

SUPPORTING INFORMATION

The anaerobe-specific ORP complex of *Desulfovibrio vulgaris* Hildenborough is encoded by two divergent operons co-regulated by σ^{54} and a cognate transcriptional regulator.

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Table S1. Bacterial strains and plasmids used in this study.

Strains or plasmid	Characteristics	Reference or source
<i>E. coli</i>		
TG1	K12, $\Delta(lac-pro)$, <i>supE</i> , <i>thi</i> , <i>hsdD5</i> (F', <i>traD36</i> , <i>proA</i> ⁺ <i>B</i> ⁺ , <i>lacI</i> ^ρ , <i>lacZAM15</i>)	Sambrook and Russell (2001)
MW3064	Donor strain for conjugation; <i>thrB1004 pro thi rpsL hsdS lacZ_M15</i>	Dehio et al (1997)
W3110 – <i>lacZ</i>	<i>RP4-1360_(araBAD)567 _dapA1341::_erm pir(wt)_</i>	This Study
W3110 – <i>lacZ rpoN</i>	<i>rrnD-rrnE, rph-1, $\Delta lacZ$</i> <i>rrnD-rrnE, rph-1, $\Delta lacZ, \Delta rpoN$</i>	This Study
<i>Desulfovibrio vulgaris</i> Hildenborough	Wild-type strain	Postgate (1984)
DVH (2103-strep)	Contains the <i>DVU2103</i> gene fused to a C-terminal Strep-TagII tag in DVH	This Study

DVH (2108-strep)	(DVH::p2103-strep) Contains the <i>DVU2108</i> gene fused to a C-terminal Strep-TagII tag in DVH (DVH::p2108-strep)	This Study
DVH (2109-strep)	Contains the <i>DVU2109</i> gene fused to a C-terminal Strep-TagII tag in DVH (DVH::p2109-strep)	This Study
DVH (Δ p2106)	DVH Δ promoter <i>DVU2106</i> ::p2106PAS	This Study
DVH (p2105:: <i>uidA</i>)	Contains the promoter region of <i>DVU2105</i> fused to the <i>uidA</i> gene	This Study
DVH (p2107:: <i>uidA</i>)	Contains the promoter region of <i>DVU2107</i> fused to the <i>uidA</i> gene	This Study
<i>Methanosarcina barkeri</i>	Wild-type strain	DSMZ 804
Plasmids		
pNotI9	Cloning vector pUC19. NdeI site replaced by a NotI site	
pNotI9Cm-Mob-XS	Contains the 1.4-kb <i>cm</i> gene and the oriT of plasmid RP4 on a 1.6-kb fragment in the pNotI9	Dolla et al (2000)
p2103-strep	Contains the <i>DVU2103</i> gene fused to a C-terminal Strep-TagII tag on an <i>SpeI-XhoI</i> 900pb fragment in the pNotI9Cm-Mob-XS vector	This Study
p2108-strep	Contains the <i>DVU2107</i> and <i>DVU2108</i> genes fused to a C-terminal Strep-TagII tag on an <i>SpeI-XhoI</i> 1230 pb fragment in the pNotI9Cm-Mob-XS vector	This Study
p2109-strep	Contains the <i>DVU2109</i> gene fused to a C-terminal Strep-TagII tag on an <i>SpeI-XhoI</i> 1500pb fragment in the pNotI9Cm-Mob-XS vector	This Study
pOK12	Cloning vector, P15A replicon, Kan ^R	
pOK2106	Contains the <i>DVU2106</i> gene fused to an N-terminal FLAG tag in the pOK12 vector	This Study
pT7.5	Cloning vector, ColeI replicon, Amp ^R	Viera and Messing, 1992
pT7-5-2105-LacZ	Contains the promoter region of <i>DVU2105</i> fused to the <i>lacZ</i> gene	This study
pT7-5-2106-LacZ	Contains the promoter region of <i>DVU2106</i> fused to the <i>lacZ</i> gene	Tabor and Richardson, 1985
pT7-5-2107-LacZ	Contains the promoter region of <i>DVU2107</i> fused to the <i>lacZ</i> gene	This Study
pET19b	Expression vector, gene expression under the control of the T7 promoter	This Study
pET2106CLIV	Contains a truncated 960 pb <i>DVU2106</i> gene encoding residues 127 to 458	This study

p2106PAS	fused to a N-terminal 10-His tag in the pET19b vector Contains a truncated <i>DVU2106</i> gene (400pb from ATG) on an <i>SpeI-XhoI</i> fragment in the pNot19Cm-Mob-XS vector	Novagen This Study
p2105-uidA	Contains the promoter region of <i>DVU2105</i> fused to the uidA gene in pNot19Cm-Mob-XS	This Study
p2107-uidA	Contains the promoter region of <i>DVU2107</i> fused to the uidA gene in pNot19Cm-Mob-XS	This Study

This Study

Postgate, J.R. (1984) in *The Sulphate-reducing bacteria*, 2nd ed; pp12-13, Cambridge University Press, Cambridge, UK.

