

Supplementary Material to

Engineering phosphate-controlled poly(3-hydroxybutyrate) production in *Vibrio natriegens* from renewable feedstocks

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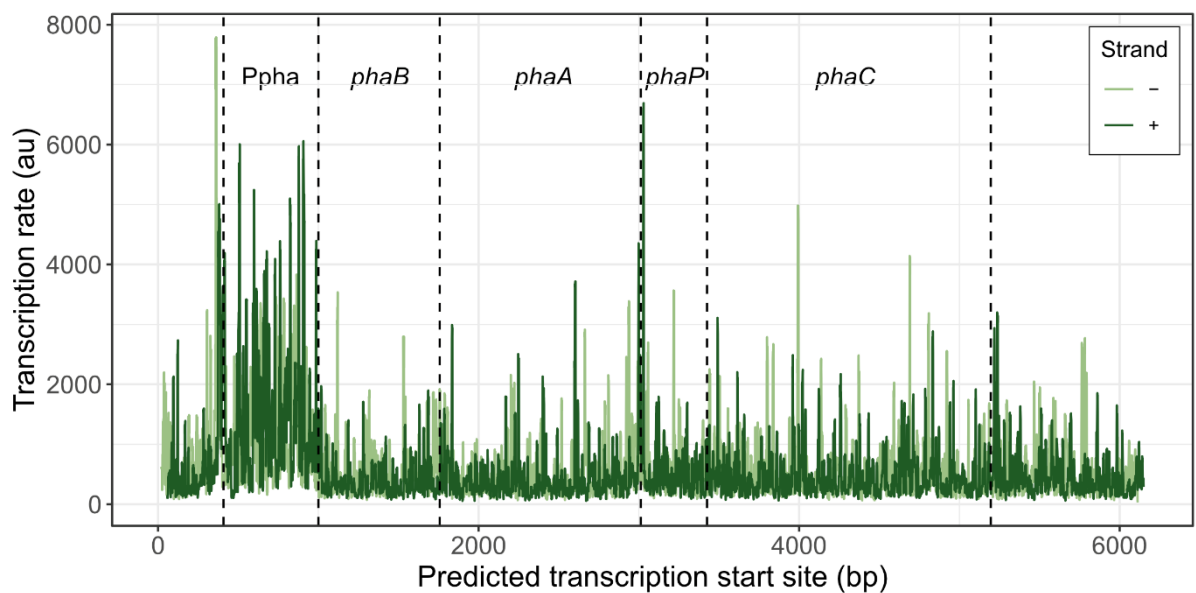
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34 **Figure S1. *In silico* prediction of transcription start sites across the *phaBAPC* operon and its**
35 **upstream region.** Transcription initiation rates were predicted using the Promoter Calculator algorithm
36 of LaFleur et al. (2022) (LaFleur et al., 2022), which scans a genomic sequence and estimates the
37 strength of σ^{70} promoters at single-base resolution. For each position along the operon locus (x-axis, in
38 bp), the predicted transcription rate (y-axis, arbitrary units) is shown for the plus (dark green) and minus
39 (light green) strands. Dashed vertical lines demarcate the predicted promoter region (P_{pha}) and the
40 operon coding sequences (*phaB*, *phaA*, *phaP*, *phaC*). The strongest predicted initiation signals form a
41 dense cluster within the P_{pha} region immediately upstream of the operon, identifying it as the dominant
42 promoter driving *phaBAPC* expression; the 592 bp segment used to construct the P_{pha} biosensor (see
43 Methods; Fig. 1A) was taken from this region. The comparatively weak, scattered peaks within the
44 downstream coding sequences likely represent minor internal sense or antisense initiation and are not
45 expected to contribute substantially to operon expression.

