Ralstonia eutropha´s Poly(3-hydroxybutyrate)(PHB) polymerase PhaC1 and PHB depolymerase PhaZa1 are phosphorylated *in vivo*

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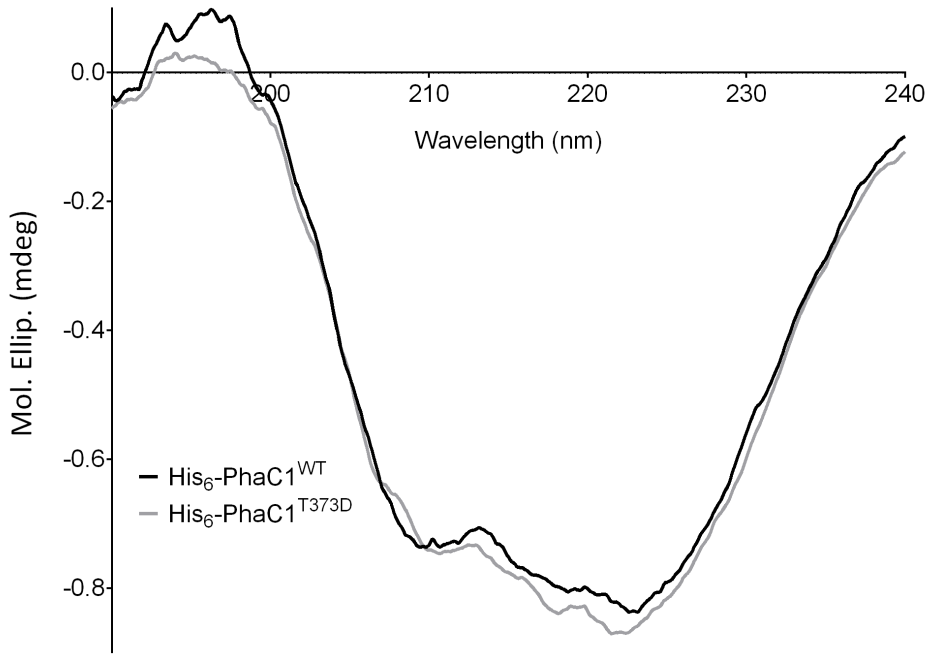
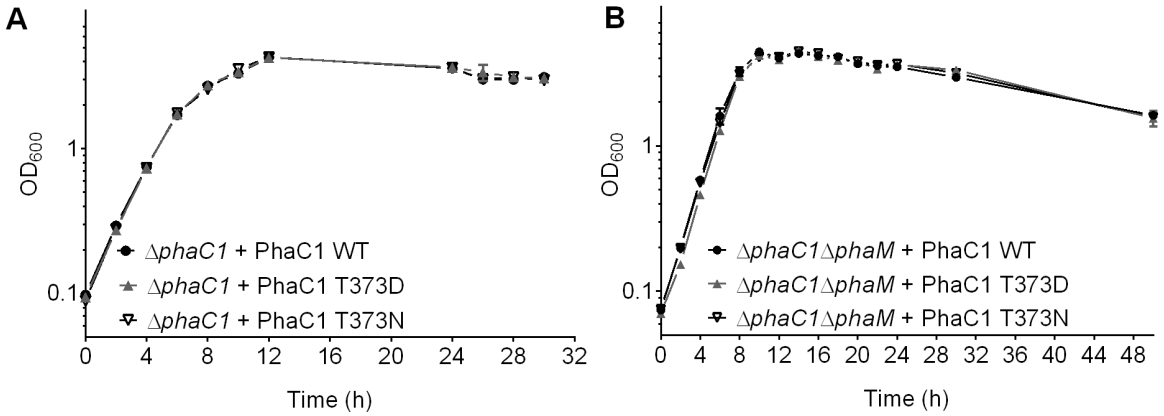
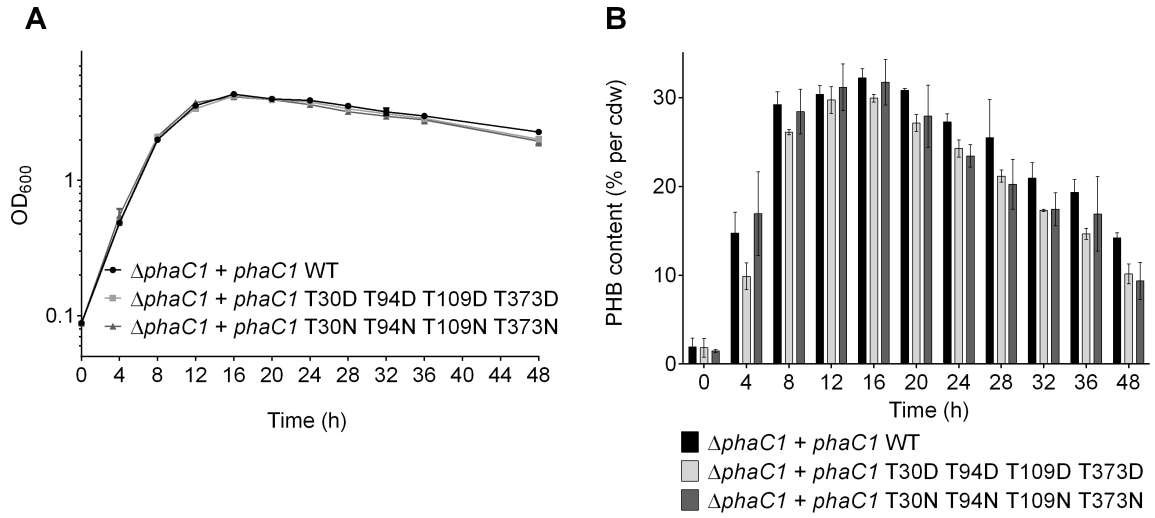
Supplementary materials



