

Activation of an Otherwise Silent Xylose Metabolic Pathway in *Shewanella oneidensis*

Supporting information

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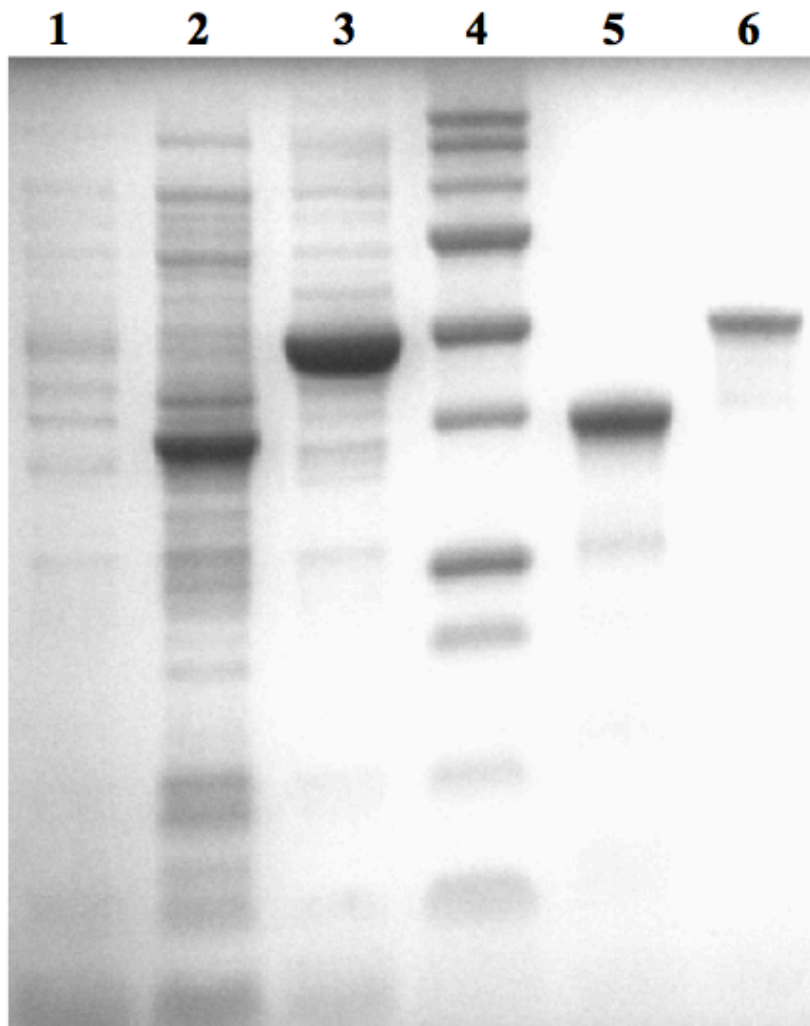


Fig. S1. SDS-PAGE for expression of recombinant SO_0900 & SO_4230 from *E. coli* JM109. Each lane was loaded with 20 g protein. Lanes: 1, Empty pQE80L plasmid crude; 2, SO_0900 crude; 3, SO_4230 crude; 4, MW marker; 5, SO_0900 pure; 6, SO_4230 pure.

