Regulation of polyhydroxybutyrate synthesis in the soil bacterium *Bradyrhizobium diazoefficiens*

Supplemental Material

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TABLE S1 Plasmids and bacterial strains used in this study.

Plasmid and strains	Relevant characteristics	Reference or source
pRK2013	ColE1 replicon, tra^+ from RK2, Km^R	1
pGEM®-T Easy	Cloning vector, Ap^R	Promega
pK18*mob*	Mob^+ Km^R, suicide in rhizobia	2
pG18*mob*2	Mob^+ Gm^R, suicide in rhizobia	3
pBBR1-MCS-4	Km^R, broad host range vector	4
pBBR1-MCS-5	Gm^R, broad host range vector	4
pHP45ΩSm	$Ap^R/Sm^R/Sp^R$, donor of Ω Sm/Sp interposon	5
pHP45ΩKm	Ap^R/Km^R, donor of Ω Km interposon	5
pCB303	Tc^R, *lacZ*, *phoA*, *oriT*	6
pIQ35	pGemT-easy with *phaR* internal fragment (244 bp), amplified with Fw1 and Rv1 (blr0227 spanning bases 224,141 to 224,384)	This work
pIQ36	pG18*mob*2 with *phaR* fragment (262 bp digested EcoRI from pIQ35)	This work
pIQ37	pBBR1-MCS5 with 1,245 bp fragment amplified with Fw1c and Rv1c carrying the entire blr0227 ORF (bases 223,562 to 224,806) cloned SmaI	This work
pIQ38	pCB303 with the entire *phaR* (1,265 bp) XbaI PstI digested from pIQ37	This work
pIQ39	pBBR1MCS4 with *phaP1* dw fragment (235 bp)	This work
pIQ40	pBBR1MCS4 with *phaP4* dw fragment (222 bp)	This work
pIQ41	pIQ39 with *phaP1* up fragment (249 bp) cloned EcoRV	This work
pIQ42	pIQ40 with *phaP4* up fragment (253 bp) cloned EcoRV	This work
pIQ43	pIQ41 with Ω Sm/Sp interposon from digested EcoRI pHP45	This work
pIQ44	pIQ42 with Ω Km interposon from digested EcoRI pHP45	This work
pIQ45	pK18*mob* with *phaP1*up-Ω Sm/Sp-*phaP1*dw fragment (2,560 bp) from digested XbaI KpnI pIQ43	This work
pIQ46	pG18*mob*2 with *phaP4*up-Ω Sm/Sp-*phaP4*dw fragment (2,817 bp) from digested XbaI KpnI pIQ44	This work
USDA 110	*Bradyrhizobium diazoefficiens* wild-type	USDA culture collection
LP 3004	*B. diazoefficiens* USDA 110 spontaneous Sm^R	7
USDA 110 *spc4*	*B. diazoefficiens* USDA 110 spontaneous Sp^R	8
9043 (*fixK*$_2$ mutant)	*B. diazoefficiens* USDA 110 *spc4* derivative, Sp^R Sm^R *fixK*$_2$::Ω	9
LP 0227 (*phaR* mutant)	*B. diazoefficiens* LP 3004 with pIQ36 inserted in blr0227	This work
LP 0227 pIQ38 (*phaR* mutant complemented)	*B. diazoefficiens* LP 0227 with pIQ38	This work
LP 5155 (Δ*phaP1*)	*B. diazoefficiens* USDA 110 with Ω Sm/Sp-cassette replacing 301 bp of bll5155	This work
LP 7395 (Δ*phaP4*)	*B. diazoefficiens* USDA 110 with Ω Km-cassette replacing 426 bp of bll7395	This work
LP 5173 (Δ*phaP1*/Δ*phaP4*)	*B. diazoefficiens* LP 7395 with Ω Sm/Sp-cassette replacing 301 bp of bll5155	This work
DH5α	*Escherichia coli supE44 ΔlacU169 (φ80 lacZΔM15) hsdR17 recA1 gyrA96 thi-1 relA1*	Bethesda Res. Lab.
S17-1	*E. coli* Sm^R Sp^R *hsdR* (RP4-2 *kan::*Tn7 *tet::*Mu; integrated into the chromosome)	10

TABLE S2 Primers used in this study.