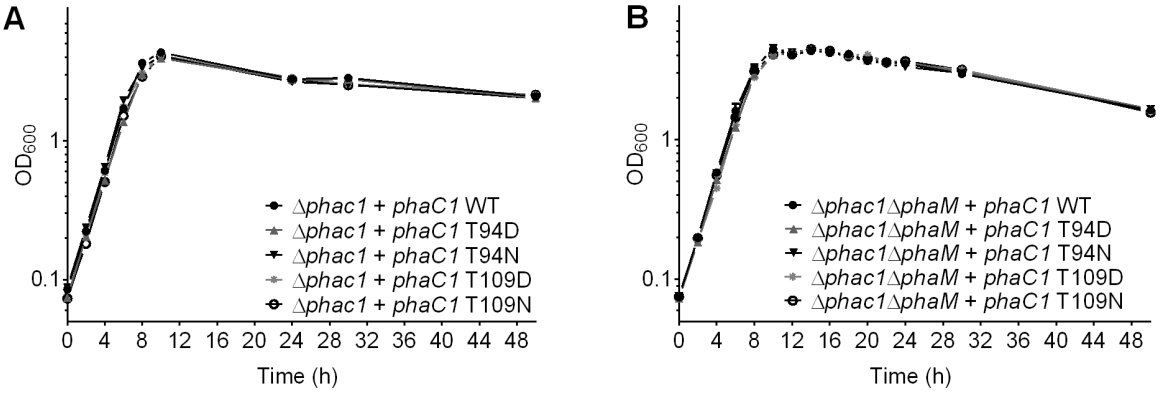
Supplementary materials Fig. S1: CD spectrum of purified His_6-$PhaC1^{WT}$ and His_6-$PhaC1^{T373D}$



