

## Supporting Information

### Experimental evolution reveals nitrate tolerance mechanisms in *Desulfovibrio vulgaris*

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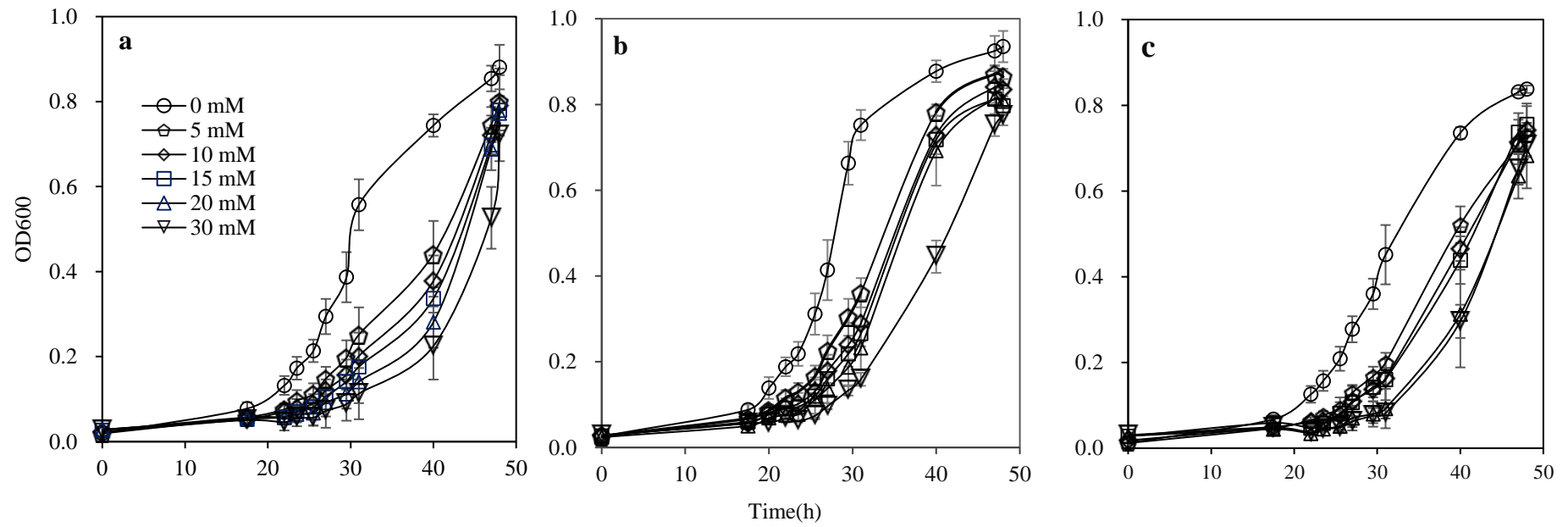


Figure S1 Determination of NaNO<sub>3</sub> concentrations for a long-term experimental evolution with ancestral populations AN2 (a), AN8 (b), and AN11 (c).

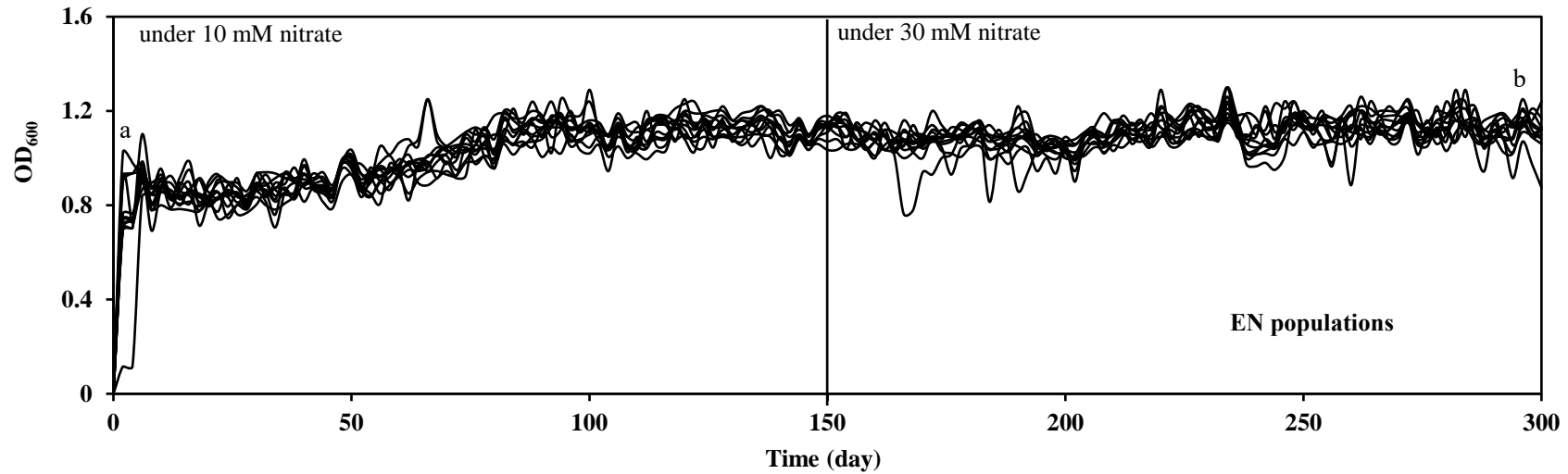


Figure S2 Growth records of 12 ancestral (AN) populations under elevated nitrate. Nitrate-evolved (EN) populations were obtained by transferring 12 ancestral DvH populations under 10 mM nitrate for 500 generations, then under 30 mM for 500 generations. Statistical significance was evaluated between biomass of first transfer and last transfer based on the Student's t-test ( $p < 0.001$ ).