Primers	Sequence (5´ to 3´)	Reference
Primers for cloning		
M13F	GTAAAACGACGGCCAGT	Universal primer
M13R	GCGGATAACAATTTCACACAGG	Universal primer
Fw1	GGTCAAGGATGGCGAAGA	This work
Rv1	GATCTGCTTGCGGAACTT	This work
Fw1c (*phaR* complementing)	ATCTGGCCCATGATGACTTC	This work
Rv1c (*phaR* complementing)	TTACCCCAGGAAGCAGTTTG	This work
Fw2	AACACGTCCAGGGAATCAAG	This work
Rv2	CGAACTGCTCTTTCCCGTAG	This work
Fw3	CCGGAATGACGAAGTACACC	This work
Rv3	TCACGATCATGGAAAATGGA	This work
Fw4	TTCTTGCTTCCAGGATGTCC	This work
Rv4	GAATGAACAGCGCGTTGAAT	This work
Fw5	TTATTTGGGCATGATCCCAT	This work
Rv5	CTCAGTTGGTTAGAGCGCCT	This work
KmF (checking)	TGTATGGGAAGCCCGATG	11
KmR (checking)	TGCCATTCTCACCGGATT	11
Sm/Sp (checking)	CGGTGGATGACCTTTTGAAT	12
FwCh1 (*phaP1* checking)	CGGTACCTGCTGTTCAATCG	This work
RvCh2 (*phaP1* checking)	GAGCCGCTATTCTGGCTAAG	This work
FwCh3 (*phaP4* checking)	GCTGCAACAGCTTGAGTACG	This work
RvCh4 (*phaP4* checking)	GTAGCCCGATGGATTCTCAG	This work
Primers for qRT-PCR		
sigA-1069F	GAGATCATCGTCGAGGTGAAG	13
sigA-1155R	GCGCTTGTTGATGTCGTAGA	13
*fixK2*_14	TGCGCAGCTACAAGCTTCTC	This work
*fixK2*_15	CTGGTGTCGATGATGGCTTC	This work
phaR-Fw	AAATCAGACCAACCCACCAC	This work
phaR-Rv	GCGTCGTAGACCAGGAAATC	This work
phaC1-Fw	CAAGCTCGCCAAGGACTATC	This work
phaC1-Rv	ACTGGTTCGACTTCCATTCC	This work
phaC2-Fw	AATTCCCTGGTCCGGTATCT	This work
phaC2-Rv	ACTCCGAGCTTGCGATAGTC	This work
phaC3-Fw	GCTCAGCCCTGAATTTATCG	This work
phaC3-Rv	TGGTATAGCCGCTTCATGTG	This work
phaP1-Fw	TCAGAGCTACGGGAAAGAGC	This work
phaP1-Rv	TTCTTGGTGTAGTCGCCGTA	This work
phaP4-Fw	AGGTGCGACTGATCCATTCT	This work
phaP4-Rv	GTCCTTGAACTTGGCGTAGC	This work

