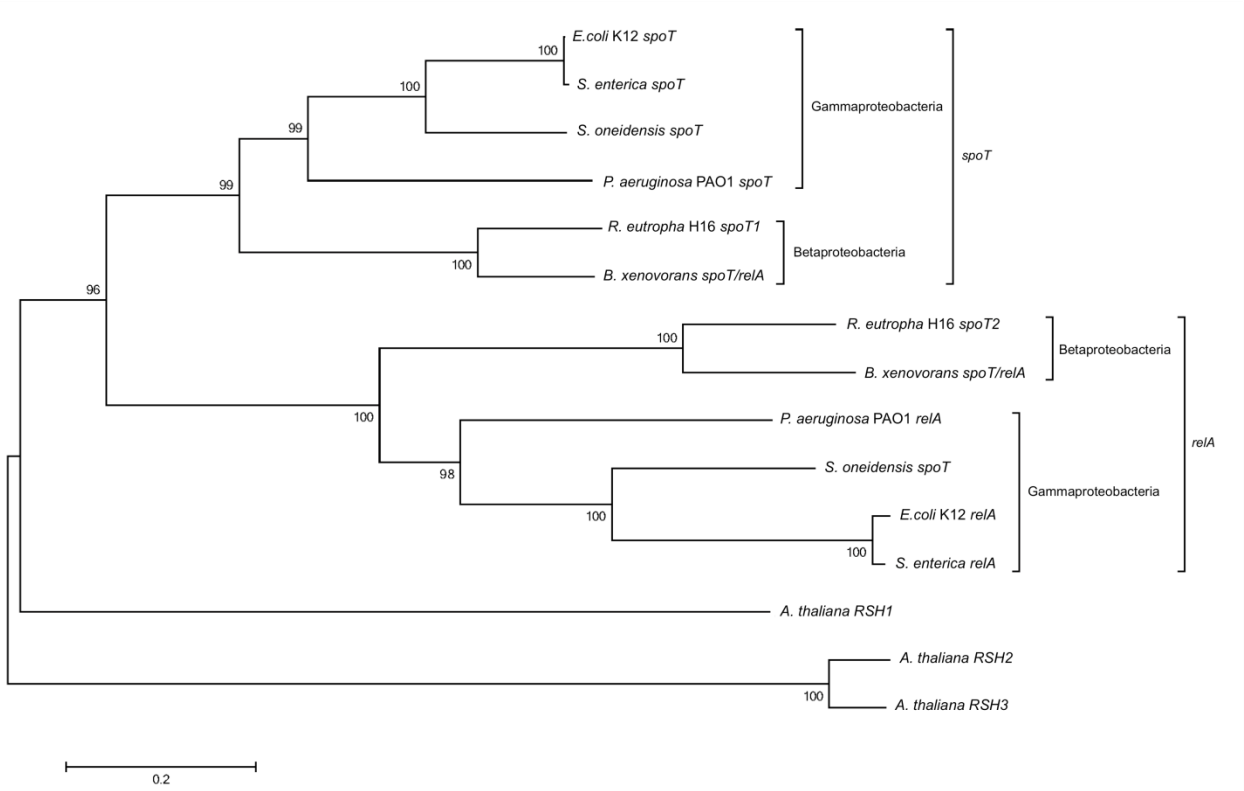
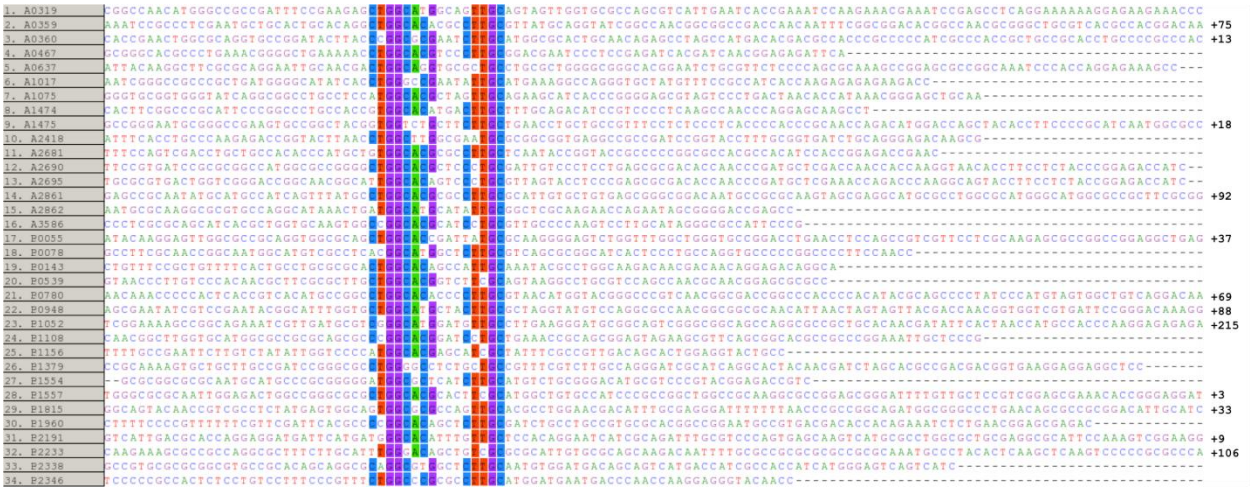
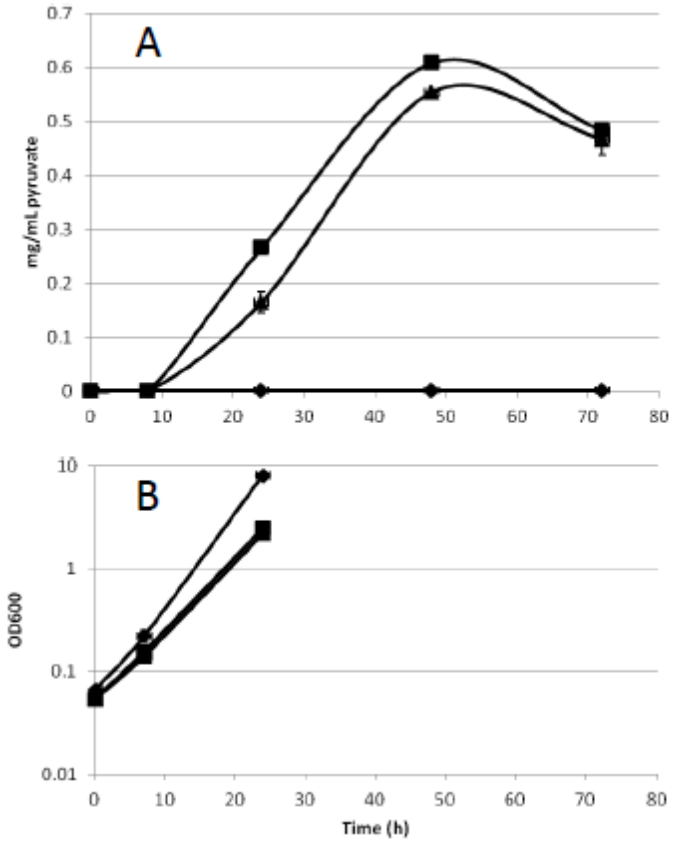
Supplementary information