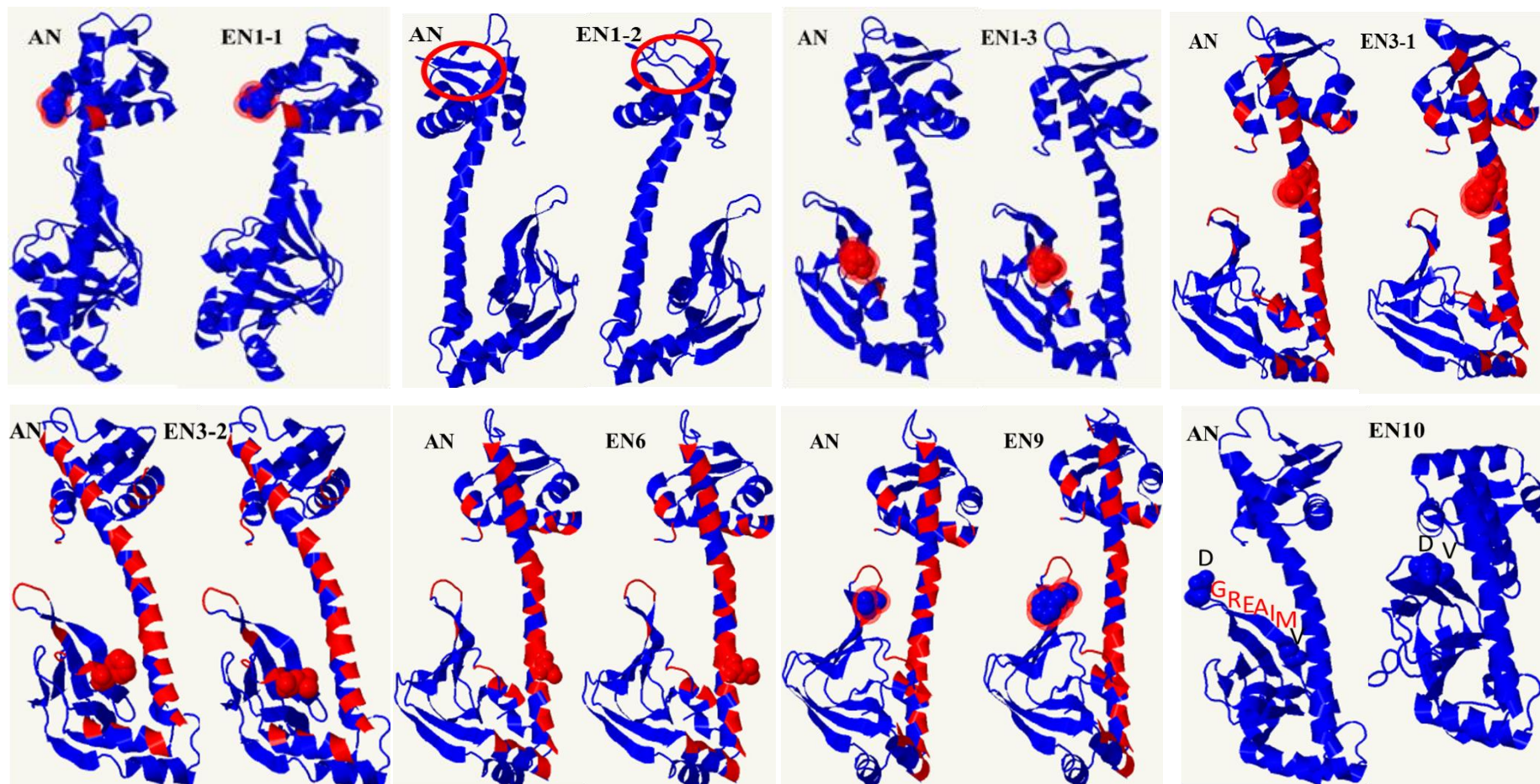


Figure S3 Changes in the tertiary structure of HcpR protein in the nitrate-evolved populations predicted by Phyre2 using Jmol [1]. The site surrounded by red lines presents the changes in the tertiary structure in HcpR.

