

Supplementary Material for:

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Genetic tools for the redirection of the central carbon flow towards the production of lactate in the human gut bacterium *Phocaeicola (Bacteroides) vulgatus*

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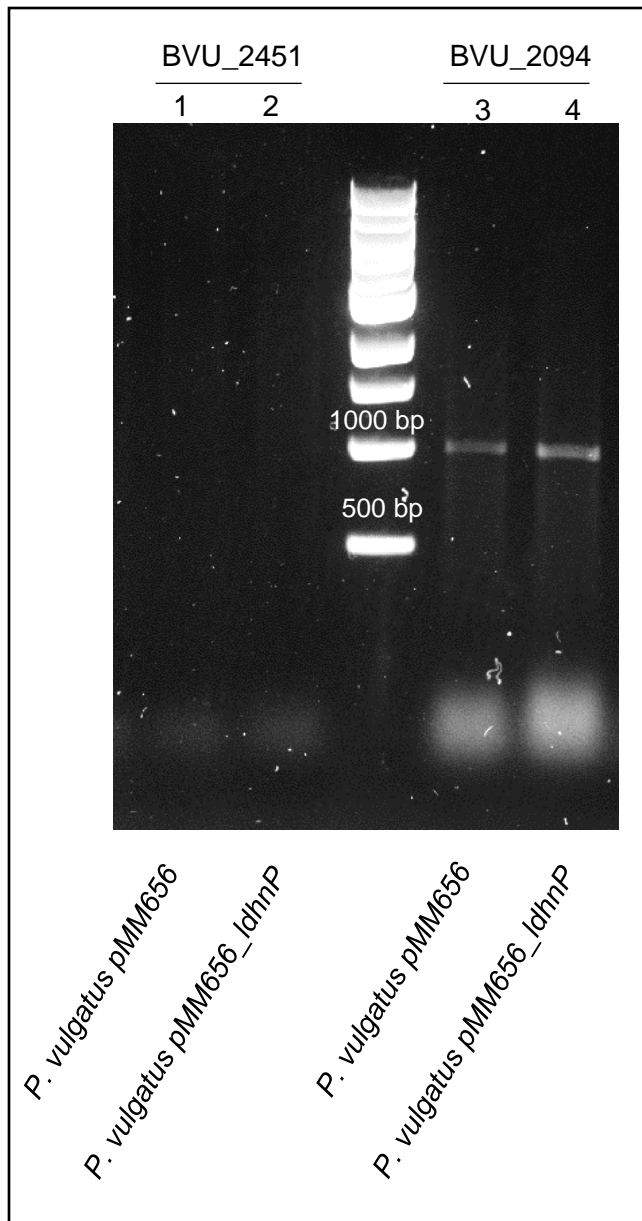


Fig. S1 PCR verification of the genomic integration of pMM656 and pMM656_ *ldhnP* into the gene BVU_2094 of *P. vulgatus*. Vector integration can occur between the attN2 site of pMM656 and one of two attPV sites at the 3' ends of the two tRNA^{Ser} genes, BVU_2451 and BVU_2094, on the *P. vulgatus* chromosome. Primer attB_inErm_rev and attB2451_for or attB2094_for were used to detect integration, with attB_inErm_rev binding in the plasmid backbone of pMM656 and attB2451_for/attB2094_for binding in the genome upstream of the corresponding attPV site. Primer pair attB_inErm_rev and attB2094_for demonstrated the integration of attN2 from pMM656 (line 3) and pMM656_ *ldhnP* (line 4) into chromosomal gene BVU_2094. Integration of the attN2 site into the corresponding locus resulted in a PCR fragment size of 1021 bp. An integration of the plasmids into the attPV site located in BVU_2451 did not occur, since the addition of primers attB_inErm_rev and attB2451_for did not lead to a DNA fragment in the PCR assay (line 1 and 2).

