Supporting Information

Figure Legends

Supplementary Figure 1: Plasmids for the synthesis of PHB. a) p83_PHB_C.nec harboring PHB synthesis genes from *Cupriavidus necator*. b) p83_PHB_B.thai harboring PHB synthesis genes from *Burkholderia thailandensis*. Both plasmids have a pMTL83151 backbone.

Supplementary Figure 2: Growth experiments with *C. ljungdahlii* wildtype, *C. ljungdahlii* [pMTL83151], and *C. ljungdahlii* [p83_tcb] for the production of 3-HB or with *C. ljungdahlii* [p83_PHB_Scaceti] for the production of PHB. a) 3-HB production under heterotrophic conditions using fructose as carbon source. b) 3-HB production under autotrophic conditions with syngas atmosphere. c) PHB production under heterotrophic conditions using fructose as substrate. d) PHB production under autotrophic growth conditions with syngas atmosphere. Bars represent quantified PHB/CDW: yellow bars = *C. ljungdahlii* [pMTL83151]; grey bars = *C. ljungdahlii* [p83_PHB_Scaceti]; Detailed figures are presented in **Supplementary Table 2**; Growth experiments were performed in triplicate.

Supplementary Figure 3: Transmission electron microscopy pictures of recombinant *C. ljungdahlii* at different time points during growth on fructose or syngas. a) *C. ljungdahlii* [p83_PHB_Scaceti] harboring genes for poly-3-hydroxybutyrate production grown on fructose; b) *C. ljungdahlii* [p83_PHB_Scaceti] harboring genes for poly-3-hydroxybutyrate production grown on syngas; c) *C. ljungdahlii* [pMTL83151] harboring empty plasmid (serving as negative control) grown on fructose; d) *C. ljungdahlii* [pMTL83151] harboring empty plasmid (serving as negative control) grown on syngas. Scale bars represent 3 µm.

Supplementary Figure 4: Fluorescence microscopy of heterotrophically grown recombinant *C. ljungdahlii* stained with lipophilic fluorescence dye Nile red to indicate presence of PHB granules in vivo and analysis of isolated PHB. a) Fluorescence microscopy pictures of *C. ljungdahlii* [p83_PHB_Scaceti] harboring genes for PHB production; b) Fluorescence microscopy pictures of *C. ljungdahlii* [pMTL83151] harboring empty plasmid and serving as negative control. Scale bars represent 10 µm.

Supplementary Table Legends

Supplementary Table 1: Genes subcloned and plasmids constructed.

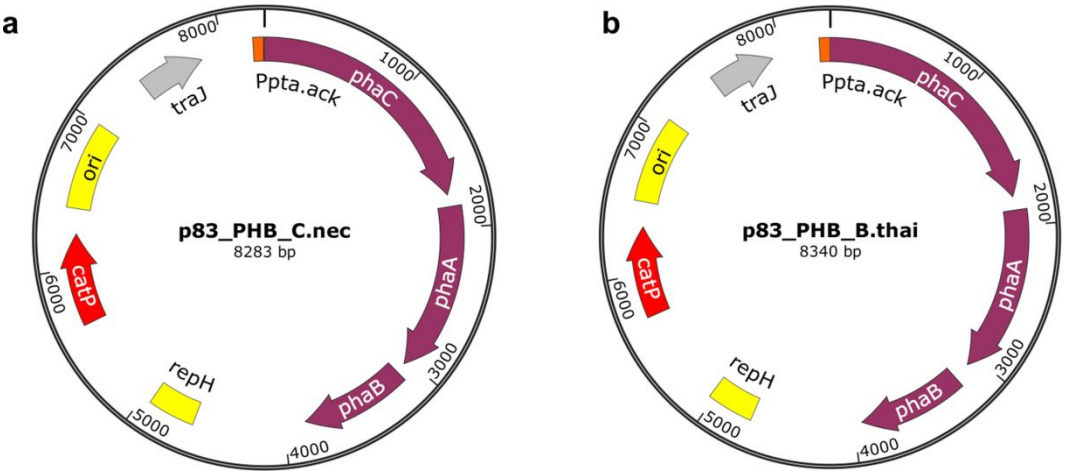
Supplementary Table 2: Primers used for plasmid construction.

Supplementary Table 3: Overview of the PHB and 3-HB gene clusters from *C. necator* ($PHB_{C.nec}$), *B. thailandensis* ($PHB_{B.thai}$), genes from *C. scatologenes* and *C. acetireducens* ($PHB_{Scaceti}$), and genes from *C. acetobutylicum* and *C. difficile* (tcb (3-HB)) with their corresponding numbers of codons, GC content, and the deviation of these codons based on the codon preference of *C. ljungdahlii* and *C. coskatii* for each amino acid.