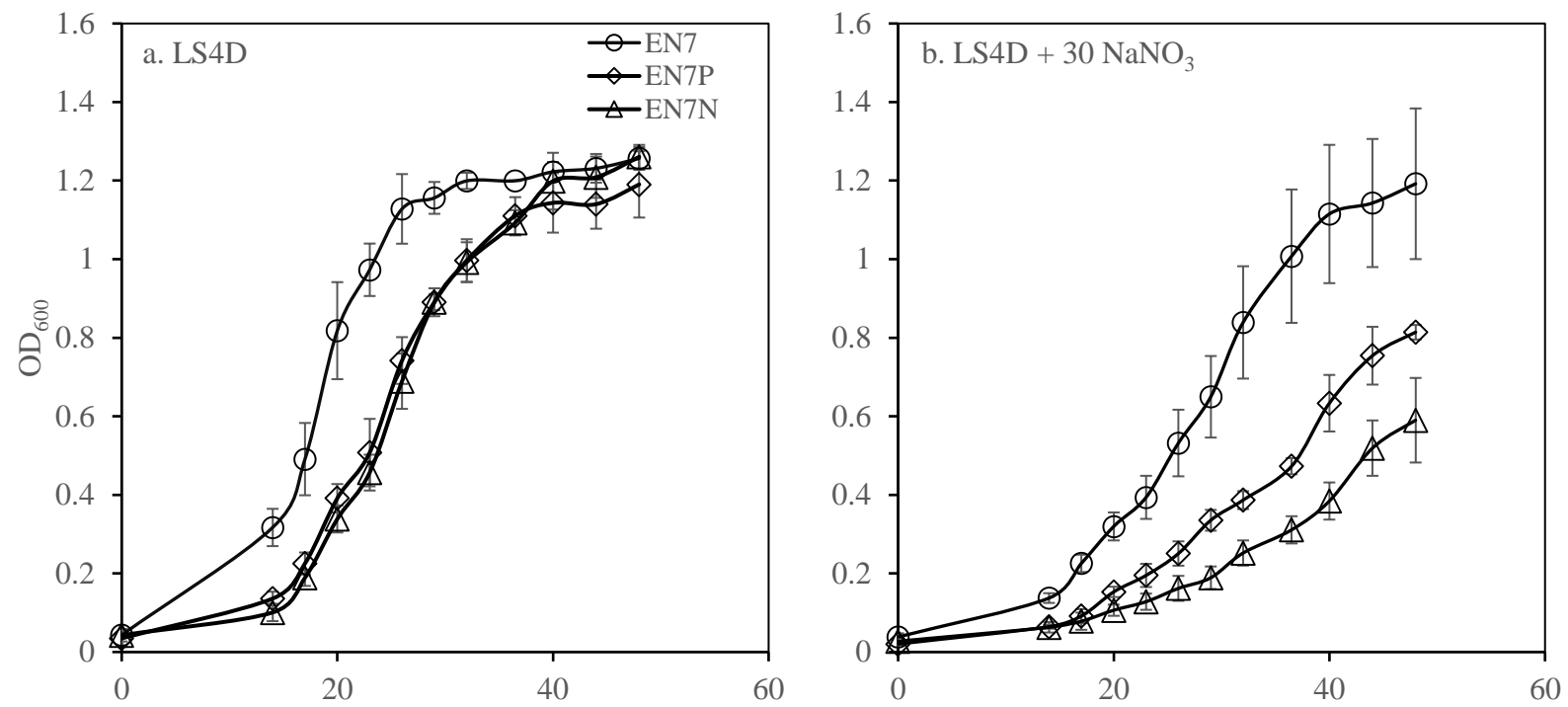


Figure S4 Growth of EN7, EN7P (EN7 with empty vector pMO719), and EN7N (EN7 with DVU2547 complement plasmid) under (a) no stress (LS4D) and (b) nitrate stress (LS4D + 30 mM NaNO<sub>3</sub>).

Table S1 Primers used to validate the randomly chosen mutations.

Primers	Sequences	Length	Populations					
			(filter score/allele frequency in the population)					
0246L	CCTCTCGACGACCTTCTCC	576	EN1	EN2	EN8	EN11		
0246R	GGTGTGGATGGCGATGAC		(27.5/77.32%)	(29.5/53.39%)	(149/21.43%)	(217/59.00%)		
0248L	CAAGATCGTCTCCGACCTG	580	EN3	EN5				
0248R	GGGGTTGATGGGTTTCGAG		(74.5/14.29%)	(12.7/10.75%)				
0251L	GTGCTCGGCCTCTACCTCTT	587	EN3					
0251R	TAGCCGAAGATGCGGTAGTT		(44/13.33%)					
1260L	CGAGGGCTTTGCACTCTATG	601	EN1	EN4				
1260R	CTGGTCGTTGAGGCGATAAT		(217/72.73%)	(215/61.84%)				
2394L	ATCCAGTCGCTGGGCACT	598	EN6	EN7	EN9			
2394R	GTAACGCATGTTCCGGTGATG		(33.5/49.44%)	(214/100%)	(107/35.63%)			
2395L	CGAGCCTCGACGATCTTATC	559	EN4	EN8	EN12			
2395R	AAGCCGTTGATGGCTGTC		(214/100%)	(217/46.21%)	(222/100%)			
2547L	GTGCAGCGTATCCTTGACCT	686	EN1	EN3	EN6	EN7	EN8	EN9
2547R	TAGAGTTGCTCCGTCCTTC		(53.5/12.5%)	(225/66.32%)	(222/98.06%)	(222/100%)	(222/98.94%)	(222/94.32%)
2571L	GTGGCCTGTTTCATCCACAC	604	EN1	EN2	EN3	EN6	EN8	EN12
2571R	GCCAGTACCGTGTTGAACG		(222/100%)	(214/100%)	(222/100%)	(222/98.96%)	(222/100%)	(222/100%)
2287L	ACTCGACCCGGTGATGCT	350	AN2	AN7	EN2	EN6	EN9	
2287R	GGTAGAAGGTGAACGCCTGA		(225/72.22%)	(225/67.07%)	(222/100%)	(222/100%)	(222/100%)	