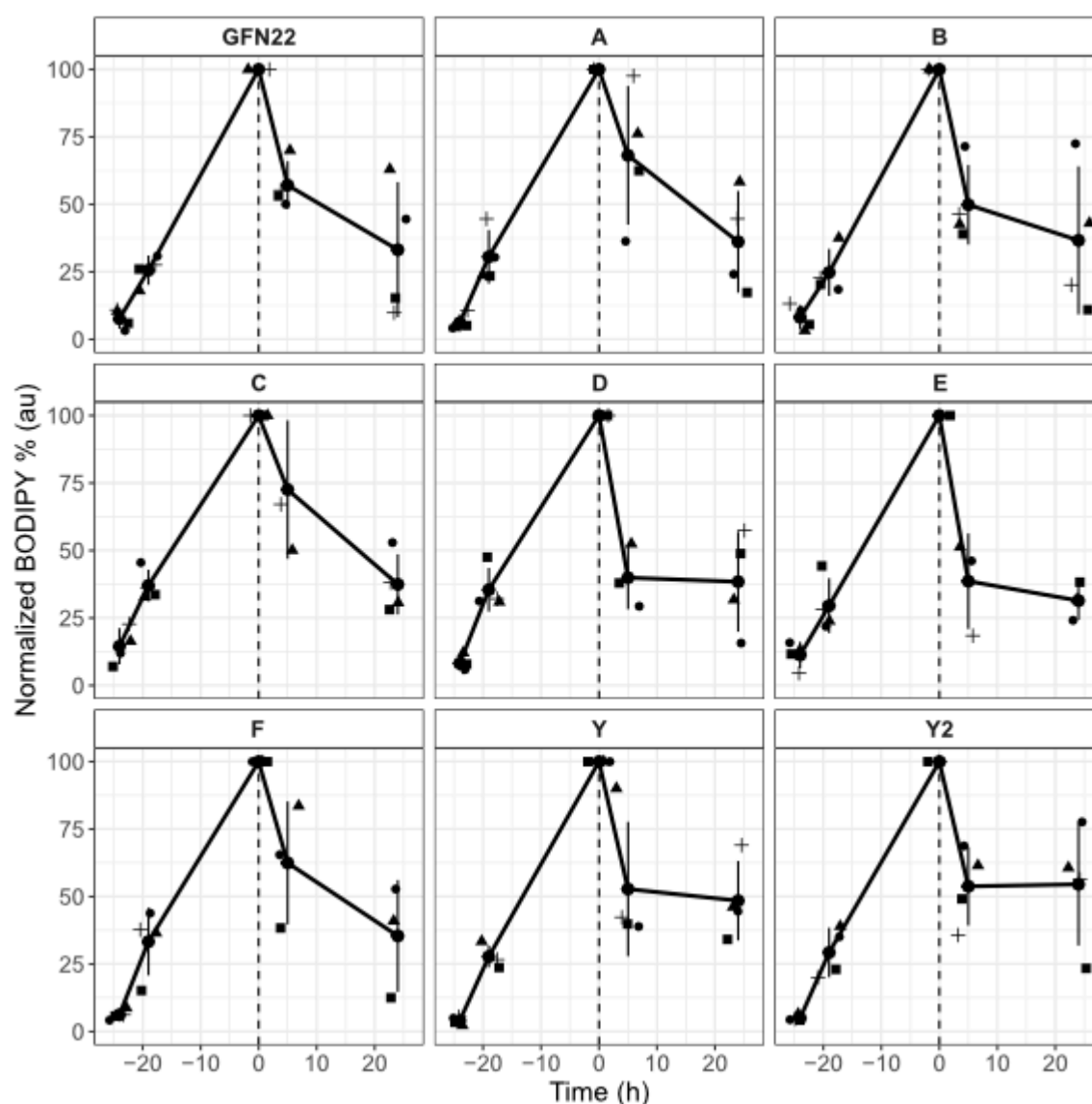


Figure S2. Characterization of candidate PHB depolymerases in *V. natriegens* by tandem starvation. Each panel shows a single strain: the parental strain GFN22; the candidate depolymerase deletion mutants A (PN96_09330), B (PN96_23160), C (PN96_18310), D (PN96_11440), E (PN96_18285), and F (PN96_15765); and the previously characterized oligomer-hydrolase mutants *phaY* (Y; PN96_21190) and *phaY2* (Y2; PN96_21745), included as controls (Politan et al., 2025). Full annotations of the knockout targets are listed in Table S2. Cells were grown in minimal medium with acetate as the sole carbon source, subjected to phosphate (Pi) starvation for 24 h to drive PHB accumulation (cf. Fig. 2A), then shifted to Pi-replete, carbon-free medium to impose carbon starvation and induce PHB mobilization. Intracellular PHB was monitored by BODIPY fluorescence, normalized to each strain's peak value (%); the dashed line marks the transition from the accumulation phase to the mobilization phase. Individual biological replicates are shown as distinct symbols.

Table S1. Reported natural PHB production across *Vibrio* species and the conditions linked to it. For each study, the table lists the strain, cultivation medium, carbon source(s) tested, the factor(s) reported to affect PHB production, and the corresponding reference. Across these reports, PHB accumulation has been attributed to carbon source, nitrogen availability, salinity (NaCl), oxygenation, temperature, pH, or quorum sensing, the last demonstrated specifically in *Vibrio harveyi* (Miyamoto et al., 1998; Sun et al., 1994). Phosphate limitation does not appear among the reported triggers in any of these studies, in contrast to the phosphate-dependent regulation of the *phaBAPC* operon described here for *V. natriegens*. NA, not available.