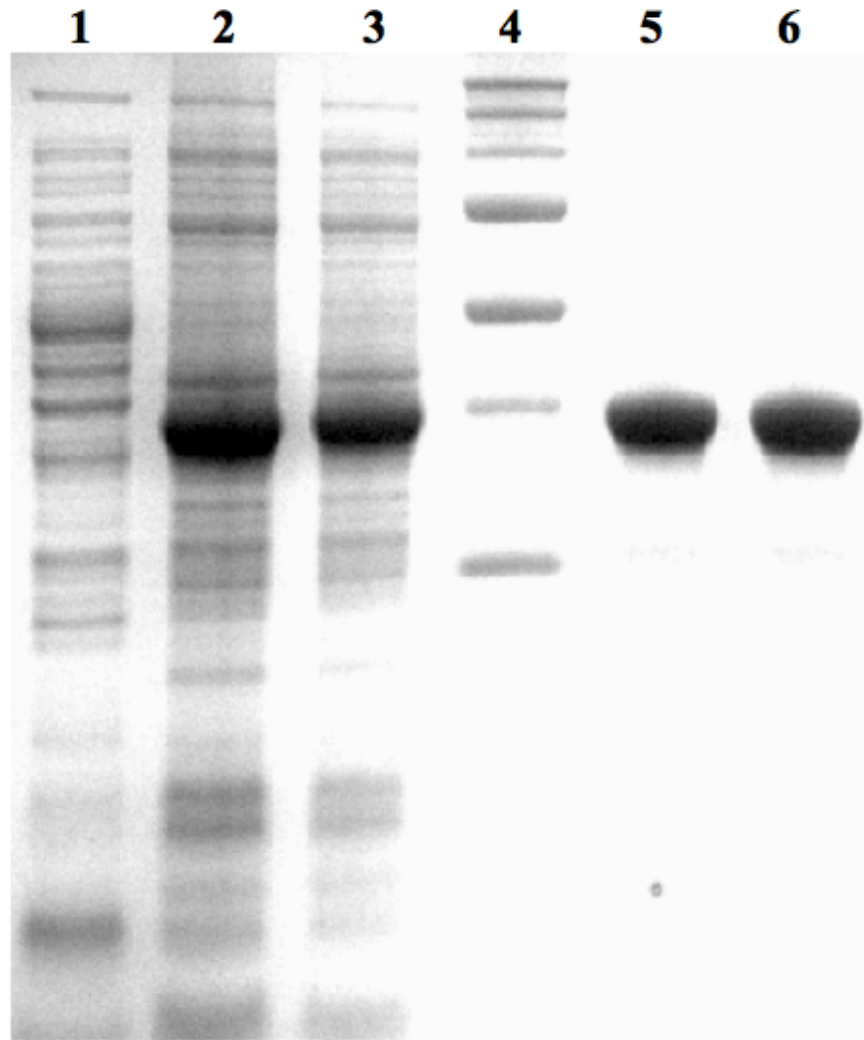


Fig. S2. SDS-PAGE for expression of recombinant SO_4673 & SO_2452 from *E. coli* JM109. Each lane was loaded with 20 g protein. Lanes: 1, Empty pQE80L plasmid crude; 2, SO_4673 crude; 3, SO_2452 crude; 4, MW marker; 5, SO_4673 pure; 6, SO_2452 pure.