Dolla, A., Pohorelic, B.K., Voordouw, J.K., and Voordouw, G. (2000) Deletion of the *hmc* operon of *Desulfovibrio vulgaris* subsp. *vulgaris* Hildenborough hampers hydrogen metabolism and low-redox-potential niche establishment. *Arch. Microbiol* **174**: 143-151.

Vieira, J., and Messing, J. (1991) New pUC-derived cloning vectors with different selectable markers and DNA replication origins. *Gene*. **100**: 189-94.

Tabor, S., and Richardson, C.C. (1985) A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. *Proc Natl Acad Sci U S A*. **82**: 1074-8.

Table S2. Primers used in this study.

Primer	Sequence
To construct plasmids for pulldown experiments	
pUC19SpeI-XhoI	5'-ACCTCAGTACACTAGTCCGGGGATCCTCTAGAGTCCG-3'
pUC19XhoI_rev	5'-GTACTCGAGGTACCGAGCTCGAATTCACTGG-3'
Nter2103SpeI	5'-TAAATCACTAGTATGAAACAGTTCGTCGTCATC-3'
2103strep_comp	5'-TAAATCACTAGTTATTTTCGAACTGCCGGTGGCTC CAAGCGTGACGAGTGCCAGCAACTGG-3'
DVU2107strep	5'-CAGAATTCGGCAGGCGGATGGCGACCTGCC-3'
DVU2108strep_comp	5'-GTGGATCCTTATTTTCGAACTGCCGGGTG GCTCCAAGCGCTCCAGTGCTCCTTCA-3'
Nter2109SpeI	5'-TAAATCACTAGTATGAGTACCGTGAACGGCGG-3'
2109strep_comp	5'-TAAATCCTCGAGTTATTTTCGAACTGCCG GGTGGCTCCAAGCGCTATGGTCGCAGGTGTGGCACCC-3'
CM1	5'-GACATGGAAGCCATCACAGA-3'
CM2	5'-ACTCCGTAGCGCTGATGT-3'
Mob_up	5'-GCCGGCGCGCCGCCATTTC-3'
Mob_down	5'-GCAGGTCGACTCTAGAGG-3'
For operon organisation	
DVU2103_dir	5'-GAAGCGCGTGTCCGGAGAC-3'
DVU2104_rev	5'-AGGCTGCTTCGCGTAATG-3'
DVU2104_dir	5'-CGTTGGGCTCCTCGACAT-3'
DVU2105_rev	5'-CTCGATGTCGTGATGTC-3'
DVU2105_dir	5'-CAGGGCATTCTTCATAGGTC-3'
DVU2106_rev	5'-CGAGGTCGACCGTGAACA-3'
F1rC	5'-GCCCGGAGACGACGGAC-3'
DVU2107_right	5'-ACAGGATGAAGGCATATTCGAT-3'
DVU2107	5'-GGCGGCACTTGGAAAGGG-3'
SaeI2108_comp	5'-GCCAGCTCTCACCAAGTGTCTTCCACGG-3'

Ndel2108	5'-CGCATATGCGAATAGTCAATCACC-3'
Mrp_comp	5'-GCCCCAGCGCACTTCGG-3'
DVU2109	5'-TGACGTACTTGCCAGCA-3'
DVU2110_comp	5'-CCTCGACACATACGGTC-3'
For real-time PCR analysis	
DVU2104_left_b	5'-GTGGACTGTGATGTCGAGGAG-3'
DVU2104_right_b	5'-AGACGAGTTCACAGAGTCCACA-3'
DVU2106_left	5'-CGACCTCTACTATCGGCTGAAT-3'
DVU2106_right	5'-ACAGGATGAAGGCATATTCGAT-3'
DVU2108_up	5'-ATTCAAGGCTGCCCAGATGG-3'
DVU2108_down+	5'-GTGTCGGCAGGGCGGAGT-3'
Nter_DVU2106	5'-TTGAATTCAATGGCCCTGCCCCGAGACAT-3'
DVU2106_rev	5'-CGAGGTCGACCCGTGAACA-3'
For identification of the transcripts start sites	
DVU2107_fwd	5'-TCCAAGACCGTTATCCCCCTGCCC-3'
DVU2107_rev	5'-CAGTGTGGGAGTCTGCCCTC-3'
DVU2105_fwd	5'-TCCGTGTGAAAGTCTCTATC-3'
DVU2105_rev(+1)	5'-CAGGGCATTCTTCATAGGTC-3'
For transcriptional fusions	
Prom2105	5'-CTCACTATAGGGAGACCCGGAATTCGAG CTCCTATGATCCCGTGTGAAAGTCTCTATCATGTG-3'
Prom2105_comp	5'-GGTCGACTCTAGAGGATCCCCGGCCATATGTGC TCCTTCCGCCGGTATGC-3'
Prom2106	5'-CTCACTATAGGGAGACCCGGAATTCGAGCTCCAT ATGTGCTCCTTCCGCCGGTATGC-3'
Prom2106_comp	5'-GGTCGACTCTAGAGGATCCCCGGCCATATGATCCCTC CGTGTGAAAGTCTCTATCATGTG-3'