Strain	Media	Carbon source	Reported regulatory factor(s)	Reference(s)
<i>Vibrio harveyi</i>	Complex medium	Glycerol	Quorum sensing	(Miyamoto et al., 1998; Sun et al., 1994)
<i>Vibrio</i> spp. (98% 16S homology to <i>V. natriegens</i>)	Marine Basal medium	Glycerol, sucrose, glucose, acetate, succinate	Carbon source	(Chien et al., 2007)
<i>Vibrio harveyi</i>	Egorova's Fish-peptone agar medium	NA	Unknown	(Boyandin et al., 2008)
<i>Vibrio</i> sp. BM-1	GYT-medium	Glucose, fructose, molasses, acetate, mannitol, glycerol	Nitrogen, carbon source, NaCl	(Wei et al., 2011)
<i>Vibrio</i> sp. KN01	Mineral salt medium	Glucose, fructose, gluconate, soybean oil	Carbon source, oxygenation	(Numata and Doi, 2012)
<i>Vibrio azureus</i> BTKB33	PHA production medium	Glucose, glycerol, lactose, mannitol, sodium acetate, starch, sucrose	Nitrogen, carbon source, oxygenation, pH, NaCl	(Sasidharan et al., 2015)

<i>Vibrio harveyi</i> MCCB 284	ZoBell's marine broth	Glycerol, sucrose, mannitol, starch, glucose, acetate, fructose, xylose	Temperature, carbon source, pH, NaCl	(Mohandas et al., 2017)
<i>Vibrio proteolyticus</i>	M9 with 5% NaCl	Glycerol, glucose, fructose, sucrose, maltose	Nitrogen, carbon source, NaCl	(Hong et al., 2019)
<i>Vibrio alginolyticus</i>	TYS medium	Glycerol, sucrose, starch, acetate, butyrate, oleic acid, linoleic acid	Carbon source, NaCl, Temperature	(Li et al., 2021)
<i>Vibrio</i> sp. ArtGut-C1	Ramsay saline glucose culture medium	Glucose	Nitrogen	(Yévenes et al., 2021)
<i>Vibrio splendidus</i> 12B01	Marine broth	Glucose	Nitrogen (during multicellular aggregation)	(Schwartzman et al., 2022)

Table S2. Candidate PHA depolymerase genes identified in the *V. natriegens* genome.

Candidates were retrieved by searching profile hidden Markov models of all PHA depolymerase homologous families from the PHA Depolymerase Engineering Database against the *V. natriegens* KEGG genome (organism code vna), retaining only genes predicted to encode an α/β -hydrolase domain (see Methods). For each candidate the table gives the single-letter code used in Fig. S2, the PN96_ locus tag, the assigned PHA depolymerase homologous family, the KEGG functional annotation, the EC number, and the KEGG Orthology (KO) identifier. Codes Y and Y2 correspond to the previously characterized oligomer-hydrolase genes *phaY* and *phaY2*, included as reference controls. Individual deletion mutants of each gene were assayed for PHB mobilization in a tandem starvation experiment (Fig. S2). Blank cells indicate that no EC number or KO identifier is assigned. ^a As listed on the PHA Depolymerase Engineering Database.

Code	Locus tag (PN96_)	Homolog Family ^a	KEGG annotation	EC NUMBER	KEGG Orthology
A	09330	Intracellular nPHAscl depolymerases (lipase box)	Histidine kinase	3.1	K01175 (esterase)
B	23160	Intracellular nPHAscl depolymerases (lipase box)	Lysophospholipase		No KO assigned
C	18310	Intracellular nPHAscl depolymerases (lipase box)	3-oxoadipate enol-lactone hydrolase	3.1.1.24	K01055 (3-oxoadipate enol-lactonase)
D	11440	Intracellular nPHAscl depolymerases (lipase box)	2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase		No KO assigned
E	18285	Extracellular dPHAscl depolymerases (catalytic domain type 1), homologous family 13	Hypothetical protein		K06889 (uncharacterized protein)
F	15765	Intracellular nPHAmcl depolymerases	Hydrolase		No KO assigned
Y	21190	Extracellular dPHAscl depolymerases (catalytic domain type 1), homologous family 13	D-(-)-3-hydroxybutyrate oligomer hydrolase (PhaY)	3.1.1.22	K07518 (hydroxybutyrate-dimer hydrolase)

Y2	21745	Intracellular nPHAscl depolymerases (lipase box)	Hypothetical protein (PhaY2)		No KO assigned
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79 **Table S3. Bacterial strains used in this study.**

Internal strain name	Plasmid	Genotype	Remarks	Reference or source
GFN22	NA	<i>V. natriegens</i> ATCC14048 Δdns $\Delta VNP1+2$	Parental strain	(Politan et al., 2025)
GFN1-057	pRP1_083	GFN22	P _{pha} promoter characterization strain with <i>lux</i> cassette	This study
GFN119	NA	GFN22 $\Delta phaBAPC$	<i>phaBAPC</i> operon deficient control strain	This study
GFN70	NA	GFN22 <i>vps::P_{pha}-phaBAPC</i>	Strain 2X, 2 chromosomal copies of the <i>phaBAPC</i> operon	This study
GFN69	NA	GFN22 <i>phaY::P_{pha}-phaBAPC vps::P_{pha}-phaBAPC</i>	Strain 3X, 3 chromosomal copies of the <i>phaBAPC</i> operon	This study
GFN100	NA	GFN22 <i>phaY::P_{pha}-phaBAPC vps::P_{pha}-phaBAPC fli::P_{pha}-phaBAPC</i>	Strain 4X, 4 copies of the <i>phaBAPC</i> operon	This study
GFN101	NA	GFN22 <i>phaY::P_{pha}-phaBAPC vps::P_{pha}-phaBAPC fli::P_{pha}-phaBAPC VNP1::P_{pha}-phaBAPC</i>	Strain 5X, 5 chromosomal copies of the <i>phaBAPC</i> operon	This study
GFN48	NA	GFN22 AE2 rep 1	GFN22 adapted to 122 mM NaOAc for 1000 generations	(Politan et al., 2025)