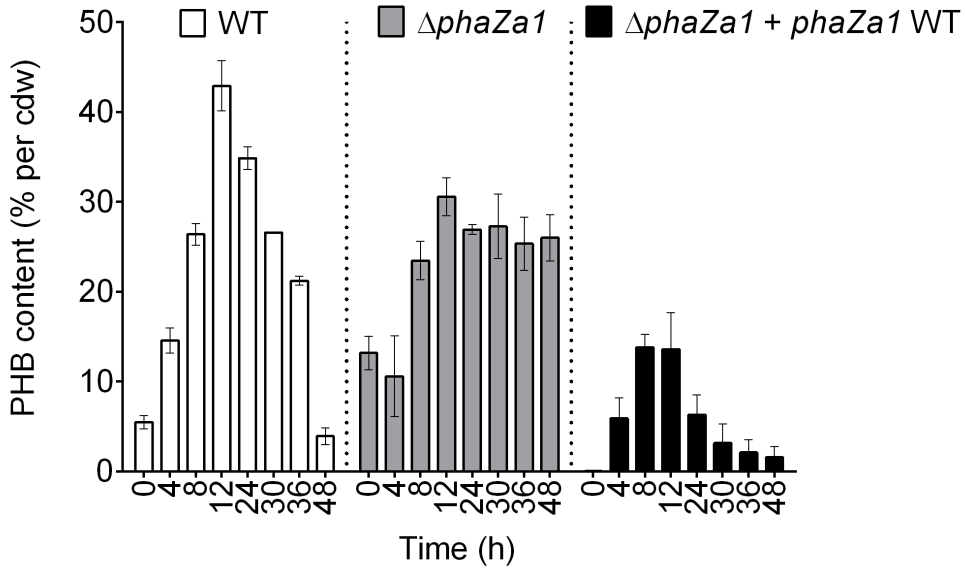
Supplementary materials Fig. S2: Growth of *R. eutropha* H16 strains (**A**) Growth of *R. eutropha* Δ*phaC1* (pBBR1MCS2_p_{phaC}_*phaC1_eyfp)*, *R. eutropha* Δ*phaC1* (pBBR1MCS2_p_{phaC}_*phaC1*_T373D_*eyfp)*, *R. eutropha* Δ*phaC1* (pBBR1MCS2_ p_{phaC}_*phaC1*_T373N_*eyfp)* on NB-medium with 0.2% gluconate. (**B**) Growth of *R. eutropha* Δ*phaC1* Δ*phaM* (pBBR1MCS2_p_{phaC}_*phaC1_eyfp)*, *R. eutropha* Δ*phaC1* Δ*phaM* (pBBR1MCS2_p_{phaC}_*phaC1*_T373D_*eyfp)*, *R. eutropha* Δ*phaC1* Δ*phaM* (pBBR1MCS2_ p_{phaC}_*phaC1*_ T373N_*eyfp)* on NB-medium with 0.2% gluconate. Error bars show the standard deviation.



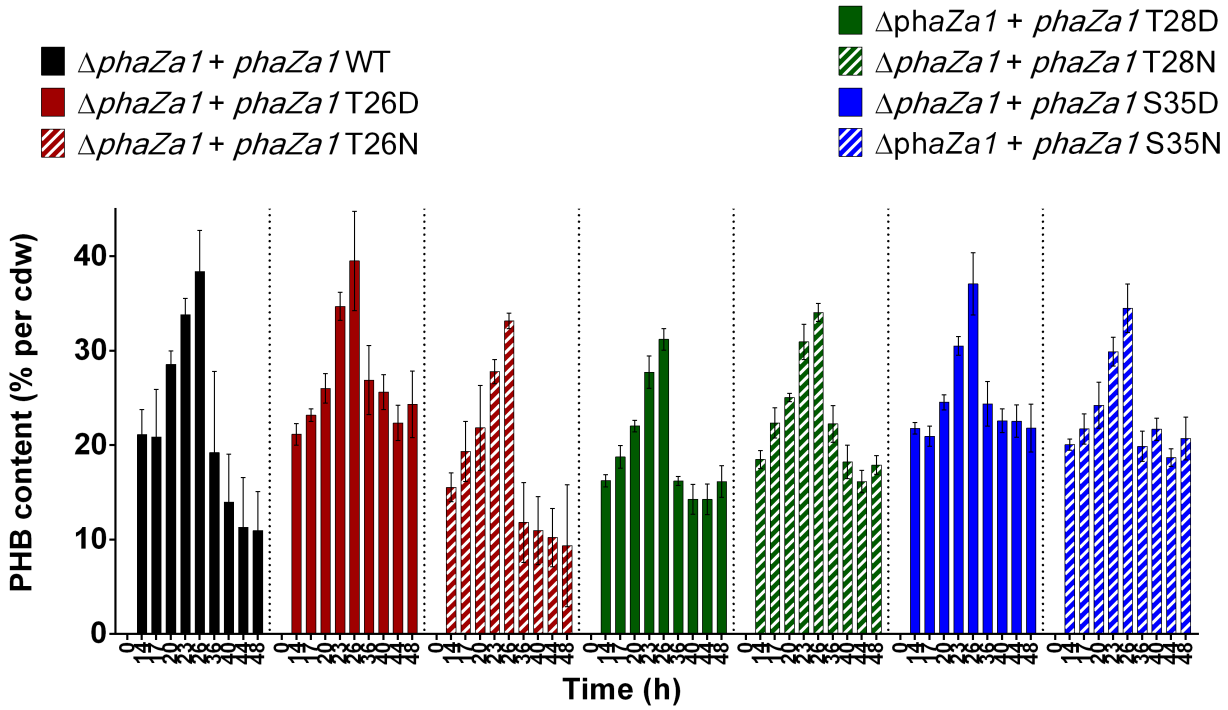
Supplementary materials Fig. S3: Growth and PHB accumulation of *R. eutropha* H16 strains over 48 h. Growth (**A**) and PHB contents (**B**) of *R. eutropha* Δ*phaC1* (pBBR1MCS2_p_{phaC}_*phaC1_eyfp)*, *R. eutropha* Δ*phaC1* (pBBR1MCS2_p_{phaC}_*phaC1*_T30D_T94D_T109D_T373D_*eyfp)* and *R. eutropha* Δ*phaC1* (pBBR1MCS2_p_{phaC}_*phaC1*_T30N_T94N_T109N_T373N_*eyfp)* on NB-medium with 0.2% Na-gluconate. Results were obtained from biological triplicates; error bars show the standard deviation.