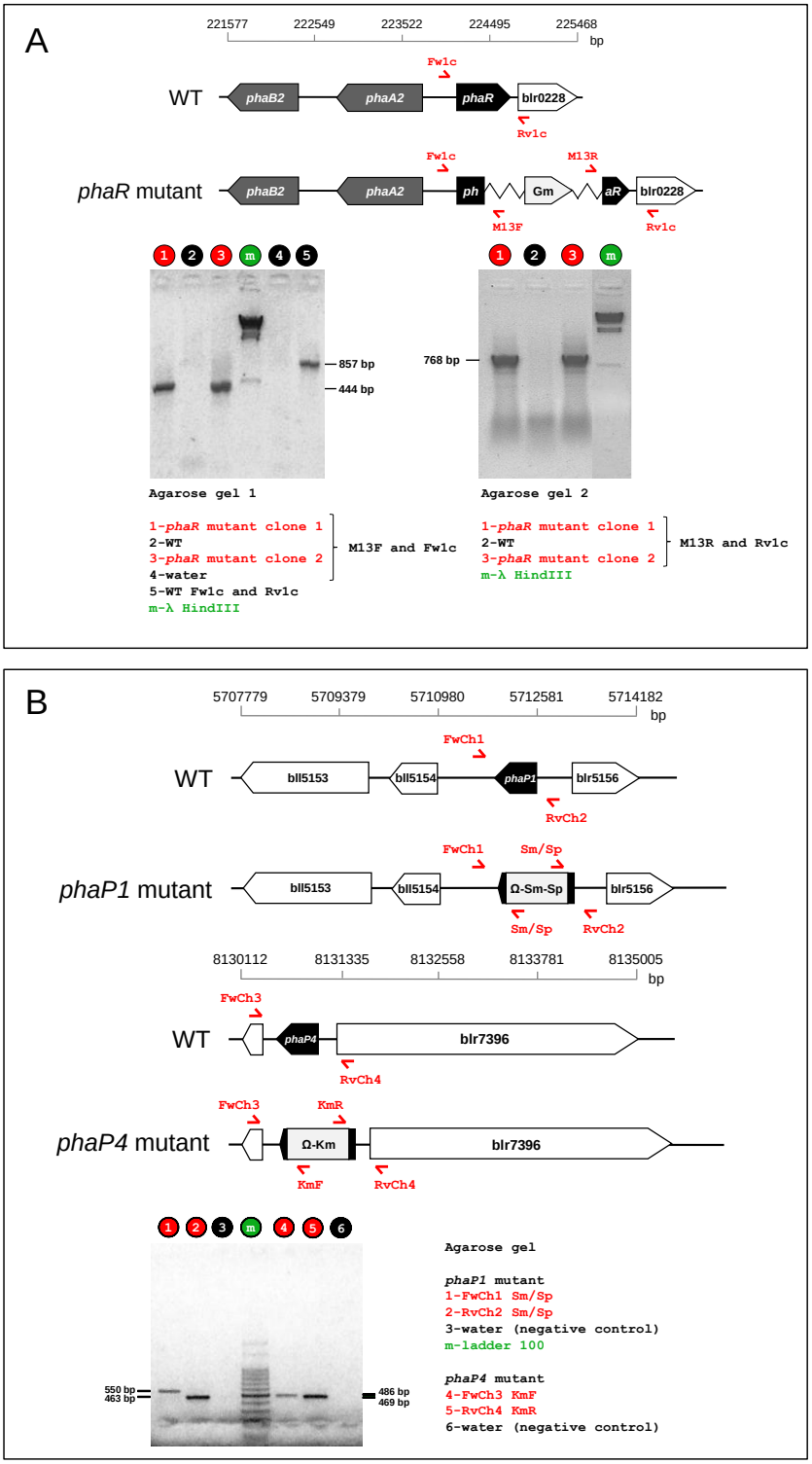

FIG S1 Genotypes of the wildtype and mutant strains for *phaR*, *phaP1* and *phaP4* regions. (A) Diagram of the region containing *phaR* (*black*), *phaA2* and *phaB2* (*grey*) in the wildtype (WT) and the *phaR* mutant. *Red* arrows indicate the primers used to confirm the insertional mutation according to the PCR results shown below the diagrams. Two independent clones were assessed in this way. (B) The same analysis was done for the selected candidates of *phaP1* and *phaP4* mutants.