GFN225	NA	GFN22 Δ PN96_09330	Depolymerase candidate A	This study
GFN227	NA	GFN22 Δ PN96_23160	Depolymerase candidate B	This study
GFN118	NA	GFN22 Δ PN96_21190	Depolymerase candidate Y (phaY)	(Politan et al., 2025)
GFN229	NA	GFN22 Δ PN96_18310	Depolymerase candidate C	This study
GFN231	NA	GFN22 Δ PN96_11440	Depolymerase candidate D	This study
GFN408	NA	GFN22 Δ PN96_18285	Depolymerase candidate E	This study
GFN409	NA	GFN22 Δ PN96_15765	Depolymerase candidate F	This study
GFN139	NA	GFN22 Δ PN96_21745	Depolymerase candidate Y2	(Politan et al., 2025)

81 **Table S4. Oligonucleotides used to assemble the tDNA plasmid for the *phaY* locus.** All sequences
 82 written as 5' -> 3'.

Plasmid	Internal Plasmid name	forward oligonucleotide	reverse oligonucleotide	PCR template	Entry vector		
phaY tDNA plasmid 5' homolog y	pRP0*_5' _phaY	GF142 5	AAAAGGTCTC GCTCGAACAG CAGACACATT AAACGGCG	GF142 6	AAAAGGTCTCC CTCACTCCCAAT CCAACGTAAC CACTAATCC	V. <i>natriegens</i> DNA	2973
phaY tDNA plasmid 3' homolog y	pRP0*_3' _phaY	GF142 7	AAAAGGTCTC ACTCGCGCTG TCCGCTGGCA GGATAATACG	GF142 8	AAAAGGTCTCC CTCAAGCTATG CCTCTAAAAAAT AATAAGCTGATT ATGAC	V. <i>natriegens</i> DNA	3131
phaY gDNA	pST_116 _phaY	GF134 1	GTCCCAGAAT CCTAGTAAAG ACTG	GF134 2	AAACCAGTCTTT ACTAGGATTCTG		pST_116

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84 **Table S5. Plasmids used in this study.**

Internal cryostock number	Internal plasmid name	Remarks	Parts
GFMC0-350	pMC0_2-3_1_Ppha+RBS native Vn	Lvl 0 P_{pha} +RBS native Vn	pMC0_V_01_Part_Entry_sfGFP, P_{pha} amplicon (see Methods)
GFMC1-019	pRP1_013	P_{pha} - <i>mScarlet</i> biosensor plasmid	pMC0_8_18_Akan (sfgfp)(Vn), pMC0_7_04_OpMB1-M, pMC0_1_02_5C1CLN, pMC0_2-3_1_Ppha+RBS native Vn, pMC0_4_12_CDSmScarlet-I (Vn), pMC0_5_03_TB0015, pMC0_6_16_3C5CSN
GFMC1-128	pRP1_037	Empty plasmid control	(Faber et al., 2025)
GFMC1-012	pRP1_011	P_{pha} - <i>phaBAPC</i> operon transcriptional unit	pMC0_8_18_Akan (sfgfp)(Vn), pMC0_7_04_OpMB1-M, pMC0_1_02_5C1CLN, pMC0_2-3_1_Ppha+RBS native Vn, pMC0_4_30_CDSphaBAPC (Vn), pMC0_5_03_TB0015, pMC0_6_16_3C5CSN
No cryostock	pRP2_tDNA_fli::pha	tDNA plasmid to replace <i>fli</i> operon with a P_{pha} - <i>phaBAPC</i> operon	pRP2_004, pRP1_011
No cryostock	pRP2_tDNA_ph aY::pha	tDNA plasmid to replace <i>phaY</i> gene with a P_{pha} - <i>phaBAPC</i> operon	pRP2_006, pRP1_011
No cryostock	pRP2_tDNA_VN P1::pha	tDNA plasmid to replace VNP1 region with a P_{pha} - <i>phaBAPC</i> operon	pRP2_008, pRP1_011