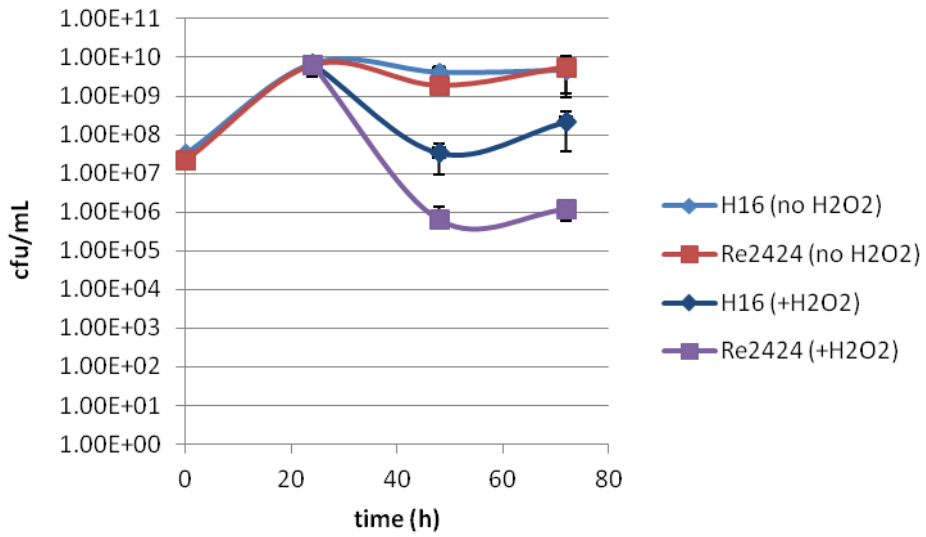
Supplemental figure S1. Phylogeny of *R. eutropha spoT1* and *spoT2*. Like most β- and γ-proteobacteria, the genome of *R. eutropha* contains two *relA-spoT* homologue genes. One of these (*spoT2*, locus tag H16_A1337) clusters with the genes characterized as *relA*, the other (*spoT1*, locus tag H16_A0955) clusters with the genes characterized as *spoT*. Sequences used for comparison belong to *Escherichia coli* K12 (NC_000913), *Salmonella enterica* (NC_010102), *Schewanella oneidensis* (NC_004347), *Pseudomonas aeruginosa* PAO1 (NC_002516), *Ralstonia eutropha* H16 (NC_008313), *Burkholderia xenovorans* (NC_007951) and *Arabidopsis thaliana* (NC_003070; NC_003074: NC_003075)