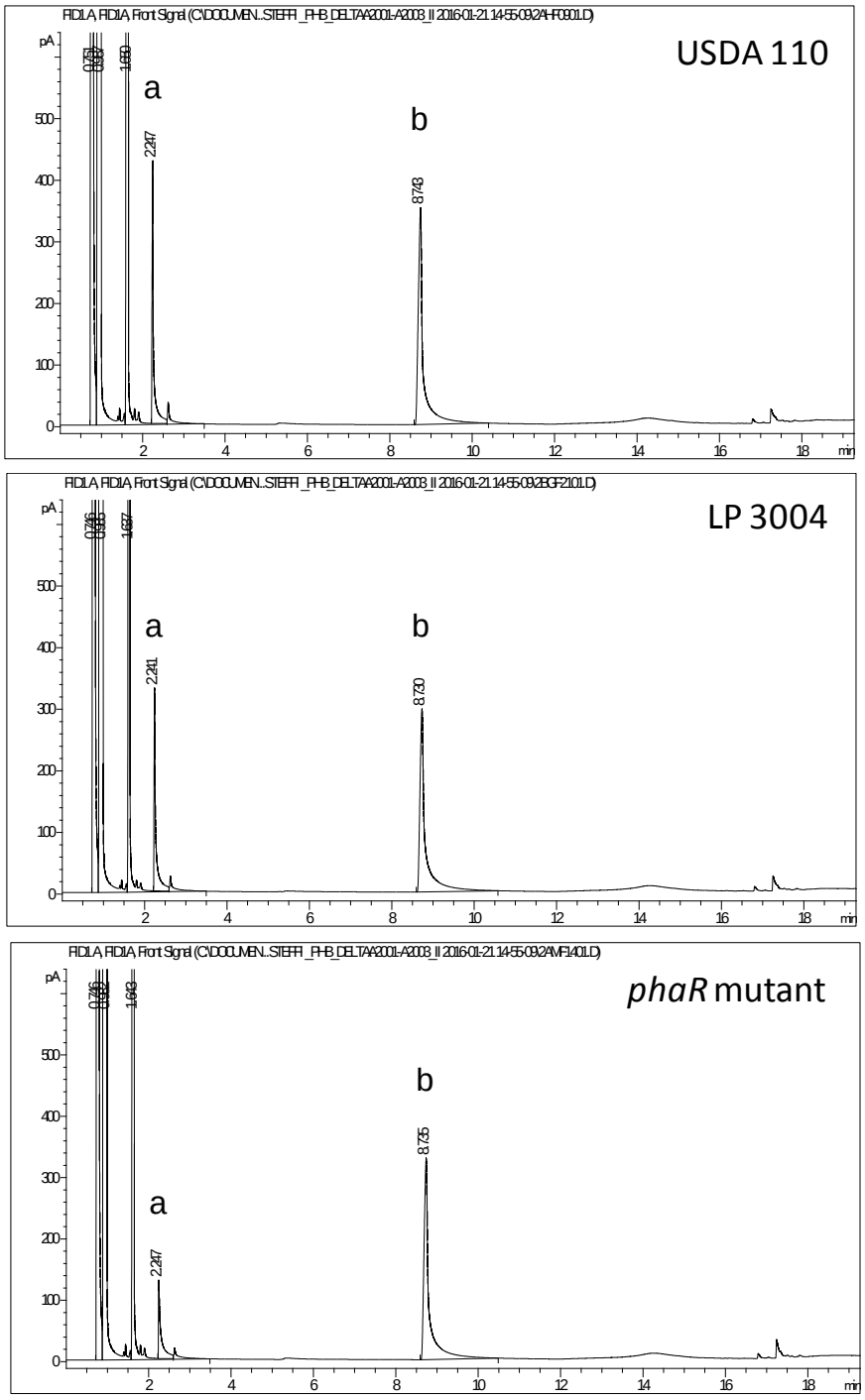

FIG S2 GC-chromatograms of *B. diazoefficiens* samples at late stationary-phase (25 days) cultured in Götz minimal medium in oxic conditions. The peak at 2.24 ± 0.006 min (a) represents 3-hydroxybutyryl-methyl esters, and the peak at 8.73 ± 0.013 min (b) represents benzoate-methyl ester (internal standard). There is no evidence of substantial amounts of 3-HV or other hydroxyalkanoic acids