No cryostock	pRP2_tDNA_vps ::pha	tDNA plasmid to replace <i>vps</i> operon with a P_{pha^-} <i>phaBAPC</i> operon	pRP2_007, pRP1_011
GFMC2-014	pST_116	NT-CRISPR single gRNA entry vector	(Stukenberg et al., 2022)
GFMC2-048	pST_116_fliR	NT-CRISPR plasmid targeting <i>fli</i> operon	(Faber et al., 2025)
GFMC2-034	pST_116_phaY	NT-CRISPR plasmid targeting <i>phaY</i> gene	(Faber et al., 2025)
GFMC2-052	pST_116_VNP1	NT-CRISPR plasmid targeting VNP1 region	(Faber et al., 2025)
GFMC2-036	pST_116_vps	NT-CRISPR plasmid targeting <i>vps</i> operon	(Faber et al., 2025)

Table S6. Oligonucleotides used to perform NT-CRISPR in this study.

Code	Locus (PN96)	Primer purpose	Primer name	Primer sequence
A	09330	gRNA_F	GF1960	GTCCAGGCCTGTTGAAAAATTACG
		gRNA_R	GF1961	AAACCGTAATTTTTCAACAGGCCT
		tDNA_UP_F	GF1962	AACTTACCCGTGTAAAGGCCA
		tDNA_UP_R	GF1963	GTTAAGCCGAAATGTGTTTATCTTATTCGTGA TGGGTTTAACG
		tDNA_DOWN_F	GF1964	CATCACGAATAAGATAAACACATTTTCGGCTT AACAAATTCACC
		tDNA_DOWN_R	GF1965	CGAAGTGGTGACGAGTGAC
		cPCR_F	GF1966	AGGAAAGAAGCACCTGC
		cPCR_R	GF1967	CACTGTTAACCAAGTAAAGTTAATGC
B	23160	gRNA_F	GF1968	GTCCCCGGGAATAAGTACCACCGG
		gRNA_R	GF1969	AAACCCGGTGGTACTTATTCCCGG
		tDNA_UP_F	GF1970	CAATGTACAAACGCTGTATAGGTATTTTCATA TTCAAGTCCTTCAC
		tDNA_UP_R	GF1971	CCAAGAATGCCCATACCTTGA
		tDNA_DOWN_F	GF1972	GTATTGGTGTATATCGCAAGTTTTCTC
		tDNA_DOWN_R	GF1973	CTTGAATATGAAAATACCTATACAGCGTTTGT ACATTGAACC
		cPCR_F	GF1974	ACGAGCAGAAATCAACTATCC
		cPCR_R	GF1975	GATCCTATCGAAGATGGCGTTTTAC
C	18310	gRNA_F	GF1976	GTCCCGTGCTAACCAACACAGGCG
		gRNA_R	GF1977	AAACCGCCTGTGTTGGTTAGCACG
		tDNA_UP_F	GF1978	CCTGAATATTTCAAACCCATGCGATCTCCTT GTTTAAGC
		tDNA_UP_R	GF1979	TCTAGCCCGACTGACTCATGC
		tDNA_DOWN_F	GF1980	GGTCTAACGGTGCAGTGAGTTTC
		tDNA_DOWN_R	GF1981	CAAGGAGATCGCATGGGTTTGAAATATTCAG GCACGCCACG
		cPCR_F	GF1982	CAACAGCAAATTCGAGCAGC
		cPCR_R	GF1983	CGCACTCCAATTACAACGAACAG
D	11440	gRNA_F	GF1992	GTCCACATTGTTGGCAAAGAACAG
		gRNA_R	GF1993	AAACCTGTTCTTTGCCAACAATGT
		tDNA_UP_F	GF1994	GCAACTTTAAGAGTAACTTTGTCTATCCGAT GTTGTCAATGAACTAAG
		tDNA_UP_R	GF1995	CTAAATTCCTCTACGGAGCTCAG
		tDNA_DOWN_F	GF1996	GGAAATAGATCACCACGGCCATC
		tDNA_DOWN_R	GF1997	CATTGACAACATCGGATAGACAAAGTTTACT CTTAAAGTTGCATTCAAC
		cPCR_F	GF1998	GATAGAGGCAAACAACGCC
		cPCR_R	GF1999	GAAGTCATTTGGGTATATACAGG
E	18285	gRNA_F	GF2000	GTCCGTTGTGGTTTCTCATCCGGG
		gRNA_R	GF2001	AAACCCCGGATGAGAAACCACAAC
		tDNA_UP_F	GF2002	GCTGGACGAATCCCTTTGATGG
		tDNA_UP_R	GF2003	GATTTGTTTACAGTGGTGCATTGCTCCTAAC TAGAGTTC