Prom2107	5'-CTCACTATAGGGAGACCGGAATTCGAGC TCCGTAGCCGGTCGACGCCAATCCCG-3'
Prom2107_comp	5'-GGTCGACTCTAGAGGATCCCCGGGCCAT GGCGTGGCGTACCTCCGGTACC-3'
pOK2106	5'-CGAGATCTATCGATGCATGCCATGGACTACAAAGACG ACGATGACAAAGGGGCCCTGCCCCGAGACATCCCCTGCG-3'
pOK_comp	5'GAAAGCTTCGAAATTCGAGCTCCCGGTATCATTTCCGTGT CGCCACGTTGCAGGATG-3'
For overexpression of DVU2106 in <i>E.coli</i> pET 2106_cliv	5'-GCCGGCCATATCGACGACGACGACAAAGACGGCCCTTC TCGACCTCCTTCCGC-3'
pET 2106_comp	5'-GTTAGCAGCCGGATCCTCGAGCATATGTTCAT TTTCGTGTCGCCACGTTGCAGGATG-3'
For gel mobility shift assay	
Prom2105	5'-CTCACTATAGGGAGACCGGAATTCGAG CTCCTATGATCCGTGTGAAAGTCTCTATCATGTG-3'
Prom2105_comp	5'-GGTCGACTCTAGAGGATCCCCGGCCATATGTGC TCCTTCCGCCGATGC-3'
Prom2106	5'-CTCACTATAGGGAGACCGGAATTCGAGCTCCAT ATGTGCTCCTTCCGCCGATGC-3'
Prom2106_comp	5'-GGTCGACTCTAGAGGATCCCCGGCCATATGATCCTC CGTGTGAAAGTCTCTATCATGTG-3'
Prom2107	5'-CTCACTATAGGGAGACCGGAATTCGAGC TCCGTAGCCGGTCGACGCCAATCCCG-3'
Prom2107_comp	5'-GGTCGACTCTAGAGGATCCCCGGGCCAT GGCGTGGCGTACCTCCGGTACC-3'
Prom2105_comp	5'-CATCTTTTCCCAACACAC-3'
Prom2106_comp	5'-GTGTGTTGGGAAAAGATG-3'

2106-A-comp_dir	5'-CATGTGGGGCATTTTGGGCCCTATTT-3'
2106-A-comp_rev	5'-AAATAGGGCGCAAATGCGCCACATG-3'
2106-B-comp_dir	5'-GACATAGGGCGTGTTTGGGCCACATG-3'
2106-B-comp_rev	5'-CATGTGGGGCAAACACGCCCTATGTC-3'
To construct p2106PAS for <i>DVU2106</i> disruption	
2106PAS	5'-AGTAGCTCGAGATGGCCCTGCCCCGAGACATCC-3'
2106PAS_comp	5'-CCGATACTAGTTCACGGTGCGTGGGGC-3'

Table S3. Identified interaction partners from the ORP gene cluster of tagged proteins. MALDI-MS scores were obtained using the MASCOT search engine and ESI-IT scores were obtained using SEQUEST.