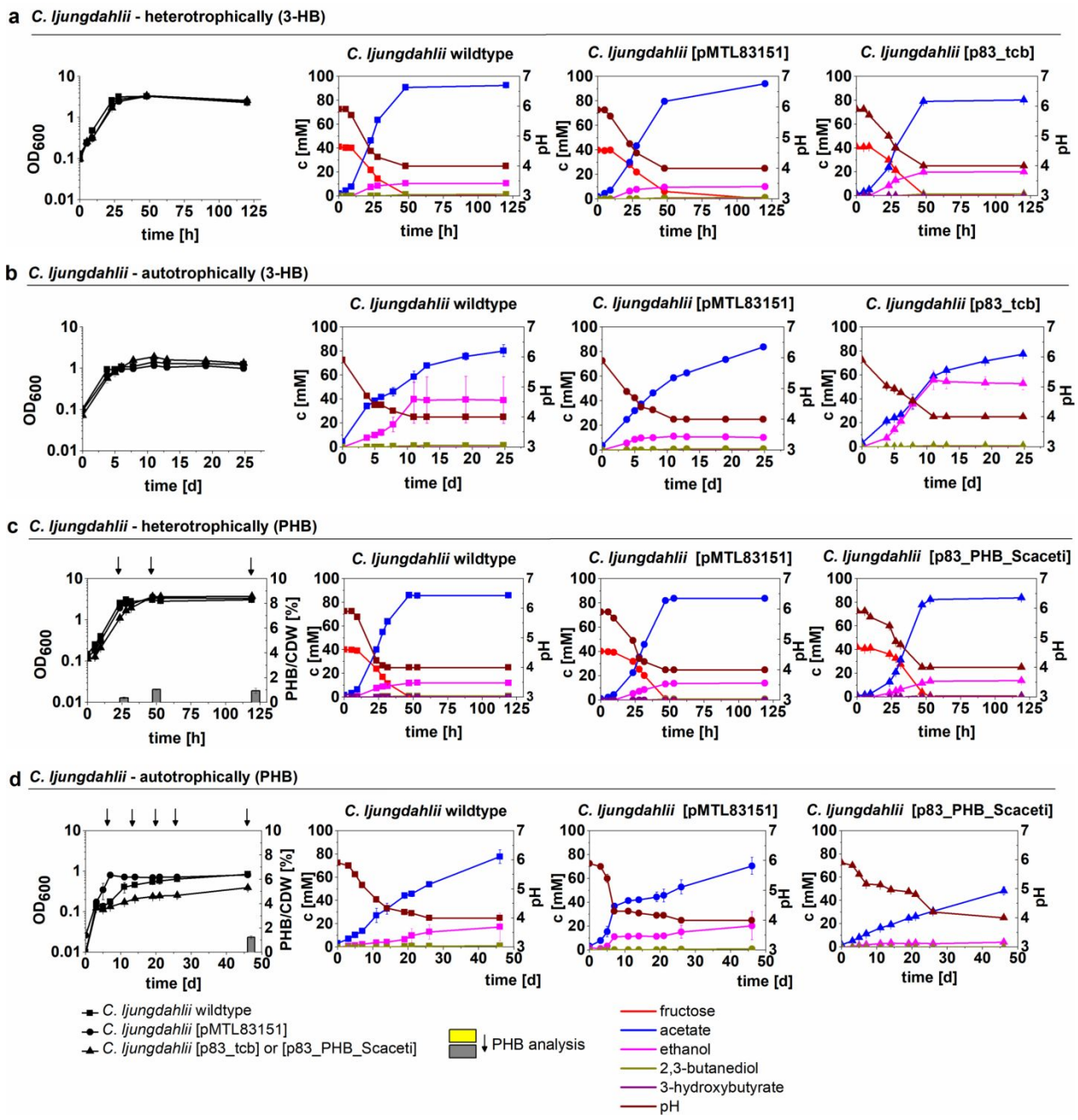
Supplementary Table 4: Max. OD_{600}, fructose consumption, and max. product formation of *C. ljungdahlii* and *C. coskatii* wildtype and recombinant strains shown in Figure 3 and Supplementary Figure 2.

