Sustainable growth of *Dehalococcoides mccartyi* 195 by corrinoid salvaging and remodeling in defined lactate-fermenting consortia

Running title: Corrinoid salvaging in dechlorinating consortia

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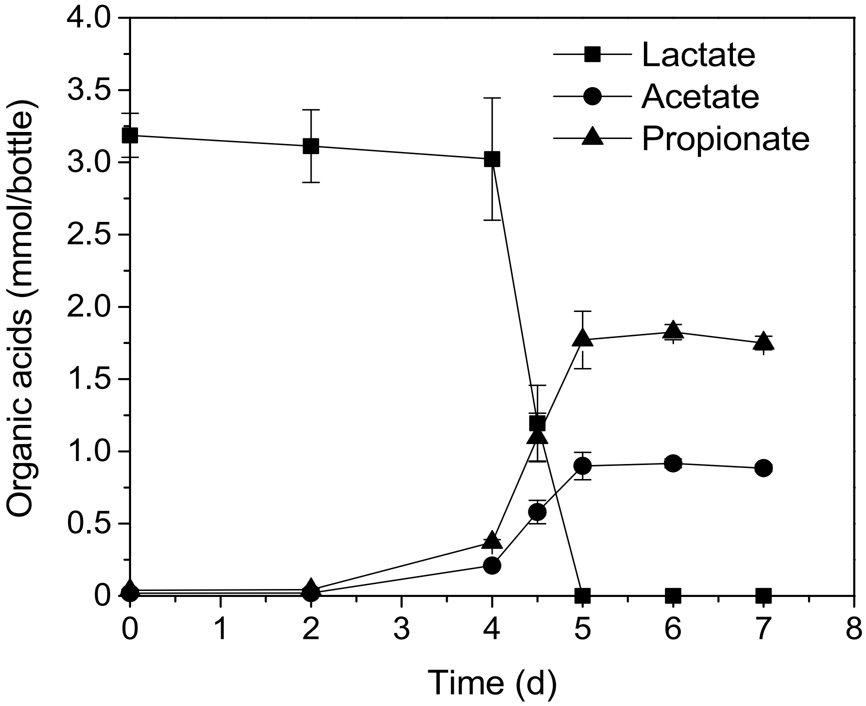

Fig. S1. Organic acids in PfR7 grown on 28 mM lactate.