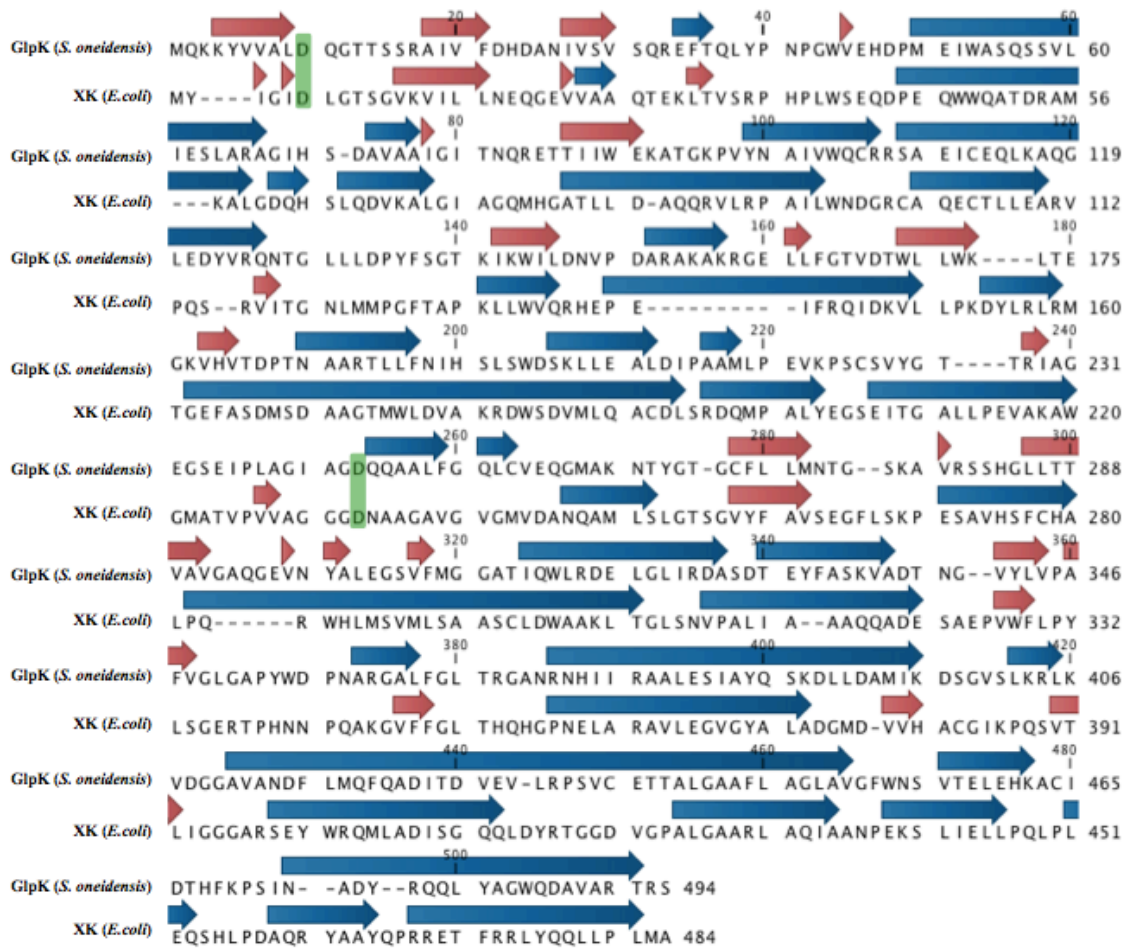


Fig. S3. Pairwise sequence alignment of glycerol kinase (from *S. oneidensis*) and xylulokinase (from *E. coli*). Blue arrows indicate alpha helix and red arrows indicate beta strands. Green shades indicate homologous residues Asp10, Asp244 (GlpK - *S. oneidensis*) and Asp6, Asp233 (XK - *E. coli*).