Supplementary Figures

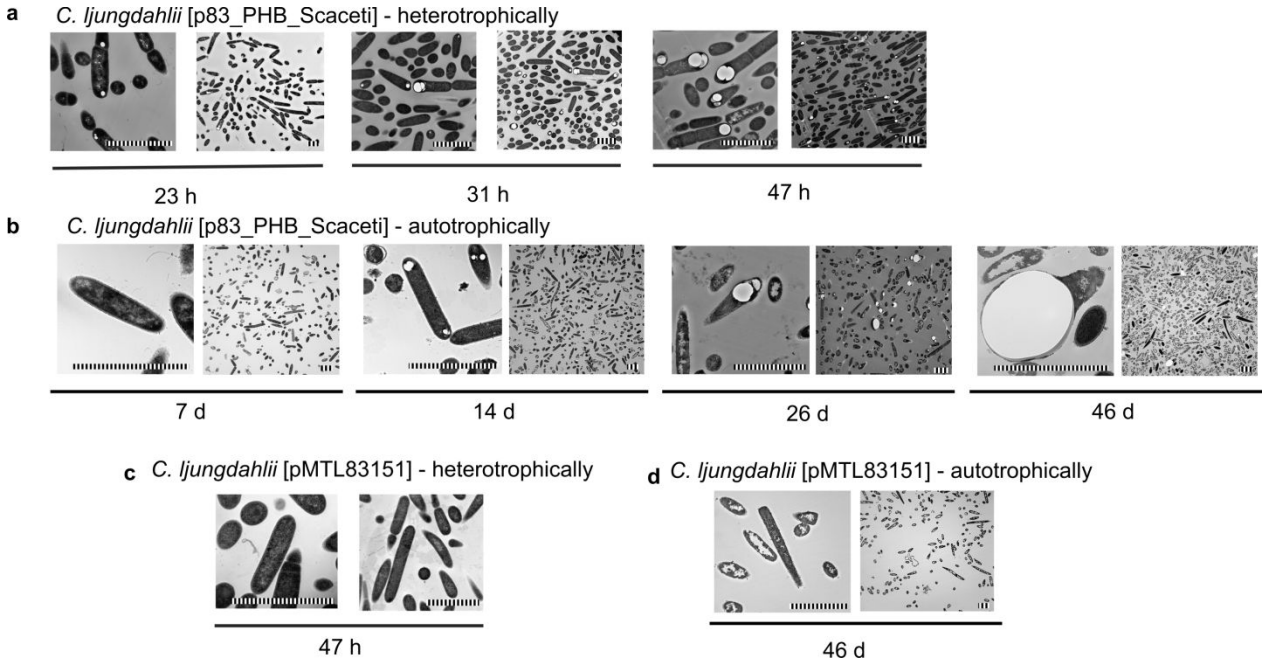
Supplementary Figure 1



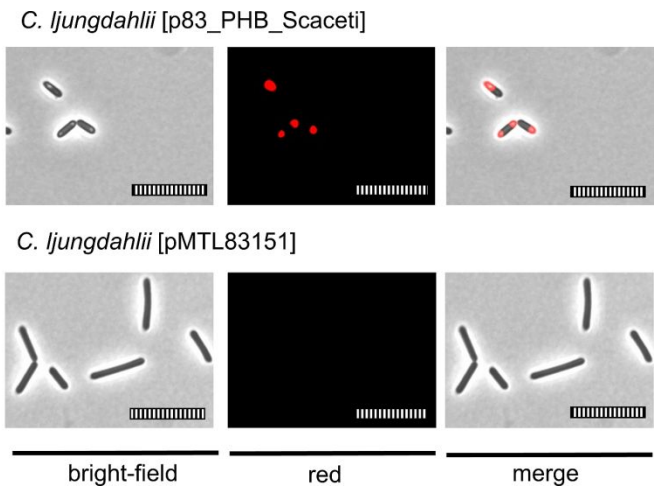
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Table 1