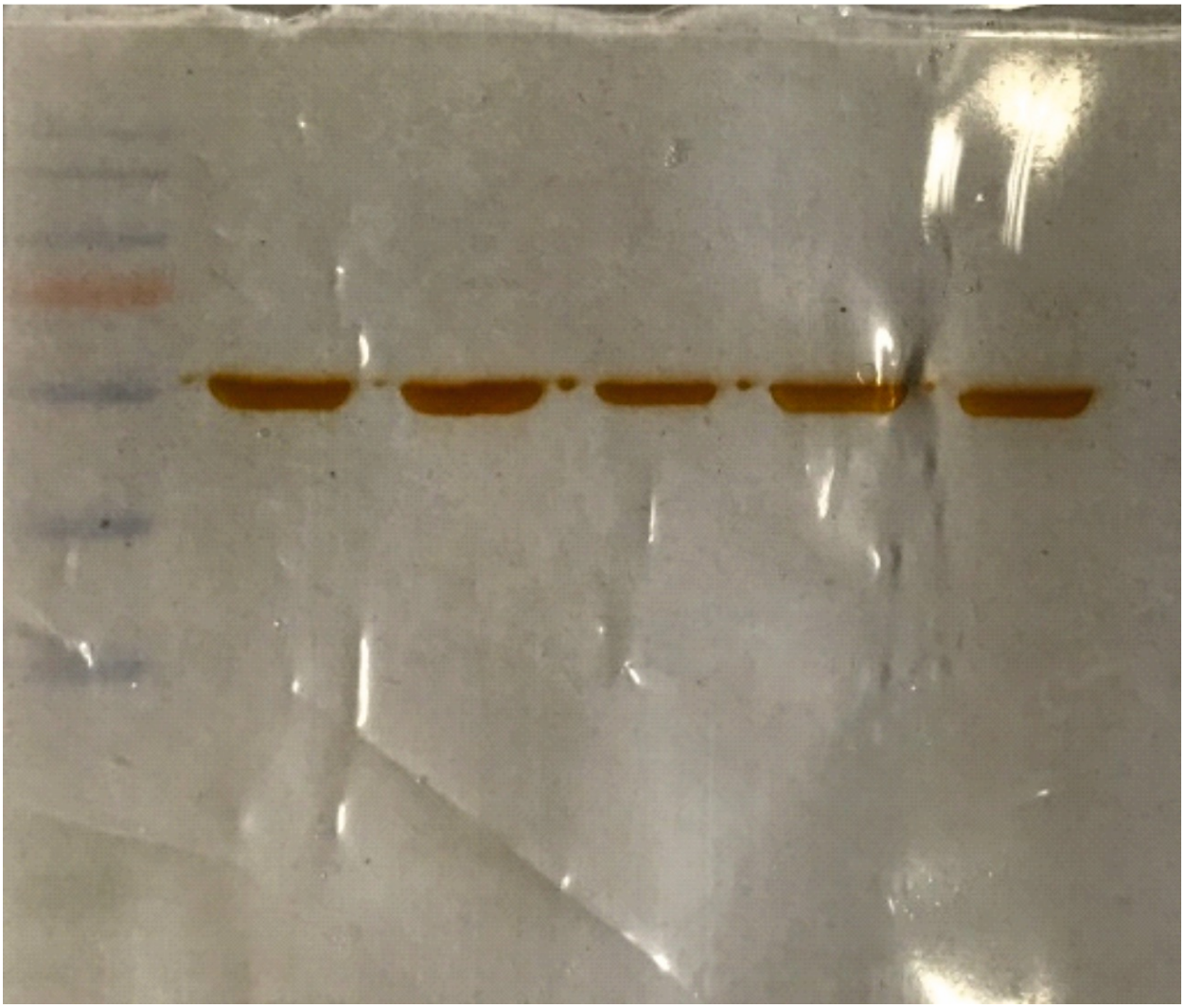
Supplementary materials Fig. S4: Growth of *R. eutropha* H16 strains (**A**) Growth of *R. eutropha* Δ*phaC* (pBBR1MCS2_p_{phaC}_*phaC1_eyfp)*, *R. eutropha* Δ*phaC1* (pBBR1MCS2_p_{phaC}_*phaC1*_T94D_*eyfp)*, *R. eutropha* Δ*phaC1* (pBBR1MCS2_p_{phaC}_*phaC1*_T94N_*eyfp)*, *R. eutropha* Δ*phaC1* (pBBR1MCS2_p_{phaC}_*phaC1*_T109D_*eyfp)* and *R. eutropha* Δ*phaC1* (pBBR1MCS2_p_{phaC}_*phaC1*_ T109N_*eyfp)* on NB-medium with 0.2% gluconate. (**B**) Growth of *R. eutropha* Δ*phaC1* Δ*phaM* (pBBR1MCS2_p_{phaC}_*phaC1_eyfp)*, *R. eutropha* Δ*phaC1* Δ*phaM* (pBBR1MCS2_p_{phaC}_*phaC1*_T94D_*eyfp)*, *R. eutropha* Δ*phaC1* Δ*phaM* (pBBR1MCS2_p_{phaC}_*phaC1*_T94N_*eyfp)*, *R. eutropha* Δ*phaC1* Δ*phaM* (pBBR1MCS2_p_{phaC}_*phaC1*_T109D_*eyfp)* and *R. eutropha* Δ*phaC1* Δ*phaM* (pBBR1MCS2_p_{phaC}_*phaC1*_T109N_*eyfp)* on NB-medium with 0.2% gluconate.