**Table S2 Mutations identified in 12 AN populations (MP\*: the number of populations with mutations).**

Gene	COG	Predicted function	Position	Reference	Alter	Type	AN1	AN2	AN3	AN4	AN5	AN6	AN7	AN8	AN9	AN10	AN11	AN12	MP*		
DVU0129	T	Sensory box protein	165841	C	A	Gene							0.15						1		
DVU0162	EF	Carbamoyl-phosphate synthase, large subunit	201043	C	G	Gene								0.50				0.23	2		
DVU0281	S	Exopolysaccharide biosynthesis protein, putative	326403	G	A	Gene	0.28	0.33	0.77		0.16	0.23	0.39		0.38			0.16	0.27	9	
DVU0448	M	GDP-mannose 4,6-dehydratase	508909	G	C	Gene		0.13											1		
DVU0467	E	Anthranilate phosphoribosyltransferase	535249	G	C	Gene	0.26	0.34	0.57	0.15	0.15	0.25	0.22		0.35		0.14	0.31	10		
DVU0564	S	ISDvu4, transposase, truncation	629934	G	GCTAC	Intergenic					0.16								1		
DVU0564	S	ISDvu4, transposase, truncation	629934	GA	AGGGCTAC	Intergenic										0.32			1		
DVU0700	NT	Methyl-accepting chemotaxis protein	778393	A	G	Gene	0.17												1		
DVU0788	M	Rod shape-determining protein MreC	874923	G	C	Gene		0.38											1		
DVU0796	E	Histidinol dehydrogenase	882512	C	G	Intergenic	0.38	0.25	0.50	0.44				0.60	0.20	0.40		0.75	8		
DVU1087	S	Hypothetical protein DVU1087	1191167	C	T	Gene		0.50	0.50			0.50						0.40	1.00	0.33	6
DVU1104	S	Baseplate assembly protein, putative	1210118	C	G	Gene		0.26												1	
DVU1204	IQ	3-oxoacyl-(acyl-carrier-protein) synthase II	1295951	G	C	Gene	0.14													1	
DVU1238	ET	Amino acid ABC transporter, periplasmic amino acid-binding protein	1327518	G	C	Gene							0.13							6	
DVU1272	NU	General secretion pathway protein E, putative	1363430	C	A	Gene	0.25			0.49					0.79	0.36		0.52	0.18	6	
DVU1349	H	Geranylgeranyl diphosphate synthase	1426830	T	G	Gene	0.49	0.66	0.50	0.38	0.86	0.80	0.62	0.24	0.28	0.99	0.30	0.58	12		
DVU1360	M	UDP-glucose 4-epimerase	1439686	CATA	CA	Gene												0.27	1		
DVU1368	R	Rhodanese-like domain-containing protein	1447894	G	A	Gene								0.30				0.26	2		
DVU1371	R	HAD family hydrolase	1449911	C	G	Gene						0.28							1		
DVU1530	J	Metallo-beta-lactamase family protein	1599469	C	T	Gene	0.35	0.37	0.61	0.14		0.21	0.37		0.43		0.17	0.28	9		
DVU1698	S	Hypothetical protein DVU1698	1773342	CGG	CG	Gene												1.00	1		
DVU1698	S	Hypothetical protein DVU1698	1773345	A	G	Gene		0.64	0.33	1.00				0.44	0.50			1.00	6		
DVU1722	S	Hypothetical protein DVU1722	1796665	C	A	Gene							0.14						1		
DVU1769	R	Periplasmic lydA	1833244	C	A	Gene												0.25	1		
DVU1819	U	Preprotein translocase subunit SecD	1884277	G	C	Gene										0.35			1		
DVU1988	R	Hypothetical protein DVU1988	2068188	T	A	Gene	0.25			0.45				0.80	0.34			0.55	5		
DVU2023	S	Hypothetical protein DVU2023	2104739	G	C	Gene	0.35	0.64	0.35	0.28	0.84	0.76	0.74	0.15	0.29	0.99	0.44	0.60	12		
DVU2063	L	Hypothetical protein DVU2063	2142086	C	G	Gene	0.18			0.55				0.74	0.34			0.57	5		
DVU2078	NT	Protein-glutamate methyltransferase CheB	2162235	C	A	Gene					0.17							0.49	2		
DVU2078	NT	Protein-glutamate methyltransferase CheB	2163270	T	C	Gene	0.18	0.13		0.52					0.73	0.26			5		
DVU2287	C	Hydrogenase, CooK subunit, selenocysteine-containing, putative	2381876	T	G	Gene	0.24	0.28	0.64	0.13		0.17	0.33		0.41				0.39	8	
DVU2287	C	Hydrogenase, CooK subunit, selenocysteine-containing, putative	2381877	G	C	Gene	0.76	0.72	0.35	0.87	0.92	0.83	0.68	0.94	0.59	0.99	0.89	0.62	12		
DVU2379	R	M16 family peptidase putative	2477501	G	T	Gene					0.15								1		
DVU2498	S	Hypothetical protein DVU2498	2609413	G	A	Gene												0.14	1		
DVU2505	D	Cell cycle protein FtsW	2616993	A	T	Gene							0.27						1		
DVU2557	H	BirA bifunctional protein	2669556	A	C	Gene	0.27	0.14		0.53				0.72	0.48		0.64	0.16	7		
DVU2617	P	Sodium/calcium exchanger family protein	2738682	C	G	Gene									0.16				1		
DVU2664	P	Phosphate ABC transporter, ATP-binding protein, putative	2775672	C	G	Gene	0.45	0.64	0.37	0.29	0.86	0.78	0.79	0.15	0.31	0.99	0.32	0.73	12		
DVU2802	K	GntR family transcriptional regulator	2905021	T	G	Gene			0.48										0.11	2	
DVU2802	K	GntR family transcriptional regulator	2905143	T	G	Gene	0.17												1		
DVU2802	K	GntR family transcriptional regulator	2905203	G	A	Gene	0.24	0.39	0.66			0.18	0.30						0.28	7	
DVU2802	K	GntR family transcriptional regulator	2905275	G	C	Gene								0.40	0.45		0.29		3		
DVU2959	S	Hypothetical protein DVU2959	3066925	CACACA	GCAC	Intergenic		1.00						1.00					2		
DVU3022	T	Sensory box histidine kinase/response regulator	3140578	A	T	Gene	1.00	1.00	1.00	1.00	1.00			1.00	1.00		1.00	1.00	9		
DVU3022	T	Sensory box histidine kinase/response regulator	3140578	A	T	Gene		1.00	1.00	1.00	1.00			1.00	1.00			1.00	7		
DVU3022	T	Sensory box histidine kinase/response regulator	3140597	T	TAT	Gene	0.83				0.86								2		
DVU3045	T	Sensory box histidine kinase/response regulator	3169435	G	C	Gene	0.29	0.24	0.62		0.12	0.23	0.27		0.35			0.26	8		
DVU3106	T	GGDEF domain-containing protein	3254670	G	C	Intergenic								0.25					1		
DVU3129	S	Hypothetical protein DVU3129	3276860	G	C	Gene					0.17							0.13	2		
Intergenic mutations could not be assigned to a gene		Sits between DVU0645 (Methyl-accepting chemotaxis protein) and DVU0646 (Precorrin-2 C20-methyltransferase) that face opposite ways + 216 bp of DVU0978 (ABC transporter, periplasmic substrate-binding protein, putative)	716874	T	A	Intergenic	0.42	0.31	0.18	0.38	0.61	0.68	0.72	0.13	0.25		0.32	0.36	11		
		Sits between DVU1176 (Hypothetical protein DVU1176) and DVU1177 (Hypothetical protein DVU1177) that face opposite ways	1268170	G	C	Intergenic								1.00				0.18	2		
		Sits between DVU1999 (Sulfate transporter family protein) and DVU2000 (Hypothetical protein DVU2000) that face opposite ways	2082600	T	A	Intergenic				0.19				0.20						2	
		Sits between DVU2112 (Hypothetical protein DVU2112) and DVU2113 (Xanthine/uracil permease family protein) that face opposite ways	2207959	G	C	Intergenic		0.28	0.52			0.24							0.29	4	
		+ 198 bp of DVU2397 (Hypothetical protein DVU2397)	2502193	G	C	Intergenic	0.43	0.65	0.30	0.40	0.86	0.84	0.70	0.16	0.22	1.00	0.31	0.56	12		
		Sits between DVU2683 (L-lactate permease family protein) and DVU2684 (Hypothetical protein DVU2684) that face opposite ways	2798285	C	G	Intergenic		0.22						0.33						1	
		+ 141 bp of DVU2885 (Alcohol dehydrogenase, iron-containing)	2982786	C	CTG	Intergenic														1	
		+ 138 bp of DVU2885 (Alcohol dehydrogenase, iron-containing)	2982789	C	CCA	Intergenic													1.00	1	
<b>No. of mutations in each population</b>							<b>24</b>	<b>24</b>	<b>18</b>	<b>22</b>	<b>16</b>	<b>15</b>	<b>18</b>	<b>22</b>	<b>25</b>	<b>8</b>	<b>22</b>	<b>26</b>			