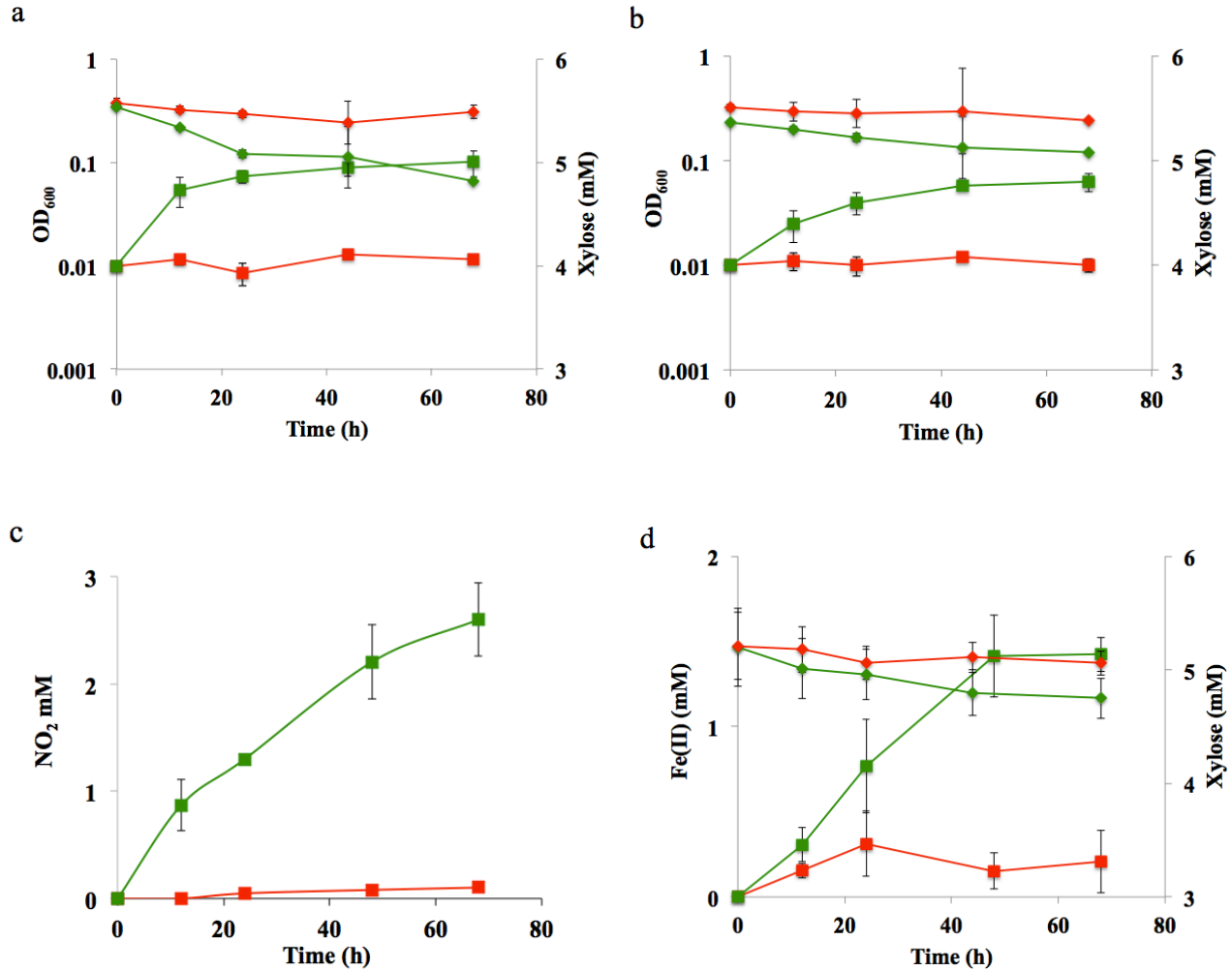


Fig S4. Anaerobic respiratory activities of strain XM1 with xylose as sole carbon and energy source. **(a)** Growth and xylose concentration profiles of *S. oneidensis* and strain XM1 in the presence of fumarate as electron acceptor in minimal media. 5 mM xylose and 10 mM fumarate were used in this experiment. Wild type; red, XM1; green, OD_{600} ; (■), xylose concentration; (◆). **(b)** Growth and xylose concentration profiles of *S. oneidensis* and strain XM1 in the presence of nitrate (NO_3) as electron acceptor in minimal media. 5 mM xylose and 5 mM nitrate were used in this experiment. Wild type; red, XM1; green, OD_{600} ; (■), xylose concentration; (◆). **(c)** Nitrite concentration profiles during growth curve of *S. oneidensis* and strain XM1 in the presence of NO_3 as electron acceptor in minimal media. 5 mM xylose and 5 mM nitrate were used in this experiment. Wild type; red (■), XM1; green (■). **(d)** Fe(II) and xylose concentration profiles during growth curve of *S. oneidensis* and strain XM1 in the presence of Fe(III) as electron acceptor in minimal media. 5 mM xylose and 10 mM Fe(III) were used in this experiment. Wild type; red, XM1; green, Fe(II); (■), xylose concentration; (◆). Error bars represent range of errors in duplicate batch reactors