Supplementary materials Fig. S5: PHB accumulation of *R. eutropha* strains over 48h. PHB content per cellular dry weight of *R. eutropha* wild type, *R. eutropha* Δ*phaZa1* and *R. eutropha* Δ*phaZa1 (*pBBR1MCS2_p_{phaC}*_phaZa1_eyfp)* on NB-medium with 0.2% gluconate (data were obtained from two biological and two technical replicates; error bars show the standard deviation).



Supplementary materials Fig. S6: PHB accumulation of *R. eutropha* H16 strains. PHB contents of *R. eutropha* Δ*phaZa1* (pBBR1MCS2_p_{phaC}_*phaZa1*_*eyfp*), *R. eutropha* Δ*phaZa1* (pBBR1MCS2_p_{phaC}_*phaZa1*_T26D_*eyfp*), *R. eutropha* Δ*phaZa1* (pBBR1MCS2_p_{phaC}_*phaZa1*_T26N_*eyfp*), *R. eutropha* Δ*phaZa1* (pBBR1MCS2_p_{phaC}_*phaZa1*_T28D_*eyfp*), *R. eutropha* Δ*phaZa1* (pBBR1MCS2_p_{phaC}_*phaZa1*_T28N_*eyfp*), *R. eutropha* Δ*phaZa1* (pBBR1MCS2_p_{phaC}_*phaZa1*_S35D_*eyfp*) and *R. eutropha* Δ*phaZa1* (pBBR1MCS2_p_{phaC}_*phaZa1*_S35N_*eyfp*) on NB-medium with 0.5% Na-gluconate. The PHB content per cellular dry weight is shown. Data were obtained from 2 biological and 2 technical replicates; error bars show the standard deviation.



Suppl. materials, Fig. S7: SDS-PAGE analysis of purified PhaC1 muteins. Left lane shows marker proteins. Gel was stained with silver.

Supplementary materials, Table S1: Summary of all PhaC1 mutants generated. For all of the mutants indicated a growth curve over 24h on NB-medium supplemented with 150µg/ml kanamycin and 0.2 % Na-gluconate (wt/vol) was recorded and the cells were stained with Nile red to visualize PHB and without any dye to visualize the eYFP-tagged PhaC1 by fluorescence microscopy.

Non-phosphorylatable	Microscopy	Phosphomimetic	Microscopy
T30A	As WT	**T30D**	As WT
T94N	As WT	**T94D**	As WT
T109N	As WT	**T109D**	As WT
T373N, T373A	As WT	**T373D, T373E**	As WT