		tDNA_DOWN_F	GF2004	GTTAGGAGCAATGCACCACTGTAAACAAATC TAAGTTAATTCTCG
		tDNA_DOWN_R	GF2005	GGACAAGCTCAAAGTGTAATGC
		cPCR_F	GF2006	GAGATCTCAAGAAGACACCACG
		cPCR_R	GF2007	GAATTAACGCTGACATATCCCACG
F	15765	gRNA_F	GF2016	GTCCGAATCGACTCTACCCAACCA
		gRNA_R	GF2017	AAACTGGTTGGGTAGAGTCGATTC
		tDNA_UP_F	GF2018	CATTTCAACGCCACCTATTGAAGCTTTACTCA CGGCTCG
		tDNA_UP_R	GF2019	TTGATTTAGGCATCGAATTGTTTGACC
		tDNA_DOWN_F	GF2020	GGTCTGGACTTTACTTATGAACCG
		tDNA_DOWN_R	GF2021	CGTGAGTAAAGCTTCAATAGGTGGCGTTGAA ATGTCTTGC
		cPCR_F	GF2022	GATTTTCATTGTCTGAAGCATTCC
		cPCR_R	GF2023	GTGTCGCCGTTGAACAATGG

ODE Model description

We describe PHB production by the 5X strain under phosphate starvation with a system of four coupled ordinary differential equations governing the time evolution of acetate (S), biomass (X), phosphate (P_i), and PHB (P). The process is represented as two phases, a phosphate-replete growth phase and a phosphate-starvation production phase, distinguished by a binary switch that depends on whether phosphate has fallen below a threshold concentration ($P_{i_{threshold}}$). The biomass-specific acetate uptake rate (q_s) follows Monod kinetics in acetate, with distinct maximum rates before and during phosphate starvation:

$$q_s = \frac{q_{s,max,g} \times S}{K_{s,g} + S} \text{ when } P_i > P_{i_{threshold}} \quad (1)$$

$$q_s = \frac{q_{s,max,p} \times S}{K_{s,p} + S} \text{ when } P_i < P_{i_{threshold}}$$

where $q_{s,max,g}$ and $q_{s,max,p}$ are the maximum acetate uptake rates before (growth phase) and during (production phase) phosphate starvation, and $K_{s,g}$ and $K_{s,p}$ are the corresponding half-saturation constants for acetate (S).

The biomass-specific phosphate uptake rate (q_{P_i}) follows Monod kinetics in phosphate, multiplied by a Monod term in acetate so that phosphate uptake falls to zero once acetate is depleted:

$$q_{P_i} = \frac{q_{P_i,max} \times P_i}{K_{P_i} + P_i} \times \frac{S}{K_s + S} \quad (2)$$

where $q_{Pi,max}$ is the maximum biomass-specific phosphate uptake rate, K_{Pi} is the half-saturation constant for phosphate (the concentration at which q_{Pi} reaches half of $q_{Pi,max}$) and $K_{\frac{s}{Pi}}$ is the half-saturation constant for acetate.

The biomass-to-acetate yield ($Y_{\frac{x}{s}}$) depends on phosphate, and the specific growth rate (μ) is defined as the product of acetate uptake and yield:

$$Y_{\frac{x}{s}} = Y_{\frac{x}{s},max,g} \times \frac{Pi}{K_{\frac{x}{Pi}} + Pi} + Y_{\frac{x}{s},max,p} \quad (3)$$

$$\mu = q_s \times Y_{\frac{x}{s}} \quad (4)$$

where $Y_{\frac{x}{s},max,g}$ and $Y_{\frac{x}{s},max,p}$ are the maximum biomass yields before and during phosphate starvation, and $K_{\frac{x}{Pi}}$ is the half-saturation constant for phosphate at which the yield reaches half of $Y_{\frac{x}{s},max,g}$. Defining μ as the product of q_s and $Y_{\frac{x}{s}}$ reproduces the biomass-specific substrate-uptake relationship of (24).

PHB productivity (q_p) is described by a Hill function of both phosphate and acetate:

$$q_p = q_{p,max} \times \frac{Pi_{threshold}^n}{Pi_{threshold}^n + Pi^n} \times \frac{s^m}{s^m + K_p^m} \quad (5)$$

where $q_{p,max}$ is the maximum PHB productivity, $Pi_{threshold}$ and K_p are the phosphate and acetate concentrations at which q_p is half-maximal, and m and n are the Hill coefficients that dictate the steepness of the q_p response to acetate and phosphate, respectively.

Finally, the dynamics of the four metabolites are given by the following mass-balance equations:

$$\frac{dX}{dt} = \mu \times X \quad (6)$$

$$\frac{dS}{dt} = -(q_s + q_p) \times X \quad (7)$$

$$\frac{dPi}{dt} = -q_{Pi} \times X \quad (8)$$

$$\frac{dP}{dt} = q_p \times X \quad (9)$$

Parameter optimization

The model contains 15 growth parameters, which were optimized to fit the model curves to the experimental data by minimizing the total weighted chi-square (χ^2):

$$\chi^2 = \sum_{i=1}^p \sum_{j=1}^4 \sum_{k=1}^3 \left(\frac{C_{i,j,k} - \hat{C}_{i,j,k}}{\sigma_{i,j,k}} \right)^2 \quad (10)$$