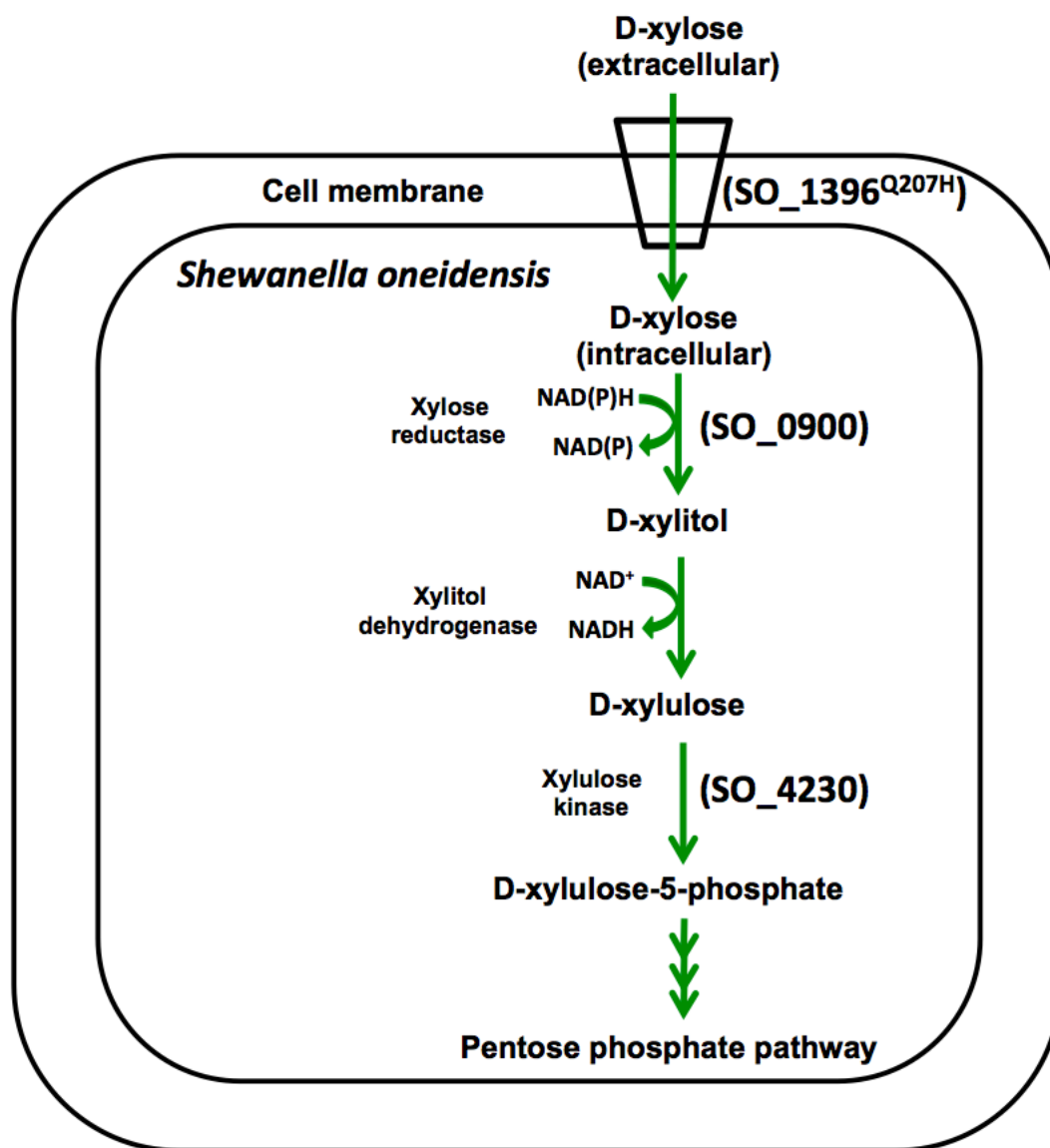


Fig. S5. Working model of the xylose metabolic pathway in strain XM1, including putative xylose transporter SO_1396^{Q207H}, putative xylose reductase SO_0900, and putative xylulose reductase SO_4230 identified in the present study

Fig. S6.