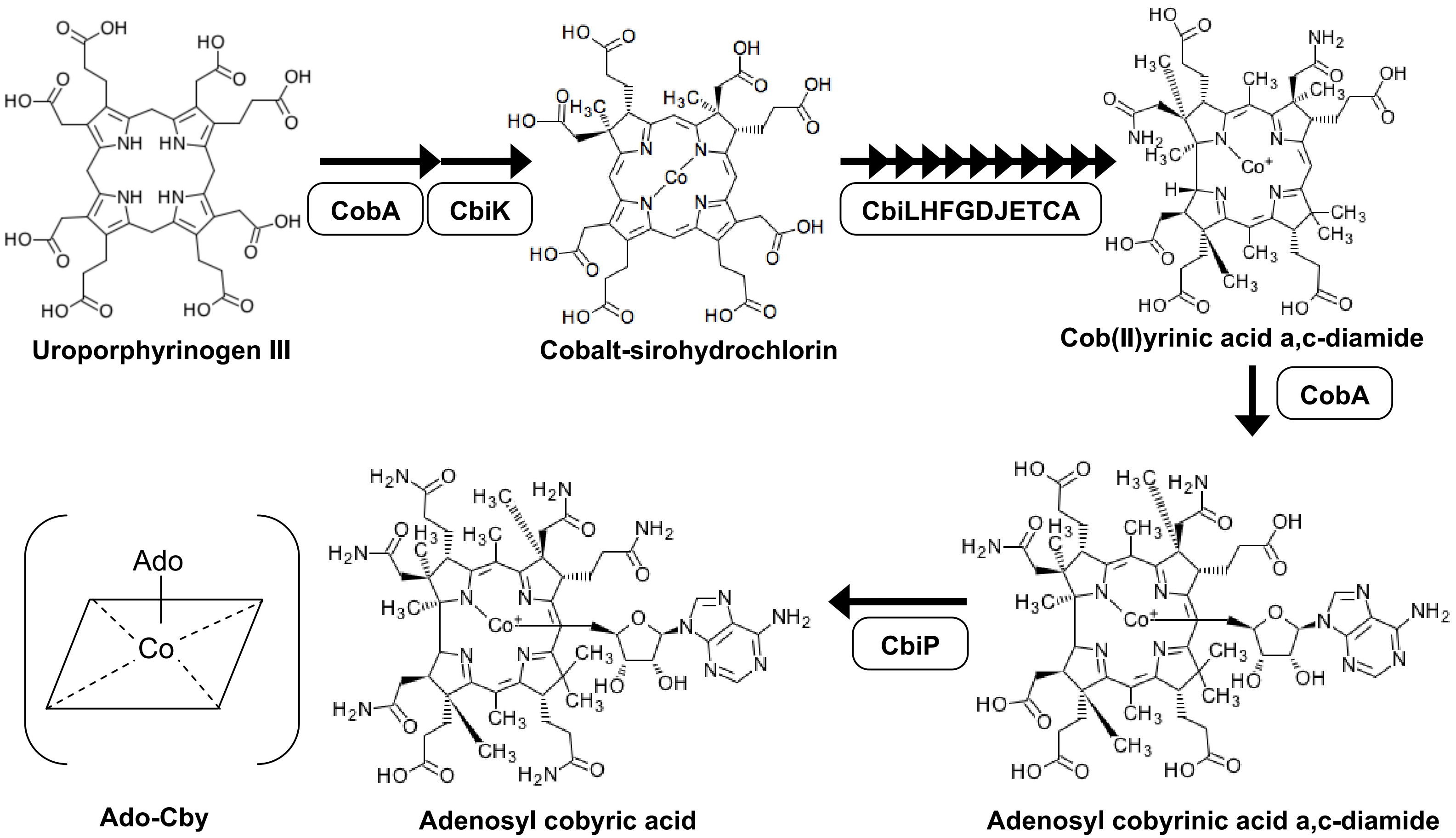

Fig. S2A. Up-stream corrinoid biosynthesis pathway

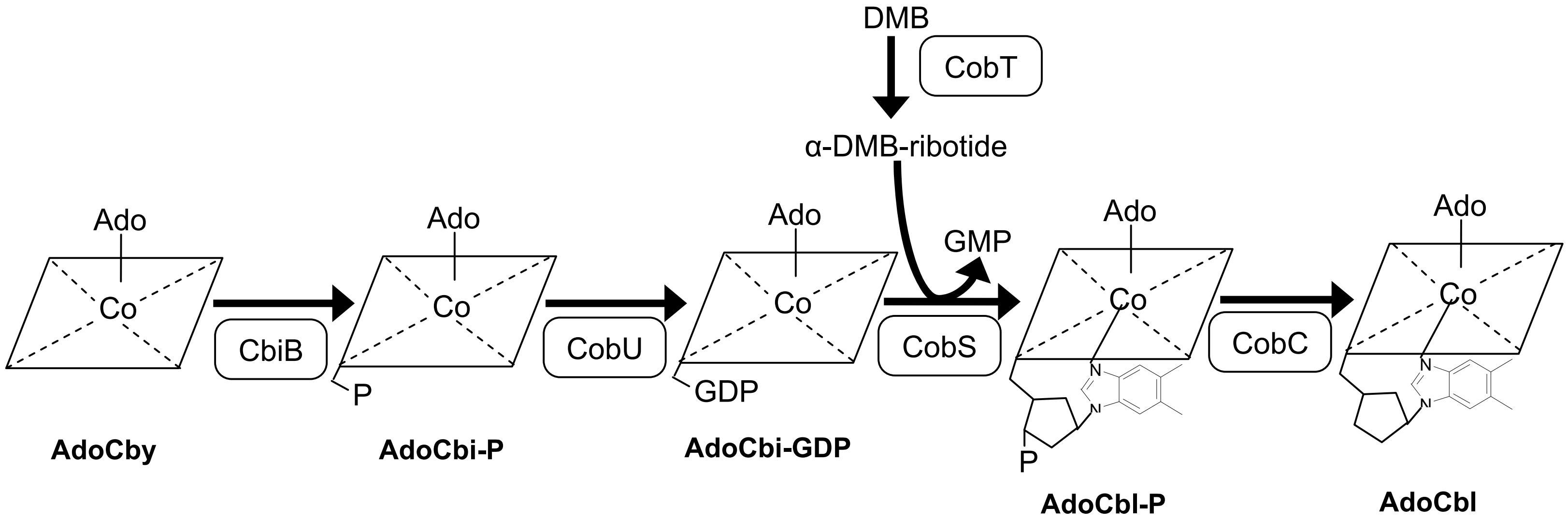

Fig. S2B. Lower ligand activation and attachment

Table S1 Primers used in qPCR targeting 16S rRNA genes

Target gene (NCBI accession No.)	Primer	Sequence 5’-3’	Annealing T (°C)	Reference
Dhc195 (NC_002936)	Forward	GGTAATACGTAGGGAAGCAAGCG	60	(1)
	Reverse	CCGGTTAAGCCGGGAAATT		
DvH (NC_002937)	Forward	AATCGGAATCACTGGGCGTA	60	(1)
	Reverse	CCCTGACTTACCAAGCAGCC		
PfR7 (NZ_AKVN01000065.1)	Forward	AGGCGACTTTCTGGACTGTG	60	This study
	Reverse	CCCCCAACACCTAGTACCCA		

Table S2 Dechlorination capability of different defined consortia after a two-week incubation

Consortium	+LAC[a] +B_{12} (74 nM)	+LAC	+LAC +DMB (36 nM)	+H_2	+H_2 +DMB (36 nM)
Dhc/DvH	√[b]	×[c]	×	N.D.[d]	N.D.
Dhc/PF	×	×	×	×	√[e]
Dhc/DvH/PF	√	×	√	N.D.	N.D.

[a] Lactate

[b] complete TCE dechlorination

[c] partial TCE dechlorination, accumulation of cDCE. The partial TCE dechlorination is probably due to the carryover of cobalamin provided in the seed culture.

[d] not done

[e] lactate was also amended in Dhc/PF as electron donor for PF