**Table S3 Mutations identified in 12 EN populations (MP\*: the number of populations with mutations)**

Gene	COG	Predicted function	Position	Reference	Alter	Type	EN1	EN2	EN3	EN4	EN5	EN6	EN7	EN8	EN9	EN10	EN11	EN12	MP*	
DVU0126	CP	ABC transporter, ATP-binding protein	162600	C	G	Gene				1.00	0.82	0.43							2	
DVU0225	S	Hypothetical protein DVU0225	262421	C	T	Gene													1	
DVU0246	S	Pyruvate phosphate dikinase PEP/pyruvate binding subunit	279123	CG	C	Gene	0.15												1	
DVU0246	S	Pyruvate phosphate dikinase PEP/pyruvate binding subunit	279507	C	A	Gene									0.59				1	
DVU0246	S	Pyruvate phosphate dikinase PEP/pyruvate binding subunit	279702	GT	G	Gene											0.47		1	
DVU0246	S	Pyruvate phosphate dikinase PEP/pyruvate binding subunit	279997	AGGGGGGG	AGGGGGG	Gene	0.77	0.53											2	
DVU0246	S	Pyruvate phosphate dikinase PEP/pyruvate binding subunit	280373	GTTGCGGT	C	Gene								0.21					1	
DVU0247	T	Response regulator	281155	AT	A	Gene							0.34						1	
DVU0247	T	Response regulator	281286	TACGA	TACGAACGA	Gene							0.50						1	
DVU0248	T	Signal transduction histidine kinase	281561	AGGGG	AGG	Gene		0.14											1	
DVU0248	T	Signal transduction histidine kinase	281703	GTT	G	Gene				0.18									1	
DVU0248	T	Signal transduction histidine kinase	282788	C	T	Gene								0.65					1	
DVU0248	T	Signal transduction histidine kinase	283136	C	T	Gene										0.18			1	
DVU0248	T	Signal transduction histidine kinase	283154	C	T	Gene				0.63									1	
DVU0248	T	Signal transduction histidine kinase	283204	G	T	Gene												0.47	1	
DVU0249	P	PtxB, putative	283782	C	T	Gene			0.17										1	
DVU0251	R	Transmembrane protein TauE like	285479	G	A	Gene			0.13										1	
DVU0251	R	Transmembrane protein TauE like	285947	C	G	Gene			0.19										1	
DVU0251	R	Transmembrane protein TauE like	285948	C	T	Gene													0.23	2
DVU0281	S	Exopolysaccharide biosynthesis protein, putative	325822	GT	G	Gene													1.00	1
DVU0281	S	Exopolysaccharide biosynthesis protein, putative	326403	G	A	Gene	1.00		1.00	1.00	0.76			1.00					5	
DVU0408	M	EAL domain-containing protein	456487	A	G	Intergenic					0.19								1	
DVU0467	E	Anthraniolate phosphoribosyltransferase	535249	G	C	Gene	1.00		1.00	1.00	0.89			1.00					5	
DVU0564	S	ISDv4, transposase, truncation	629931	C	A	Intergenic							0.48						0.22	2
DVU0564	S	ISDv4, transposase, truncation	629934	GA	TAGGGCTAG	Intergenic	0.21						0.73						2	
DVU0564	S	ISDv4, transposase, truncation	629934	G	GCTAC	Intergenic									0.29				1	
DVU0596	KT	DNA-binding response regulator LytR	664509	C	T	Gene	0.11												1	
DVU0597	T	regulatory protein LytS	665366	C	T	Intergenic			0.12										1	
DVU0597	T	Regulatory protein LytS	665986	C	T	Gene						0.42							1	
DVU0597	T	Regulatory protein LytS	666383	C	T	Gene				0.11			0.12						2	
DVU0597	T	Regulatory protein LytS	666479	A	C	Gene												0.25	1	
DVU0796	E	Histidinol dehydrogenase	882512	C	G	Intergenic	0.80	0.44		1.00	0.29	0.38		0.40	1.00		0.40		8	
DVU0797	S	Hypothetical protein DVU0797	883424	C	T	Gene								0.38					1	
DVU0799	S	Hypothetical protein DVU0799	885159	G	GTCGGAA CCGTGTA GGTICTTG CCG	Gene								0.55					1	
DVU799	S	Hypothetical protein DVU799	885499	G	A	Gene								0.48					1	
DVU0847	C	Adenylylsulfate reductase	934997	C	T	Gene							0.99						1	
DVU0942	P	FUR family transcriptional regulator	1034641	A	C	Gene										1.00			1	
DVU0942	P	FUR family transcriptional regulator	1034711	A	T	Gene			1.00	0.88					1.00				3	
DVU0942	P	FUR family transcriptional regulator	1034799	A	T	Gene											1.00		1	
DVU0942	P	FUR family transcriptional regulator	1034881	G	A	Gene							1.00						1	