$$\chi_v^2 = \chi^2 / df \quad (11)$$

where $C_{i,j,k}$ and $\hat{C}_{i,j,k}$ are the experimental and predicted concentrations, respectively, of the four metabolites j (phosphate, acetate, biomass, and PHB) for the three experimental groups with different starting phosphate levels k , at each timepoint i , and $\sigma_{i,j,k}$ is the experimental standard deviation. In Equation 11, df is the number of degrees of freedom in our dataset, and the reduced chi-square (χ_v^2) serves as the goodness-of-fit indicator. Parameter bounds were set to a range of 1.0×10^{-6} to 20.0 (in their respective units shown in Table S7), except for the Hill coefficients n and m (bounded between 1.0 and 5.0) and the biomass yield (bounded between 0 and 2.0). The optimized parameter values contributing to the fitted curves are listed in Table S7.

Table S7. Optimized parameters of the ODE model. The parameters defined in the ODE model (see Supplementary Methods) were estimated by fitting the model to the experimental data with LMFIT and are reported here with their confidence intervals. A wide confidence interval indicates a poorly constrained parameter, whose value can vary substantially without materially changing the fitted curves; a narrow confidence interval indicates a parameter that is tightly constrained and precisely determined by the fit.

Parameters (Unit)	Optimized Value $\pm$ Confidence Interval (relative error %)
$q_{S,max,g}$ (mol/mol biomass/h)	7.83 ± 0.76 (8%)
$q_{S,max,p}$ (mol/mol biomass/h)	0.651 ± 0.540 (83%)
$K_{S,g}$ (mol/L)	0.0794 ± 0.0016 (2%)
$K_{S,p}$ (mol/L)	0.0547 ± 0.0633 (116%)
$Y_{\frac{x}{s},max,g}$ (mol biomass/mol)	0.111 ± 0.027 (25%)
$Y_{\frac{x}{s},max,p}$ (mol biomass/mol)	0.228 ± 0.021 (9%)
$q_{p,max}$ (mol/mol biomass/h)	0.134 ± 0.055 (41%)
$q_{Pi,max}$ (mol/mol biomass/h)	0.00963 ± 0.00086 (9%)
$Pi_{threshold}$ ($\times 10^{-6}$ mol/L)	6.54 ± 0.77 (13%)
K_{Pi} ($\times 10^{-6}$ mmol/L)	8.62 ± 4.06 (47%)
n	1.00 ± 0.00 (0%)
m	3.00 ± 0.87 (29%)
K_p (mol/L)	0.0789 ± 0.0183 (23%)
$K_{\frac{x}{Pi}}$ (mol/L)	0.00250 ± 0.00124 (49%)
$K_{\frac{s}{Pi}}$ ($\times 10^{-5}$ mol/L)	1.56 ± 2.91 (187%)

In principle, fitting an ODE model to data should return a unique optimum for every parameter. In practice, the parameters of biological rate laws are often mathematically coupled, so the

data constrains only certain combinations of them rather than each parameter individually. To assess this, we computed the pairwise correlations between the 15 fitted parameters (Fig. S3). A coefficient near zero (white) means two parameters act independently, so the fit pins down each one uniquely. A coefficient approaching +1 (red) indicates strong positive coupling: an increase in one parameter can be offset by a proportional increase in the other with no change in the fitted curves or in the chi-square. A coefficient approaching -1 (blue) indicates the same coupling with opposite sign, where an increase in one parameter must be compensated by a decrease in the other.