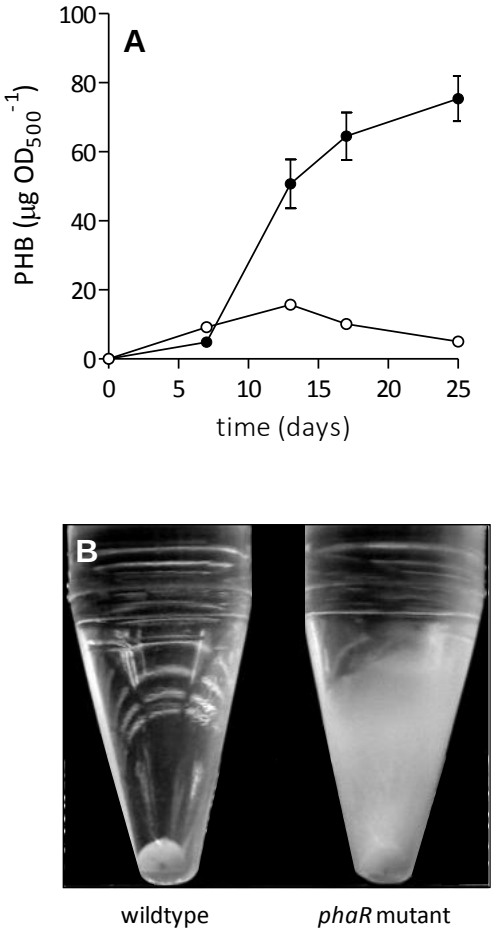

FIG S3 PHB and EPS accumulation in *B. diazoefficiens*. (A) Time-course of PHB accumulation of wildtype (black circles) and *phaR* mutant (white circles) in Götz minimal medium under oxic conditions. (B) Cell pellets at late-stationary phase (25 days). Note the compactness of the wildtype in comparison with the mucoidy of *phaR* mutant.

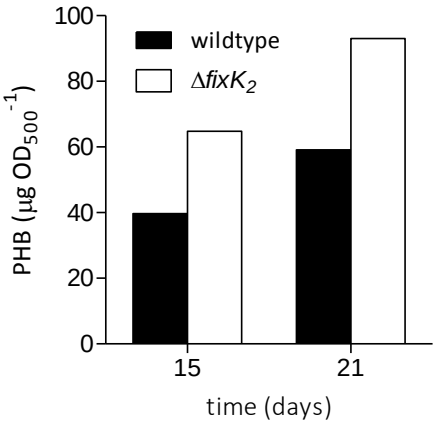

FIG S4 PHB accumulation of *B. diazoefficiens* wildtype (*black bars*) and Δ*fixK*$_2$ (*white bars*) at middle-stationary phase of growth in Götz minimal medium under oxic conditions.

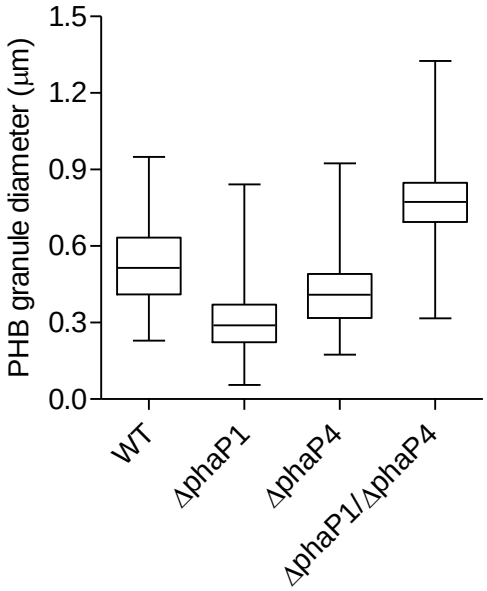

FIG S5 PHB granule diameters in wildtype and their *phaP* mutant derivatives at middle-stationary phase (17 days) of growth cultured in Götz minimal medium under oxic conditions. Each data point represents the mean of 176 (wildtype), 411 (Δ*phaP1*), 151 (Δ*phaP4*), and 132 (Δ*phaP1*/Δ*phaP4*) individual measurements of PHB granule diameters.

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