DVU2571	P	Ferrous iron transport protein B	2685074	A	C	Gene												1.00	1	
DVU2571	P	Ferrous iron transport protein B	2685298	T	C	Gene			1.00										1	
DVU2571	P	Ferrous iron transport protein B	2685402	CTCAAGG GCGGCCTC GGCCCGTT CGGCATC AAGGCGG GC	CTCAAGG GCGGC	Gene		1.00											1	
DVU2571	P	Ferrous iron transport protein B	2685709	G	A	Gene	1.00												1	
DVU2571	P	Ferrous iron transport protein B	2685734	G	A	Gene								1.00					1	
DVU2571	P	Ferrous iron transport protein B	2685977	A	T	Gene							0.99						1	
DVU1087	S	Hypothetical protein DVU1087	1191167	C	T	Gene			0.40		0.50				0.75		0.57	0.33	5	
DVU1204	IQ	3-oxoacyl-(acyl-carrier-protein) synthase II	1295686	C	T	Gene							1.00						1	
DVU1204	IQ	3-oxoacyl-(acyl-carrier-protein) synthase II	1295951	G	C	Gene	1.00												1	
DVU1204	IQ	3-oxoacyl-(acyl-carrier-protein) synthase II	1296057	C	G	Gene				1.00	0.92								2	
DVU1204	IQ	3-oxoacyl-(acyl-carrier-protein) synthase II	1296081	T	C	Gene									0.99				1	
DVU1204	IQ	3-oxoacyl-(acyl-carrier-protein) synthase II	1296268	C	T	Gene		1.00											1	
DVU1204	IQ	3-oxoacyl-(acyl-carrier-protein) synthase II	1296277	C	T	Gene												1.00	1	
DVU1204	IQ	3-oxoacyl-(acyl-carrier-protein) synthase II	1296453	T	A	Gene			0.96										1	
DVU1208	I	Putative glycerol-3-phosphate acyltransferase PlsX	1299241	T	G	Gene								0.43		1.00			2	
DVU1260	I	Outer membrane protein P1, putative	1346776	TGG	TG	Gene				0.16									1	
DVU1260	I	Outer membrane protein P1, putative	1347079	CTATAAT GTCCGCA TCCA	C	Gene				0.62									1	
DVU1260	I	Outer membrane protein P1, putative	1347740	CT	C	Gene	0.73												1	
DVU1272	NU	General secretion pathway protein E, putative	1363430	C	A	Gene												1.00	1	
DVU1349	H	Geranylgeranyl diphosphate synthase	1426830	T	G	Gene		1.00		0.15	1.00	1.00		1.00	1.00			1.00	7	
DVU1371	R	HAD family hydrolase	1449911	C	G	Gene								1.00					1	
DVU1408	S	Hypothetical protein DVU1408	1477702	A	G	Gene	0.18												1	
DVU1469	J	30S ribosomal protein S1	1551144	C	T	Gene								0.56					1	
DVU1469	J	30S ribosomal protein S1	1551147	C	T	Gene							0.98						1.00	2
DVU1530	J	Metallo-beta-lactamase family protein	1599469	C	T	Gene	1.00		0.99	1.00	0.83			1.00					5	
DVU1545	S	Hemolysin-type calcium-binding repeat/calx-beta domain-containing protein	1620196	C	T	Gene							0.52						1	
DVU1576	I	4-diphosphocytidylyl-2C-methyl-D-erythritol kinase	1658379	CGTATG	CG	Gene												0.94	1	
DVU1588	F	Hypoxanthine phosphoribosyltransferase	1671284	CGGGGGG	CGGGGGG	Gene							0.59						1	
DVU1634	S	Hypothetical protein DVU1634	1717112	C	T	Gene					0.13								1	
DVU1698	S	Hypothetical protein DVU1698	1733445	A	G	Gene	1.00			1.00			1.00	1.00	1.00		0.75	1.00	7	
DVU1785	U	MarC membrane protein	1848460	A	C	Gene							0.97						1	
DVU1785	U	MarC membrane protein	1848475	G	A	Gene												0.94	1	
DVU1862	T	GGDEF domain-containing protein	1929597	CA	C	Gene						0.29							1	
DVU1862	T	GGDEF domain-containing protein	1930141	C	T	Gene			0.57	0.16									2	
DVU1862	T	GGDEF domain-containing protein	1930239	CA	C	Gene								0.42					1	
DVU1862	T	GGDEF domain-containing protein	1930258	A	T	Gene			0.31										1	
DVU1988	R	Hypothetical protein DVU1988	2068188	T	A	Gene												1.00	1	
DVU2023	S	Hypothetical protein DVU2023	2104739	G	C	Gene		1.00			0.24	0.99	1.00		1.00	1.00		1.00	7	
DVU2063	L	Hypothetical protein DVU2063	2142086	C	G	Gene													1	
DVU2078	NT	Protein-glutamate methyltransferase CheB	2162235	C	A	Gene										1.00			1	
DVU2078	NT	Protein-glutamate methyltransferase CheB	2162370	T	C	Gene												1.00	1	
DVU2284	S	Hypothetical protein DVU2284	2374803																	