PvLDH	MAYKIAFYDTPKYDERSFTEANEKFG-FDIRYYKGHLNMNNVVLTKGVDVVICI	FV NDTAD	59	
EcLDH	--MKLAVYSTKQYDKKYLQQVNESFG-FELEFFDFLLTEKTAKTANGCEAVCI	FV NDDGS	57	
PaLDH	--MRILFFSSQAYDSESFQASNHRHG-FELHFQQAHLQADTAVLAQGFVVCA	FV NDDLS	57	
LcLDH	--MKIIAYGARVDEIQYFKQWAKDTG-NTLEYHTEFLDENTVEWAKGFDGINS	LQTT	57	
LpLDH	--MKIIAYAVRDDRPFDFDTWMKENPDVEVKLVPELLTEDNVDLAKGFDGADV	YQ QKDYT	58	
LdLDH	-MTKIFAYAIREDKPFLEKWEWDAHKDVEVEYTDKLLTPETVALAKGADGVVV	YQ QLDYT	59	
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PvLDH	AEVIRAMADNGVKLLALRC	AG YNNVDLAAT-AGKMKV	RV PAYS	118
EcLDH	RPVLEELKKHGKVI	IALRCA GF NNVDLDAAKELGLKV	RV PAYD	117
PaLDH	RPVLERLAAGGTRLVALRS	AG YNHVDLAAAEALGLPV	HV PAYS	117
LcLDH	AGVFEKMHAYGIKFLTIRN	VG TDNIDMTAMKQYGIRLS	NV PAYS	117
LpLDH	AEVLNKLADGKVNISLRN	VG VDNLDVPTVKARGLNIS	NV PAYS	118
LdLDH	AETLQALADNGITKMSLRN	VG VDNIDMAKAKELGFQIT	NV PVYS	119
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PvLDH	RKI PRATMRTRDGNFSL-HGLMGDFMHGKTAGI	I GTGKIA KILIQILRGFGMNVLAY	DLY	177
EcLDH	RRIHRA YQRTRDANFSL-EGLTGFTMYGKTAGVI	GTGKIG VAMLRILKGFGRMLLAF	DPY	176
PaLDH	RRLHRA YNRTREGDFSL-HGLTGFDLHGKRVGVI	GTGQIG ETFARIMAGFGCELLAY	DPY	176
LcLDH	RNMKGVAQQLQAGDYEKAGTFIGKELGQQT	VGVM GTGHIG QVAIKLFGFGAKVIA	DPY	177
LpLDH	RQTPMFNKKLAKQDFRW-APDI AKELNMTVGVI	GTGRIG RAAIDIFKGF GAKVIGY	DVY	177
LdLDH	RQDKAMDEKVARHDLRW-APTIGREVRDQV	GV GTGHIG QVFMQIMEGFGAKVIA	YDIF	178
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PvLDH	PDYNFAREHQVYVYCTLDELYHSSDIISLHCP	LTEQTKYLINDYSISKMKDGVMII	NTGRG	237
EcLDH	PSAAA-LELGV EYVDLPTLFSESDVISLHCP	LTPENYHLLNEAAFEQMKNGVMIV	NTSRG	235
PaLDH	PNPRI-QALGGRYLALDALLAESDVISLHCP	LADTRHLIDAQRLATMKPGAMLIN	NTGRG	235
LcLDH	PMKGD-HP-DFDYV SLEDLDFKQSDVIDLHVP	PIEQNTHINEAAFNLMPGAIVIN	TARP	235
LpLDH	RNAEL-EKEGMYVDTLDELYAQADVI	TLHVPALKDNYHMLNADAFSKMKD	GAYILN FARG	236
LdLDH	RNPEL-EKKGYVDSLDDLYKQADVISLHVP	DVPANVHMINDESI AKMKQDVVIV	NSRG	237
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PvLDH	QLIHTNALIEGLKTKKVG YAGLDVYEE	EE EPYFYEDKSDKIIDDDTLARLLS	FNNVIV TSH	297
EcLDH	ALIDSQA AIEALKNQKIGSLGMDVYEN	ER DLFFEDKSNVDIQDDVFRRLS	ACHNVLF TGH	295
PaLDH	ALVNAAAALIEALKSGQLG YLGLDVYEE	EAD IFFEDRS DQPLQDDVLRLLS	FPNVV TAH	295
LcLDH	NLIDTQAMLSNLKSGKLAGV GIDTYEY	ET EDLLNLAKHGSFKDPLWDEL	LGMNVV LPH	295
LpLDH	TLIDSEDLIKALDSGKVAGAA	LV TYEY ET KIFNKDLEGQTI	DDKVFMMNLFNRDNV LTPH	296
LdLDH	PLVDTDVAIRGLDSGKIFGYAM	DV YEG EV GFINEDWEGKEFP	DARLADLIARPNV LTPH	297
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PvLDH	QAF FTKEAMTNIAHTTLQNVKDFAESRSLVNEVAVGRV	335		
EcLDH	QAF LTAEALTSISQTTLQNLNLEKGETCPNELV----	329		
PaLDH	QAF LREALAAIADTTLDNIAAWQDGTFRNRVRA----	329		
LcLDH	IAYTETAVHNMVYFSLQHLVDFLTKGETSTEVTGPAK	333		
LpLDH	TA FYTETAVHNMVHVMNSNKQFIETGKADTQVKFD--	332		
LdLDH	TA FYTTHAVRNMVVKAFDNNLELVEGKEAETPVKVG--	333		
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Fig. S2 Sequence alignment of representative D-LDHs. The amino acid sequences of the D-LDHs from *P. vulgatus* (PvLDH; A6L392), *P. aeruginosa* (PaLDH; Q9I530), *E. coli* (EcLDH; P52643), *L. delbrueckii*, (LdLDH; P26297) and *L. pentosus* (LpLDH; P26298) as well as the D-hydroxyisocaproate dehydrogenase from *Lactobacillus casei* (LcHDH; A0A0E2BVW2) were aligned using the program Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). The characteristic motifs for D-LDHs are marked according to Furukawa et al. (2018): Amino acids of the substrate-binding site are shown in red and the residues involved in the coenzyme binding are colored in blue. The two hinge regions between the catalytic and the NAD-binding domains are boxed.