Table S3 Electron balance in different defined consortia

					eeq[b] (mmol)			
Defined consortia	Lactate (mmol)	Propionate (mmol)	Acetate (mmol)	Formate (mmol)	Total available[c]	Dechlorination	H_2	Biosynthesis[d]
Dhc195/DvH (+B_{12})	0.82 (0.18)[a]	-	0.52	0.50	1.6	**0.40**	1.6	0.1-0.4
Dhc195/DvH/PF (+B_{12})	0.66 (0)	0.28	0.30	0.05	0.86	**0.42**	0.6	0.1-0.8
Dhc195/DvH/PF (+DMB)	0.73 (0)	0.29	0.34	0.10	1.3	**0.36**	0.7	0.1-0.7

[a] The value outside of parentheses indicates total lactate added, the value in parentheses indicates lactate remained.

[b] Electron equivalent

[c] Values are calculated from lactate fermentation to acetate and hydrogen as shown in equation 1) and 2) below.

[d] Values are calculated using the cell numbers measured by qPCR described in the method section in each of the consortia and based on the stoichiometry of biosynthesis as shown in equation 7) below with the assumptions that Dhc195 is a sphere with a diameter of 0.5 µm (2), DvH and PF are rod shaped with a size of (0.5-1) × (3-5) (3) and (0.6-1) µm × (2-7) µm (4), respectively, a wet density of 1 g/mL and 0.2 g of dry weight per gram of wet weight (5).