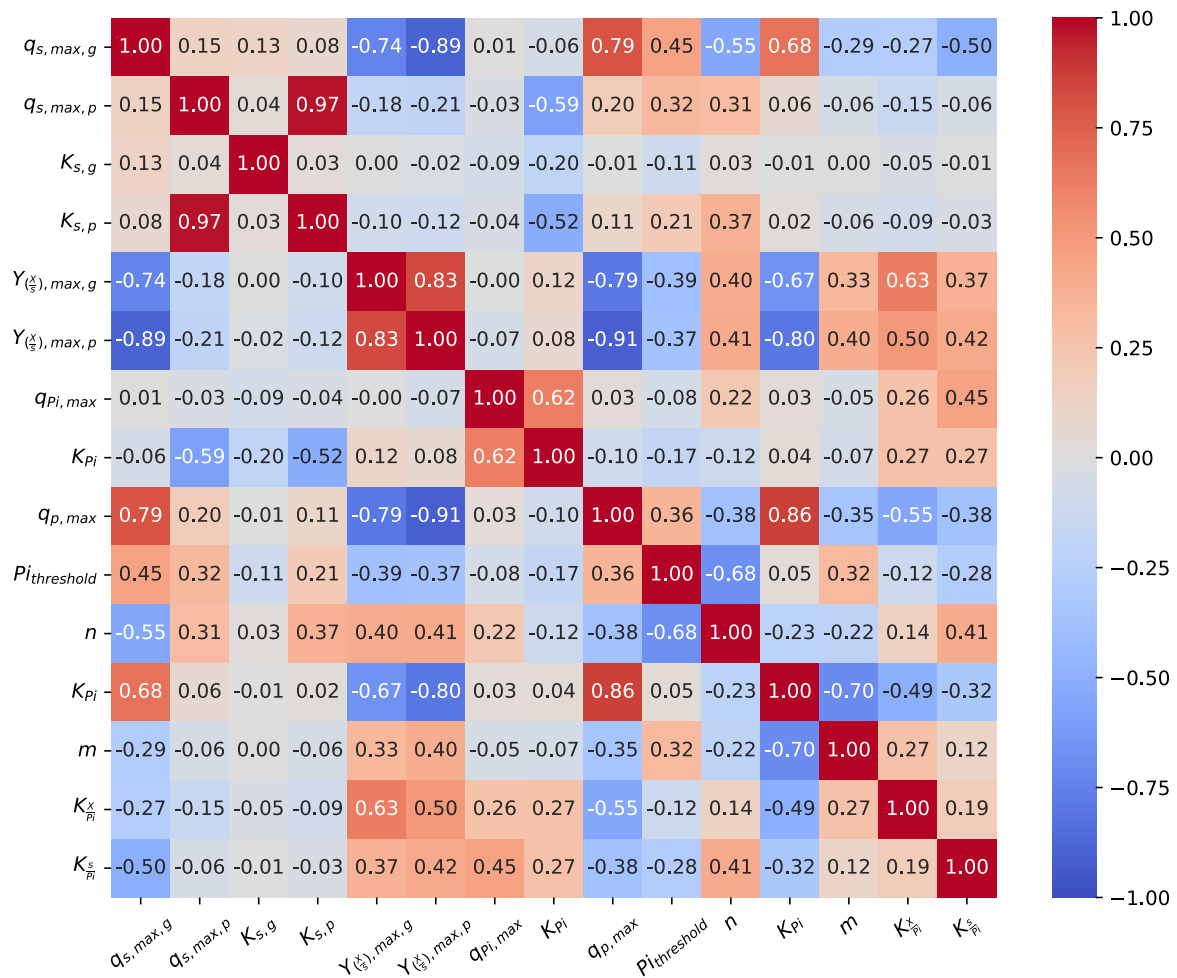


Figure S3. Pairwise correlations between the optimized ODE-model parameters. Heatmap of the correlation coefficients between the 15 fitted growth parameters (Table S7), obtained from the covariance matrix of the fit. Colors run from -1.0 (blue, perfect negative correlation) through 0 (white, no correlation) to +1.0 (red, perfect positive correlation), with the diagonal equal to 1.0 by definition. Coefficients near zero indicate parameters that the data constrain independently, so the fit determines each one uniquely. Coefficients approaching ± 1 indicate parameters that are identifiable only in combination: a strong positive value marks a pair constrained only as a ratio, whereas a strong negative value marks a pair constrained only as a product. For such pairs the optimization recovers the ratio or

product but not the individual parameter values (see Supplementary text). Parameter symbols and units are defined in Table S7.

Among the strongest correlations, two can be traced directly to the model equations and illustrate the two ways parameters become coupled. A strong positive correlation arises when two parameters enter the rate laws only as a ratio. In the production phase, once acetate falls below $K_{S,p}$, the acetate uptake term in Equation 1 reduces to $q_S \approx q_{S,max,p}/K_{S,p} \times S$, so the data constrains only the ratio $q_{S,max,p}/K_{S,p}$; the two parameters are correspondingly positively correlated ($r = 0.97$), because either can be raised with no effect on the fit provided the other is raised in proportion. A strong negative correlation arises when the data constrains only the product of two parameters. The final PHB titer is governed by the product of productivity and biomass, since $\frac{dP}{dt} = q_p \times X$ (Equation 9) and the biomass that accumulates scales with the growth-phase biomass yield through Equations 3 and 4. The PHB data therefore constrain the product $q_{p,max} \times Y_{X/S,p,max}$ rather than either factor alone, so a higher productivity can be exactly compensated by a lower yield, and the two parameters are anticorrelated ($r = -0.91$). For both types of pair, the optimization recovers only the underlying ratio or product, so re-running the fit from different initial guesses returns different absolute values for the individual parameters while leaving the fitted curves and chi-square identical. This coupling is also the origin of the large confidence intervals in Table S7, for instance the relative errors of 83% and 116% for $q_{S,max,p}$ and $K_{S,p}$. The weakly correlated parameters, by contrast, are well constrained and can be read as effective kinetic values, whereas the strongly coupled parameters should be interpreted only as lumped combinations. Importantly, this non-identifiability does not affect the model's predictive use, since the macroscopic metabolite dynamics, and hence the predicted phosphate optimum, depend only on the well-determined parameter combinations.