**Table S5** Mutated functional genes with newly acquired mutations in EN populations (MP\*: the number of populations with mutations)

Gene	COG	Predicted function	MP*	EN1	EN2	EN3	EN4	EN5	EN6	EN7	EN8	EN9	EN10	EN11	EN12	
DVU0126	CP	ABC transporter, ATP-binding protein	2				1.00	0.82								
DVU0225	S	Hypothetical protein DVU0225	1						0.43							
DVU0246	S	Pyruvate phosphate dikinase PEP/pyruvate binding subunit	5	0.77	0.53					0.48	0.21	0.59		0.47		
DVU0247	T	Response regulator	1						0.50							
DVU0248	T	Signal transduction histidine kinase	6			0.14	0.63	0.18			0.65	0.14	0.18		0.47	
DVU0249	P	PtxB, putative	1			0.17										
DVU0251	R	Transmembrane protein TauE like	3			0.19				0.34					0.23	
ΔDVU0246-0253		Functional loss of DVU0246-0251	3					0.28					0.33		0.32	
ΔDVU0246-0256																
ΔDVU0240-0270																
DVU0281	S	Exopolysaccharide biosynthesis protein, putative	1												1.00	
DVU0596	KT	DNA-binding response regulator LytR	1	0.11												
DVU0597	T	regulatory protein LytS	5			0.12 <sup>a</sup>	0.11		0.42	0.12				0.25		
DVU0797	S	Hypothetical protein DVU0797	1								0.38					
DVU0799	S	Hypothetical protein DVU0799	1								0.55					
DVU0847	C	Adenylylsulfate reductase	1							0.99						
DVU0942	P	FUR family transcriptional regulator	6				1.00	0.88		1.00		1.00	1.00	1.00		
DVU2571	P	Ferrous iron transport protein B	6	1.00	1.00	1.00			0.99		1.00				1.00	
DVU1204	IQ	3-oxoacyl-(acyl-carrier-protein) synthase II	7		1.00	0.96	1.00	0.92		1.00		0.99		1.00		
DVU1208	I	Putative glycerol-3-phosphate acyltransferase PlsX	2								0.43		1.00			
DVU1260	I	Outer membrane protein P1, putative	2	0.73			0.62									
DVU1408	S	Hypothetical protein DVU1408	1	0.18												
DVU1469	J	30S ribosomal protein S1	3						0.98		0.56				1.00	
DVU1545	S	Hemolysin-type calcium-binding repeat	1						0.52							
DVU1576	I	4-diphosphocytidyl-2C-methyl-D-erythritol kinase	1											0.94		
DVU1588	F	Hypoxanthine phosphoribosyltransferase	1						0.59							
DVU1634	S	Hypothetical protein DVU1634	1					0.13								
DVU1785	U	MarC membrane protein	2						0.97					0.94		
DVU1862	T	GGDEF domain-containing protein	4			0.68	0.16	0.29			0.42					
DVU2284	S	Hypothetical protein DVU2284	1						0.20							
DVU2394	T	Sigma-54 dependent transcriptional regulator	5			0.59		0.16	0.49	1.00		0.36				
DVU2395	T	Sensor histidine kinase	5				1.00				0.46	0.54	0.45		1.00	
DVU2396	C	Alcohol dehydrogenase, iron-containing	3	0.72	0.98				0.42							
DVU2402	C	Heterodisulfide reductase, A subunit	1		0.96											
DVU2405	C	Alcohol dehydrogenase, iron-containing	2					0.86						1.00		
DVU2406	S	Hypothetical protein DVU2406	1							0.29						
DVU2411	S	EF hand domain-containing protein	1						0.42							
DVU2543	C	Hydroxylamine reductase	2				0.99	0.79								
DVU2547	T	Transcriptional regulator, putative	7	0.74		0.66			0.99	1.00	0.99	0.94	0.72			
DVU2548	I	Acyl carrier protein phosphodiesterase	3		0.93									0.71 <sup>a</sup>	1.00	
DVU2747	S	Hypothetical protein DVU2747	1		0.57											
DVU2894	T	Sigma-54 dependent transcriptional regulator	1	0.14												
DVU2950	S	Sensory box protein/GGDEF domain protein	1		1.00											
DVU3022	T	Sensory box histidine kinase/response regulator	2									1.00		1.00		
DVU3190	S	Hypothetical protein DVU3190	1											0.96		
DVU3264	C	Fumarate hydratase	1					0.19								
DVU3304	S	Hypothetical protein DVU3282	2	0.23	0.14											