1) $\underset{\text{(lactate)}}{CH_3CHOHCOO^-} + H_2O \rightarrow \underset{\text{(acetate)}}{CH_3COO^-} + \underset{\text{(formate)}}{HCOO^-} + H^+ + H_2$ $\Delta G^{0'} = -5.3$ kJ/mol (6)

2) $\underset{\text{(formate)}}{HCOO^-} + H_2O \rightarrow H_2 + HCO_3^-$ $\Delta G^{0'} = 1.4$ kJ/mol (6)

3) $\underset{\text{(lactate)}}{3CH_3CHOHCOO^-} \rightarrow \underset{\text{(acetate)}}{CH_3COO^-} + \underset{\text{(propionate)}}{2CH_3CH_2COO^-} + CO_2 + H_2O$ $\Delta G^{0'} = -56.6$ kJ/mol (6)

4) $\underset{\text{(TCE)}}{C_2HCl_3} + 2H_2 \rightarrow \underset{\text{(VC)}}{C_2H_3Cl} + 2H^+ + 2Cl^-$ $\Delta G^{0'} = -149.7$ kJ/mol (7)

5) $\underset{\text{(TCE)}}{C_2HCl_3} + 3H_2 \rightarrow \underset{\text{(Ethene)}}{C_2H_4} + 3H^+ + 3Cl^-$ $\Delta G^{0'} = -219.9$ kJ/mol (7)

6) $\underset{\text{(lactate)}}{3CH_3CHOHCOO^-} + 3H_2O + \underset{\text{(TCE)}}{C_2HCl_3} \rightarrow \underset{\text{(acetate)}}{3CH_3COO^-} + \underset{\text{(formate)}}{3HCOO^-} + \underset{\text{(ethene)}}{C_2H_4} + 6H^+ + 3Cl^-$ $\Delta G^{0'} = -235.8$ kJ/mol derived from 1) + 5)

7) $1/8\ \underset{\text{(acetate)}}{CH_3COO^-} + 1/20\ NH_4^+ + 3/40\ CO_2 \rightarrow 3/40\ HCO_3^- + 3/40\ H_2O + 1/20\ \underset{\text{(cells)}}{C_5H_7O_2N}$ (8)

Table S5 Presence of genes involved in cobalamin biosynthesis pathway in the genome of PfR7, DvH and Dhc195

		PfR7[a]	DvH[b]	Dhc195[c]
Cobalamin biosynthesis enzymes		Locus tag	Locus tag	Locus tag
CobA	uroporphyrin-III C-methyltransferase	FR7_3128	DVU0734	×
CbiK	cobalt chelatase	FR7_0449	DVU0650/ DVU1365	×
CbiL	precorrin-2 C20-methyltransferase	FR7_0463	DVU0646	×
CbiH	precorrin-3B C17-methyltransferase	FR7_3506	DVU3170	×
CbiF	precorrin-4 C11-methyltransferase	FR7_3508	DVU2748	×
CbiG	cobalamin biosynthesis protein	FR7_3507	DVU3169	×
CbiD	cobalt-precorrin-6A synthase	FR7_3511	DVU2750	×
CbiJ	precorrin-6x reductase	FR7_3505	×	×
CbiE	precorrin-6y C5,15-methyltransferase	FR7_3510	×	DET0240, DET0296
CbiT	precorrin-6y C5,15-methyltransferase	FR7_3509	×	×
CbiC	Precorrin-8x methylmutase	FR7_3504	DVU3087	×
CbiA	cobyrinic acid a,c-diamide synthase	FR7_3503	DVU0405, DVU3086	DET0128
CobA	cob(I)alamin adenosyltransferase	FR7_3530	DVU1403	DET0245, DET1139, DET1224
CbiP	cobyric acid synthase	FR7_3501	DVU0816	DET0936
CbiB/ CobD	cobalamin biosynthesis protein	FR7_3500	DVU2237	DET0654/ DET0688, DET1138
CobU	adenosylcobinamide-phosphate guanylyltransferase	FR7_3502	DVU1007	DET0660/ DET0694
CobT	nicotinate-nucleotide dimethylbenzimidazole-P phophoribosyl transferase	FR7_2880	DVU3279	DET0657/ DET0691
CobC	alpha-ribazole-5'-P phosphatase	FR7_0550, FR7_2039	×	DET0659/ DET0693
CobS	cobalamin 5'-phosphate synthase	FR7_3499	DVU0914	DET0658/ DET0692
CbiZ	adenosylcobinamide amidohydrolase	×	×	DET0653/ DET0687

[a]*Pelosinus fermentans* strain R7; [b]*Desulfovibrio vulgaris* Hildenborough; [c]*Dehalococcoides mccartyii* strain 195

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