Bait	Locus interactors	M_r (kDa)	Method of identification	Score	Peptide (hits)	% Coverage
2103-strep	DVU2108	12.5	ESI-IT	40.22	15	
	DVU2104	32	MALDI-MS	79	12	39
	DVU2109	51	MALDI-MS	86	9	31
2108-strep	DVU2103	31	MALDI-MS	176	18	81.2
	DVU2104	32	MALDI-MS	89	11	35.5
2109-strep	DVU2108	12.5	ESI-IT	30.2	7	
	DVU2105	17	MALDI-MS	62	7	51
	DVU2103	31	MALDI-MS	109	14	71
	DVU2104	32	MALDI-MS	116	11	37

Table S4 Recurrent copurified proteins defined as “contaminants”

ORF number	Name
DVU0811	Dna K
DVU0847	Adenylsulfate reductase
DVU0881	Elongation factor G
DVU1834	Pyruvate carboxylase
DVU1976	GroEL
DVU2920	Elongation factor TU
DVU0958, DVU1303, DVU1306, DVU1309, DVU1320, DVU1328, DVU2518, DVU3150	Ribosomal proteins

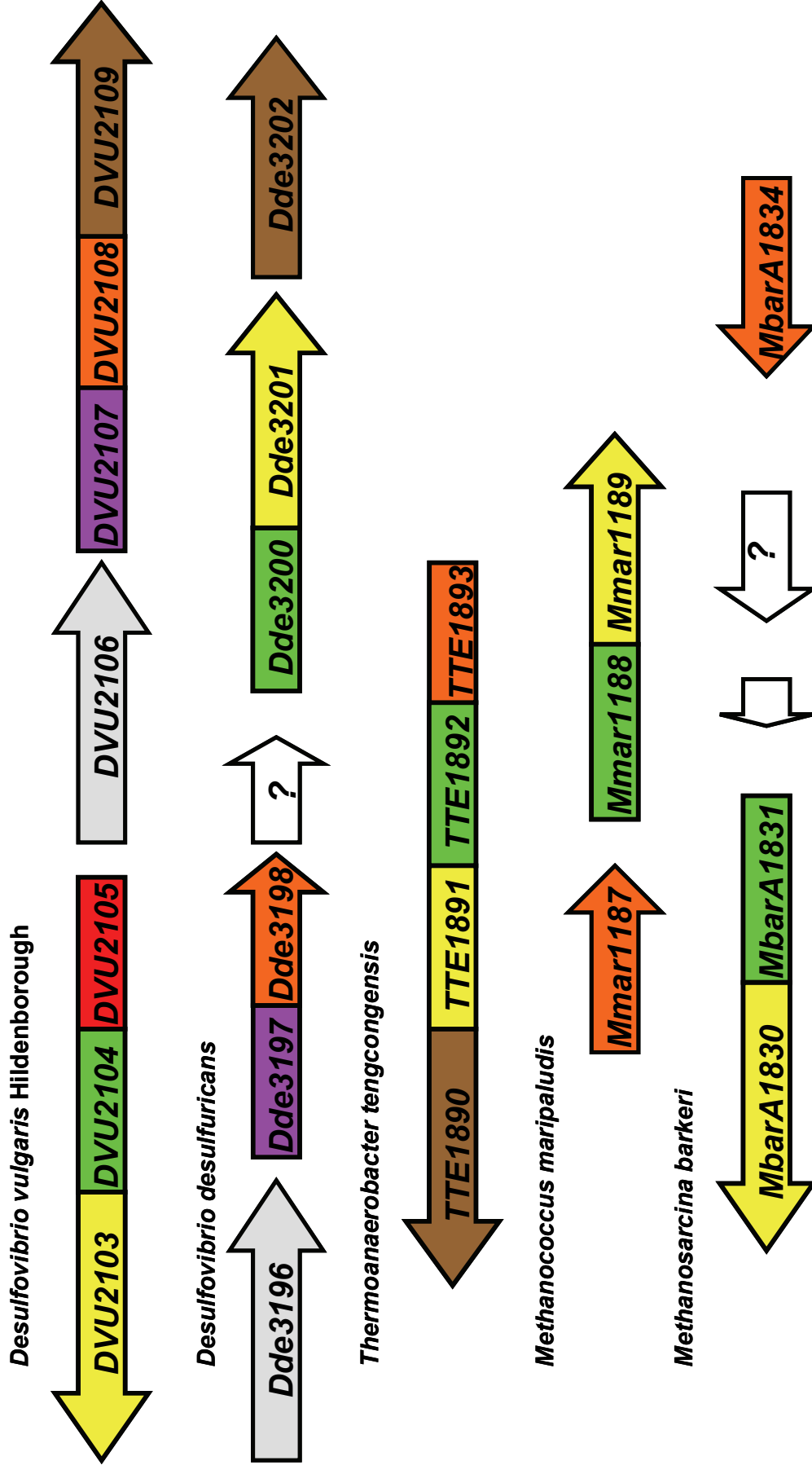


Figure S1. *orp* gene cluster organization in various microbial species using STRING (von Mering et al., 2005) and phylogenetic analysis of DVU2103, DVU2104 and DVU2108 genes and their homologues published by Scholten (Scholten et al., 2007). Homologues of DVU2103 (yellow), DVU2104 (green), DVU2105 (red), DVU2106 (grey), DVU2107 (purple), DVU2108 (orange) and DVU2109 (brown) are indicated. Genes are not drawn to scale. The direct boarding of ORFs indicates overlapping of start and stop codons, while space between ORFs indicates intergenic region larger than 1 base pair.