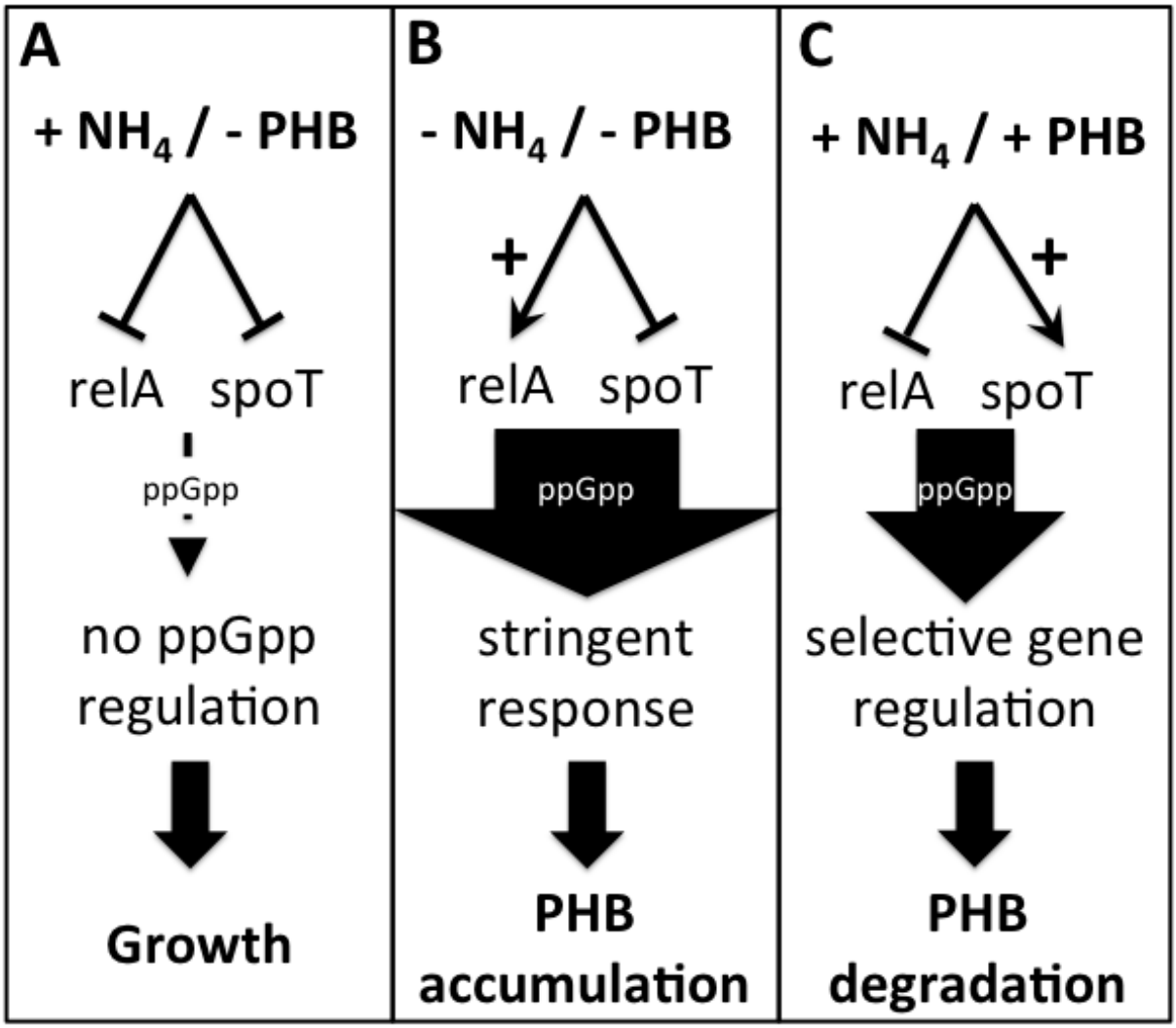
Supplemental figure S2. Alignment of promoter regions of the most strongly upregulated genes under nitrogen stress. The 34 sequences shown are potential promoters for 76 out of 96 genes that are upregulated over 50-fold during nitrogen stress. Highlighted nucleotides indicate the -12 and -24 boxes, are conserved in 50% or more of the shown sequences and were identified using the weighted consensus sequence (-28) YTGGCACGNNNNTTGCW (-10) for σ^{54} promoters (1). Sequences are truncated at the predicted start codon. The number behind the sequences indicates the distance to the start codon. Nucleotides are color coded (adenine - green; thymine – red; cytosine – blue; guanine – purple).