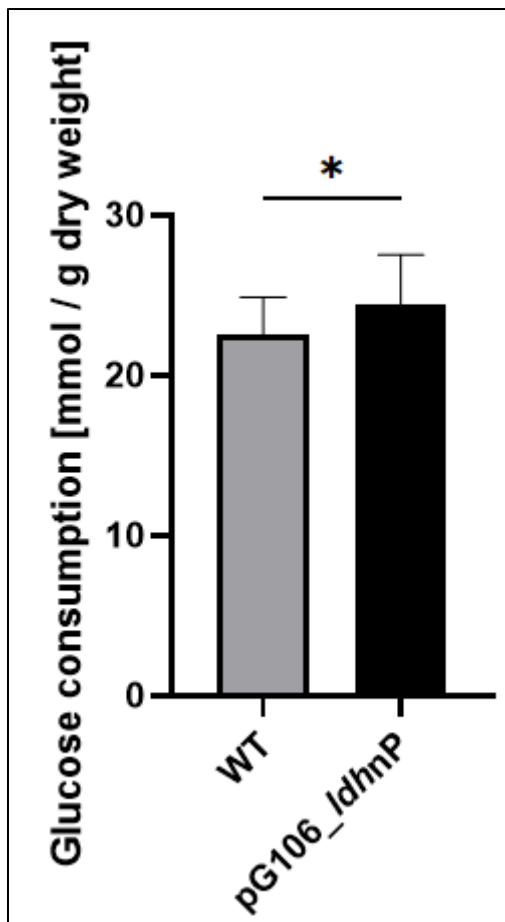


Fig. S3 Substrate consumption. *P. vulgatus* WT (gray) and *P. vulgatus* pG106_IdhnP (black) were grown in minimal medium with glucose as substrate. Cultures were harvested and the supernatants analyzed by HPLC. The amount of glucose consumption was correlated to the dry weight of the corresponding cultures. Values represent the average of at least 16 different cultures for each strain. For significance analysis data sets were analyzed by t-test using GraphPad Prism 8.0.2.263. * = $p \leq 0.05$.