Fievet et al., Figure S1

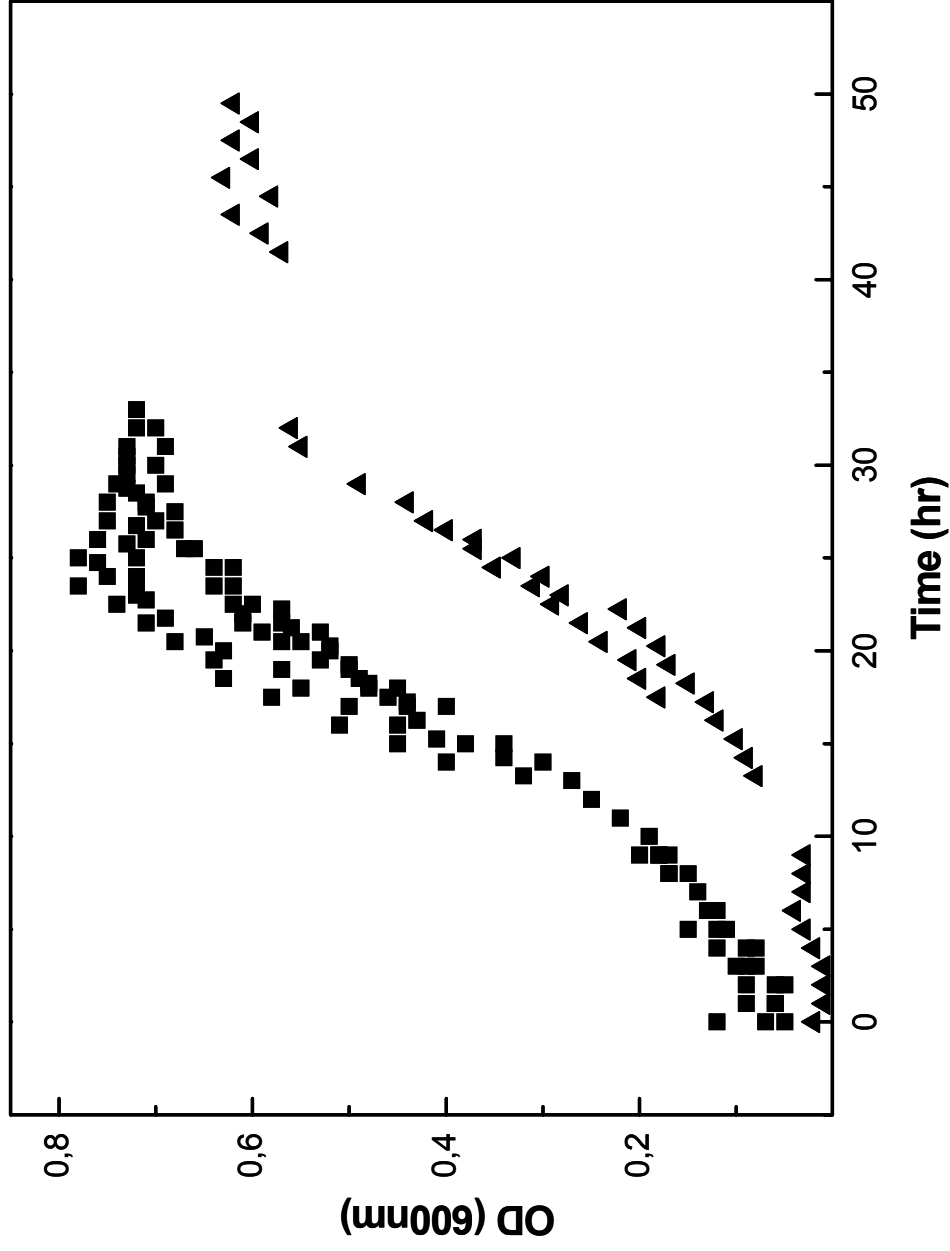


Figure S2: Inactivation of DVU2106 affects the growth phenotype.
Square: DVH Wild-type and triangles: DVH p2106::2106PAS strain

Fievet et al., Figure S2