Supplemental Figure S3. Secretion of pyruvate into the growth medium by *R. eutropha* strains that do not synthesize PHB. *R. eutropha* H16 (wild type, diamonds), Re2411 (Δ*spoT2*, boxes), and Re2061 (Δ*phaCAB*, triangles) were incubated in minimal medium containing 0.1 % (w/v) NH_4Cl, 2 % (w/v) sodium gluconate, and supplemented with 10 g/mL gentamicin. Throughout the course of the experiment, culture supernatants were analyzed by HPLC for the presence of pyruvate (A). Pyruvate concentrations present in the culture supernatant were determined by comparison with a standard curve. Over the first 24 h, cell growth (OD_{600} values) of the cultures was analyzed (B). Strain Re2061 was constructed using previously described methods (2) using primers listed in Supplemental Table 1. Cultures were performed in triplicate.



Supplemental Figure S4. Hydrogen peroxide challenge of *R. eutropha* H16 (WT, diamonds) and Re2424 (Δ*rpoS*, boxes). Cells were incubated in TSB medium supplemented with 10 µg/mL gentamicin. At 24 h, cultures were split and 3 % (v/v, final concentration) of hydrogen peroxide was added to half of the cultures and buffer was added to the other half. Every 24 h, 1 mL aliquots of culture were serially diluted in 0.85% (w/v) NaCl and plated on TSB agar plates supplemented with 10 µg/mL gentamicin to quantify viable colony forming units per mL culture (cfu/mL).



Supplemental Figure S5. A schematic potential overview of proposed ppGpp regulation of the PHB cycle. (A) In the presence of a nitrogen source, ppGpp synthase activities of both RelA and SpoT are inhibited, no ppGpp-dependent gene regulation occurs and normal growth progresses. (B) Depletion of the nitrogen source causes amino acid shortage, thus activating RelA-dependent ppGpp synthase activity and initiating the stringent response and PHB accumulation. (C) Renewed availability of the nitrogen source in the presence of intracellular PHB initiates SpoT-dependent ppGpp synthase activity, leading to a lower intracellular ppGpp concentration and the observed selective gene regulation. Thickness of ppGpp arrow represents putative intracellular ppGpp concentrations.

Supplemental Table 1. List of primers used in this work.

DS spoT2-del BamH1 1 (Fw)	5’ - TATAGGATCCCATGCCCCGACCTTTGTC - 3’
DS spoT2-del XhoI 2 (Rv)	5’ - ATTACTCGAGCATGTCACAACGCCTGCATC - 3’
DS spoT2-del XhoI 3 (Fw)	5’ - TGTTCTCGAGTGATGCTATACTTGCGGCTTCG - 3’
DS spoT2-del XbaI 4 (Rv)	5’ - TTACTCTAGAGGCAGCGTGATTGCGAT - 3’
DS spoT2-del test (Fw)	5’ - GTCGATCCCAAGGCCGATA - 3’
DS spoT2-del test (Rv)	5’ - CAGCGGTAGTAGTTCGCGG - 3’
DS rpoS-del BamHI 1	5’ - TTATGGATCCTCACCGATGGCTCGATGAAG - 3’
DS rpoS-del XhoI 2 (Rv)	5’ - TTATCTCGAGCATGAACCCTCACTGTGGCG - 3’
DS rpoS-del XhoI 3 (Fw)	5’ - TTATCTCGAGAGGAAGGACGCTGTTCTATGACC - 3’
DS rpoS-del XbaI 4 (Rv)	5’ - GAATTCTAGAATCGCCAGCAGGTCCATCA - 3’
DS rpoS-del test Rv	5’ - CTGTAGCGCGCGATCAGTTC - 3’
DS rpoS-del test Fw	5’ - ATATTGCGGGCAAGAAGGG - 3’
rpoScompFW	5’ - GAACGTCGACATGCCACGCCAGAAAA - 3’
rpoScompRV	5’ - GGTCTCTAGATCATAGAACAGCGTCC - 3’
phaCABdel1	5’ - ACTAGGATCCAGATGCGAGCGCTGCATAC - 3’
phaCABdel2	5’ - GCCGTTAATTAAGATTTGATTGTCTCTCTGCC - 3’
phaCABdel3	5’ - CTTGTTAATTAACCTGCCGGCCTGGTTCAACC - 3’
phaCABdel4	5’ - AATTGGATCCGAGCACCGATGGCCACGACC - 3’
phaCABdelchkFW	5’ - CTATCGGAATGGACGCAAG - 3’
phaCABdelchkRV	5’ - GAAACGATTCGCGGGCCTT - 3’