Plasmid/production purpose	Gene abbreviation	Locus	Gene originating from	Gene encoding for
p83_PHB_C.nec/ PHB production	*phaC*	H16_A1437	*Cupriavidus necator*	PHA synthase
	phaA	H16_A1438		β-ketothiolase
	PhaB	H16_A1439		acetoacetyl-CoA reductase
p83_PHB_B.thai/ PHB production	*phaC*	BTHAA_RS07305	*Burkholderia thailandensis*	PHA synthase
	phaA	BTHAA_RS07310		β-ketothiolase
	PhaB	BTHAA_RS07315		acetoacetyl-CoA reductase
p83_tcb/ 3-HB production	*thlA*	CA_C2873	*Clostridium acetobutylicum*	thiolase A
	ctfA/ctfB	CA_P0163/ CA_P0164		acetoacetyl-CoA:acetate/butyrate CoA transferase
	bdhA	CDIF630_02933	*Clostridioides difficile*	3-hydroxybutyrate dehydrogenase
p83_PHB_Scaceti/ PHB production	*thlA*	CSCA_2635	*Clostridium scatologenes*	thiolase A
	hbd	CSCA_2636		3-hydroxybutyryl-CoA dehydrogenase
	crt	CSCA_2637		crotonase
	phaJ	CLAOCE_21160	*Clostridium acetireducens*	(*R*)-enoyl-CoA hydratase
	phaEC	CLAOCE_21150/21140		PHA synthase

Supplementary Table 2

Primer	Sequence (5'→3')	Application
C.nec_phaCAB_F1_ XhoI	GAAAAACTCGAGATGGCGACCGGCAAAGGCGC	Amplification of *phaCAB* gene cluster
C.nec_phaCAB_R1_ NheI	GAAAAAGCTAGCTCAGCCCATATGCAGGCCGCCG	
B.thai_phaCAB_F1_ Eco147I	ATTTTTAGGCCTATGCAACAGTTGTTCGAG	
B.thai_phaCAB_R1_ NheI	ATTTTTGCTAGCTCAGCCCATATGCAAG	
C.lju_Prom_F1_MluI	ACGCGTGTAAAGTATTGACTAGCAAAAT	Amplification of $P_{pta\text{-}ack}$ from *C. ljungdahlii*
C.lju_Prom_F1_XhoI	CTCGAGTTCCTCCCTTTAAATTTAACAC	
C.lju_pta-ack_F1_KpnI	GGTACCGTATTGACTAGCAAAATTTTTTG	
C.lju_pta-ack_R1_Eco147	AGGCCTTTCCTCCCTTTAAATTTAACAC	
Pep_thlA_fw	TTCGCCGTAATCTATAATTAGA	Amplification of *thlA*, *ctfA/B*, and *bdhA* from *C. difficile*
Pep_bdhA_rev	CTCATAAATATCCCTCCTTAGT	
j5_bdhA_fw	GGAAACAGCTATGACCGCGGCCGCCTATTGTGCAGTATATCCTCCATCTAGTAAGC	Construction of p83_tcb
j5_bdhA_rev	CCAATGAACTTAGACCCATGGCTGTTTAGGTACCTGGAGGAAATGAAAATGGTTAAAGATAAAG	
j5_scat7_fw	TATACTTGCCCCCATCCTCAGGTCCTCCCTTTAAATTTAACAC	Construction of p83_PHB_Scaceti
j5_scat7_rev	ACTGGATATTAAAGAGATCTTGACTCGAGAAGTGAGGAGGGATAAAATGGGG	
J5_phaJEC_fw	CCCTCCTCACTTCTCGAGTCAAGATCTCTTTAATATCCAGTCCG	
j5_phaJEC_rev	GGAGGACCTGAGGATGGGGGCAAGTATAGATTATCTTG	

Supplementary Table 3

Recombinant genes	Total no. of codons	GC content [%]	Deviation of codons from recombinant genes sorted by the percentage of preferred codons from *C. ljungdahlii* and *C. coskatii* for each amino acid							
			0 % to < 10 %		10 % to < 20 %		20 % to < 30 %		≥ 30 %	
			no.	%	no.	%	no.	%	no.	%
$PHB_{C.nec}$	1228	66.6	214	17.4	522	42.5	209	17.0	283	23.0
$PHB_{B.thai}$	1236	66.8	370	29.9	381	30.8	213	17.2	272	22.0
$PHB_{Scaceti}$	1814	30.2	17	0.9	79	4.4	233	12.8	1485	81.9
***tcb* (3-HB)**	1089	35.2	20	1.8	66	6.1	167	15.3	836	76.8

