Supplementary Material for:

Applied Microbiology & Biotechnology

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

Rebecca Lück and Uwe Deppenmeier*

Institute of Microbiology and Biotechnology, University of Bonn, Meckenheimer Allee 168, 53115 Bonn, Germany

*For correspondence. E-mail: udeppen@uni-bonn.de; Tel. +49-228-735590; Fax +49-228-737576.

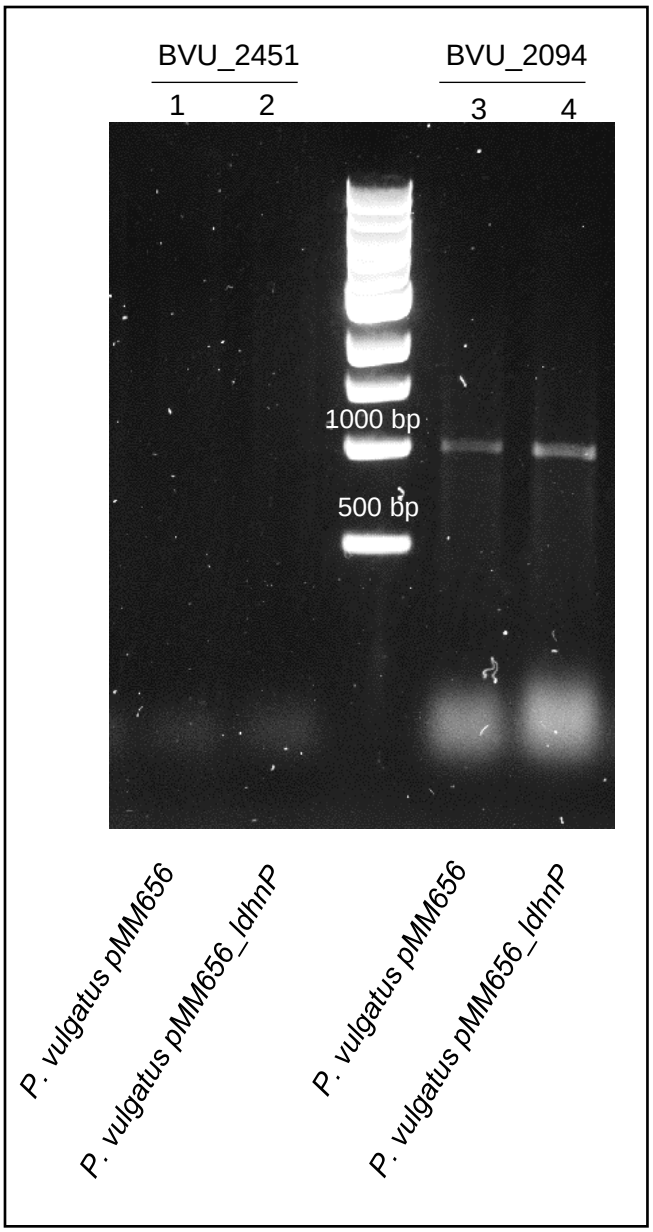

Fig. S1 PCR verification of the genomic integration of pMM656 and pMM656_*ldh*nP into the gene BVU_2094 of *P. vulgatus*. Vector integration can occur between the attN2 site of pMM656 and one of two attPV sites at the 3′ ends of the two $tRNA^{Ser}$ genes, BVU_2451 and BVU_2094, on the *P. vulgatus* 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_*ldh*nP (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).

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.

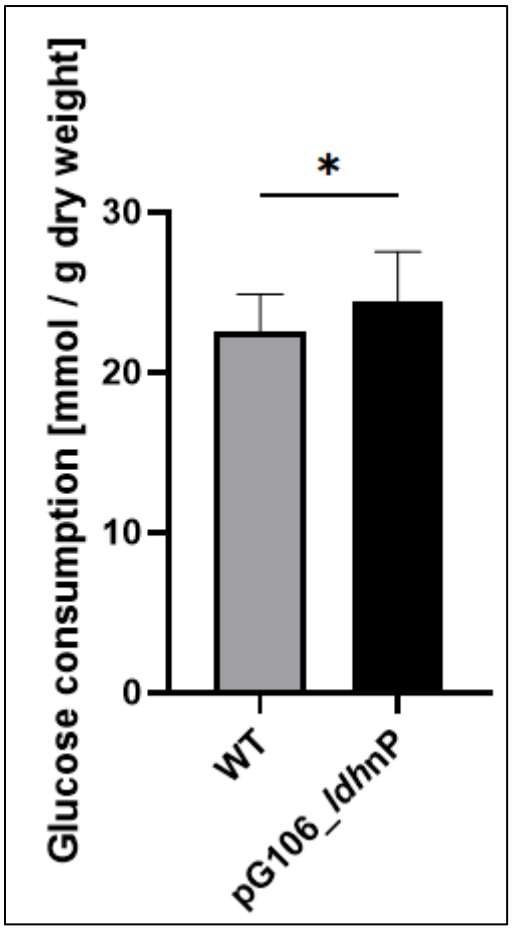

Fig. S3 Substrate consumption. *P. vulgatus* WT (gray) and *P. vulgatus* pG106_*ldh*nP (black) were grown in minimal medium with glucose as substrate. Cultures were harvested and the supernatants analyzed by HPLC. The amount of glucose consumption was correlated to the dry weight of the corresponding 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. * = $p \leq 0.05$.