Restriction sites underlined

Supplemental Table 2. Gene expression of β-ketothiolase homologs (showing similarity to PhaA).

Locus tag	Growth with fructose and nitrogen	PHB Production	PHB Utilization	P value
H16_A0170	8.2	8.0	7.0	0.001
H16_A0462	5.4	5.6	5.0	0.246
H16_A1528	6.0	7.1	5.9	0.754
H16_A1713	6.6	7.1	6.6	0.900
H16_A1720	2.9	3.1	3.3	0.146
H16_A1887	4.3	4.4	4.6	0.513
H16_B0200	5.3	5.6	5.2	0.419
H16_B0381	2.8	3.0	3.0	0.0468
H16_B0662	4.2	4.3	4.7	0.0646
H16_B0668	2.9	2.8	3.2	0.28
H16_B0759	5.8	7.6	4.7	0.0687
H16_B1369	3.0	3.4	3.2	0.445
H16_B1771	6.6	7.2	6.6	0.938

Supplemental Table 3. Gene expression of Acetoacetyl-CoA reductase homologs (showing similarity to PhaB).

Gene locus tag	Growth with fructose and nitrogen	PHB production	PHB utilization	P value
H16_A0743	7.8	7.2	7.3	0.284
H16_A0931	6.7	6.2	5.8	0.00776
H16_A1267	3.8	3.9	3.8	0.892
H16_A1287	3.8	4.1	3.9	0.584
H16_A1325	8.8	8.0	8.5	0.111
H16_A1334	8.6	8.2	7.1	0.00345
H16_A1531	4.8	5.5	4.9	0.617
H16_A1814	3.6	3.7	3.9	0.486
H16_A2152	3.7	3.7	4.2	0.0399
H16_A2567	9.2	7.0	7.9	0.00108
H16_A3164	8.0	5.0	5.6	0.00191
H16_A3487	6.9	6.4	6.5	0.269
H16_B0062	7.6	7.5	6.9	0.00826
H16_B0101	5.2	5.1	5.1	0.48
H16_B0201	5.0	5.1	5.8	0.0311
H16_B0361	3.6	3.9	3.8	0.322
H16_B0385	4.0	3.8	4.3	0.147
H16_B0394	2.7	2.7	3.0	0.223
H16_B0601	5.1	4.8	4.8	0.0361
H16_B0651	3.2	3.1	3.4	0.353
H16_B0663	3.8	3.8	3.9	0.103
H16_B0666	2.9	3.0	3.0	0.093
H16_B0687	4.6	5.7	4.4	0.224
H16_B0713	4.9	7.6	5.4	0.00489
H16_B1075	3.0	3.2	3.3	0.0228
H16_B1240	7.0	6.1	5.9	0.0223
H16_B1297	3.8	4.9	4.8	0.111
H16_B1334	4.1	4.1	4.5	0.153
H16_B1442	2.9	3.2	2.9	0.907
H16_B1696	3.6	3.9	3.9	0.395
H16_B1834	4.1	5.1	4.0	0.412
H16_B1904	4.3	4.7	5.0	0.0181
H16_B2339	2.8	6.1	3.1	0.029
H16_B2510	5.9	5.5	6.2	0.0238

References:

1. **Barrios, H., B. Valderrama, and E. Morett.** 1999. Compilation and analysis of sigma(54)-dependent promoter sequences. Nucleic Acids Res **27:**4305-13.
2. **Brigham, C. J., C. F. Budde, J. W. Holder, Q. Zeng, A. E. Mahan, C. Rha, and A. J. Sinskey.** 2010. Elucidation of beta-oxidation pathways in Ralstonia eutropha H16 by examination of global gene expression. J Bacteriol **192:**5454-64.