Supplementary Table 4

	Strain	OD_{600}	Fructose	Acetate	Ethanol	2,3-BD	3-HB	PHB
3a	***C. coskatii* WT**	3.4 ± 0.17	32.0 ± 0.61	80.3 ± 0.12	0 ± 0	0 ± 0	0 ± 0	- -
	***C. coskatii* [pMTL83151]**	3.4 ± 0.13	30.6 ± 0.60	81.1 ± 0.48	1.8 ± 0.16	0 ± 0	0 ± 0	- -
	***C. coskatii* [p83_tcb]**	3.6 ± 0.22	33.5 ± 0.99	64.9 ± 0.14	0 ± 0	0 ± 0	21.7 ± 0.27	- -
3b	***C. coskatii* WT**	0.63 ± 0.03	0 ± 0	54.2 ± 3.33	2.9 ± 0.12	0 ± 0	0 ± 0	- -
	***C. coskatii* [pMTL83151]**	0.52 ± 0.08	0 ± 0	39.4 ± 3.13	3.8 ± 1.60	0 ± 0	0 ± 0	- -
	***C. coskatii* [p83_tcb]**	0.40 ± 0.05	0 ± 0	34.5 ± 2.57	1.4 ± 0.00	0 ± 0	0.98 ± 0.12	- -
3c	***C. coskatii* WT**	3.0 ± 0.17	30.8 ± 1.36	69.1 ± 0.33	0 ± 0	0 ± 0	0 ± 0	- -
	***C. coskatii* [pMTL83151]**	2.7 ± 0.09	36.0 ± 0.80	76.5 ± 0.53	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	***C. coskatii* [p83_PHB_Scaceti]**	2.4 ± 0.11	25.4 ± 2.31	56.5 ± 0.87	0 ± 0	0 ± 0	0 ± 0	3.4 ± 0.29
3d	***C. coskatii* WT**	0.41 ± 0.02	0 ± 0	58.1 ± 1.02	2.2 ± 0.29	0 ± 0	0 ± 0	- -
	***C. coskatii* [pMTL83151]**	0.37 ± 0.04	0 ± 0	50.2 ± 1.78	1.8 ± 0.12	0 ± 0	0 ± 0	0 ± 0
	***C. coskatii* [p83_PHB_Scaceti]**	0.43 ± 0.02	0 ± 0	53.2 ± 2.54	2.6 ± 0.21	0 ± 0	0 ± 0	1.2 ± 0.12
Sup. 2a	***C. ljungdahlii* WT**	3.3 ± 0.19	41.1 ± 0.31	92.6 ± 1.73	10.5 ± 0.17	0 ± 0	0 ± 0	- -
	***C. ljungdahlii* [pMTL83151]**	3.3 ± 0.09	39.8 ± 0.42	94.0 ± 1.41	10.1 ± 0.83	0 ± 0	0 ± 0	- -
	***C. ljungdahlii* [p83_tcb]**	3.4 ± 0.18	40.9 ± 0.40	80.2 ± 1.57	20.1 ± 0.57	0 ± 0	0 ± 0	- -
Sup. 2b	***C. ljungdahlii* WT**	1.4 ± 0.20	0 ± 0	80.2 ± 5.00	38.9 ± 19.4	1.4 ± 0.19	0 ± 0	- -
	***C. ljungdahlii* [pMTL83151]**	1.2 ± 0.04	0 ± 0	83.9 ± 1.19	10.3 ± 2.22	0.97 ± 0.09	0 ± 0	- -
	***C. ljungdahlii* [p83_tcb]**	1.9 ± 0.13	0 ± 0	77.4 ± 0.41	52.7 ± 5.18	0.93 ± 0.05	0 ± 0	- -
Sup. 2c	***C. ljungdahlii* WT**	3.1 ± 0.26	40.1 ± 0.78	85.8 ± 1.27	12.1 ± 0.42	0.97 ± 0.05	0 ± 0	- -
	***C. ljungdahlii* [pMTL83151]**	3.4 ± 0.07	40.2 ± 1.11	83.7 ± 1.68	13.9 ± 0.33	1.00 ± 0.0	0 ± 0	0 ± 0
	***C. ljungdahlii* [p83_PHB_Scaceti]**	3.7 ± 0.11	41.9 ± 1.29	83.9 ± 0.41	13.7 ± 0.05	0.93 ± 0.05	0.7 ± 0	1 ± 0.04
Sup. 2d	***C. ljungdahlii* WT**	0.84 ± 0.05	0 ± 0	77.7 ± 5.9	17.3 ± 12.0	1.17 ± 0.05	0 ± 0	- -
	***C. ljungdahlii* [pMTL83151]**	0.82 ± 0.05	0 ± 0	70.5 ± 7.07	20.4 ± 11.7	1.0 ± 0.22	0 ± 0	0 ± 0
	***C. ljungdahlii* [p83_PHB_Scaceti]**	0.39 ± 0.06	0 ± 0	48.2 ± 3.20	4.0 0.31	0 ± 0	0 ± 0	1.2 ± 0.12