<sup>a</sup> intergenic mutation identified within 100 bp of the upstream of start codon of the gene

Table S6 Newly arising mutations occurred more than once in the EN populations in addition to those in six gene groups.

Gene	Products	COG	Mutation position	Nucleotide change	Allele frequency	Populations
DVU0126	ABC transporter, ATP-binding protein	CP	<b>162600</b>	<b>C → G</b>	<b>100</b>	<b>EN4</b>
					<b>100</b>	<b>EN5</b>
DVU1260	outer membrane protein P1, putative	I	1347740	CT → C	72.73	EN1
			1346776	TGG → TG	16.28	EN4
			1347080	-17:TATAATGTCGGCATCCA	61.84	EN4
DVU1469	30S ribosomal protein S1	J	1551144	C → T	50.56	EN8
			<b>1551147</b>	<b>C → T</b>	<b>98.02</b>	<b>EN6</b>
					<b>100</b>	<b>EN12</b>
DVU1785	MarC membrane protein	U	1848460	A → C	96.83	EN6
			1848475	G → A	93.67	EN11
			1929985	△258 bp	25.10	EN5
			1930123	△146 bp	67.50	EN3
DVU1862	GGDEF domain-containing protein	T	<b>1930141</b>	<b>C → T</b>	<b>56.67</b>	<b>EN3</b>
			1930258	A → T	31.11	EN3
			1929597	CA → C	28.99	EN5
			1930239	CA → C	42.11	EN8
DVU3022	response regulator	T	<b>3140595</b>	<b>T → G</b>	<b>81.82</b>	<b>EN6</b>
					<b>99.04</b>	<b>EN11</b>
DVU3304	Signal transduction histidine kinase	T	3482319	G → A	23.47	EN1
			3481721	G → A	13.08	EN2

Table S7 Newly arising mutations (single nucleotide polymorphisms) in DVU1204 (*fabF*) and DVU1208 (*plsX*) of EN populations.

Gene	Populations	Allele frequency (%)	Mutation position	Nucleotide change	Amino-acid change
DVU1204 <i>fabF</i>	EN2	100	1296268	C → T	Ala → Thr
	EN3	96	1296453	T → A	Gln → Leu
	EN4	100	1296057	C → G	Gly → Ala
	EN5	90	1296057	C → G	Gly → Ala
	EN7	100	1295686	C → T	Gly → Ser
	EN9	99	1296081	T → C	His → Arg
	EN11	100	1296277	C → T	Ala → Thr
DVU1208 <i>plsX</i>	EN8	43	1299241	T → G	Silent mutation
	EN10	100	1299241	T → G	Silent mutation

Table S8 Newly arising mutations in DVU0924 (*fur*) and DVU2571 (*feoB*) of EN populations.

Gene	Populations	Mutation type	Allele frequency (%)	Mutation position	Nucleotide change	Amino-acid change
DVU0924 <i>fur</i>	EN4	SNP	100	1034711	A → T	Glu → Asp
	EN5	SNP	88	1034711	A → T	Glu → Asp
	EN7	SNP	100	1034881	G → A	Arg → Gln
	EN9	SNP	100	1034711	A → T	Glu → Asp
	EN10	SNP	100	1034641	A → C	Asp → Ala
	EN11	SNP	100	1034799	A → T	Asn → Tyr
DVU2571 <i>feoB</i>	EN1	SNP	100	2685709	G → A	Ser → Phe
	EN2	Deletion	100	2685414	-27: CTCGGCCCGTTCGGCATCAAGGGCGGC	nine amino acids loss
	EN3	SNP	100	2685298	T → C	Lys → Arg
	EN6	SNP	99	2685977	A → T	Phe → Ile
	EN8	SNP	100	2685734	G → A	Arg → Cys
	EN12	SNP	100	2685074	A → C	Phe → Val

Table S9 Newly arising mutations in two component system *LytR/LytS* (DVU0596/DVU0597) of EN populations.

Gene	Populations	Allele frequency (%)	Mutation position	Nucleotide change	Amino-acid change
DVU0596 <i>lytR</i>	EN1	11	664509	C → T	Pro → Leu
	EN3	12	665366	C → T	four bases upstream of the start codon
DVU0597 <i>LytS</i>	EN4	11	666383	C → T	Ala → Val
	EN6	40	665986	C → T	Leu → Phe
	EN7	12	666383	C → T	Ala → Val
	EN11	25	666479	A → C	Asn → Thr



## References

1. Kelley LA, Mezulis S, Yates CM, Wass MN. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc. 2015;**10**:845-858.