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Amazonensis 1 INIIFMGNSLBEGYIPTIPAAASDVPLEVFSRLLLEGRFISLFLMLFGVGLVIQYQRF---
Woodyi 1 MNIYFNGNAVSGYASHEISPFHDTLVELFSNFFLEGRFISLFLMLFGVGLVQFNRYSKQ
Piezotolerans 1 MNIYFNGNSFSGYANHETVPEHDTVIMLFSNFFLEGRFISLFLSMLFGVGLAIQYQNKLSAL
Haliotis 1 LNVSEFMGVTELYGAPFPEFHLDATITETLSNFFLEGRFISLFLSMLFGVGLMIQQQNFBAR
Algae 1 LNVSEFMGVTELYGAPFPEFHLDATITETLSNFFLEGRFISLFLSMLFGVGLMIQQQNFBAR
Frigidimarina 1 LNIYFMGNSEFSGYAPHEVQPTSDIILDFSNFFLEGRFISLFLSMLFGVGLMIQYQR-QQM
W3-18-1 1 MNIYFMGISFYGYAPHEMPELSDHEIKVFNHFFIEGRFISLFLSMLFGVGLMIQYQRFSAK
Putrefaciens 1 MNIYFMGIIFSGYAPHEQPLSDHEIKVFSNFFIEARFISLFLSMLFGVGLMIQYQRFSAK
Baltica 1 MNIYFMGIIFSGYAPHEQPLSDHEIKVFSNFFIEARFISLFLSMLFGVGLMIQYQRFSAK
MR-1 1 MNIYFMGISFYGYAPHQIPLLMDQILOTFSNFFIEGRFISLFLSMLFGVGLMIQYQRFTAK
XM1 1 MNIYFMGISFYGYAPHQIPLLMDQILOTFSNFFIEGRFISLFLSMLFGVGLMIQYQKFTAK
ANA-3 1 MNIYFMGISFYGYAPHQIPLLSDQVLEAFSNFFIEGRFISLFLSMLFGVGLMIQYQRFSDK
MR-4 1 MNIYFMGISFYGYAPHQIQLLSDQVLEAFSNFFIEGRFISLFLSMLFGVGLMIQYQRFSDK
MR-7 1 MNIYFMGISFYGYAPHQIPLLSDQVLEAFSNFFIEGRFISLFLSMLFGVGLMIQYQRFSDK
consensus 1 .....*.....*.....*.....*.....*.....*.....*.....*.....*.....*.....

Amazonensis 58 -GQVEP-----IKRRLKLLMLFGLLHGIEIWPGDVLLSYGSLAMVALRYLDAPLDHIIH
Woodyi 61 NIDRAYKQ-----IKSRLKWLIVFGAVHGVIVWISGDVLFAYGLSGFLALCYLKLQNKLI
Piezotolerans 61 DGARYQT-----MKSRLKWLIVFGVIHGIEIWYGDILETYALGGFLALCYLQDNKKLL
Haliotis 61 GLEPEPL-----IRSRLKWLIVFGALHSLFIWPGDILLTYISICGFLAWHYRNLEIQAQQ
Algae 61 GLEPEPL-----IRSRLKWLIVFGALHSLFIWPGDILLTYISICGFLAWHYRNLEIQAQQ
Frigidimarina 60 GMDTATTSQRNKRIRKRLNWLIAFGVIHAFIEIPGDILLTYGVSGLAERYINLSADELI
W3-18-1 61 GLNAYPL-----LRSRLKWLIVFGLIHGIIWPGDILFTYISGFLALCYKDLISIGELK
Putrefaciens 61 GLEAYSL-----LRSRLKWLIVFGLIHGIEIWSGDILETYISGFLALCYKDLISVBAIK
Baltica 61 GLEAYPL-----LRSRLKWLIVFGLIHGIIWPGDILLTYISGFLALCYKDLISVBAIK
MR-1 61 GLAAYPL-----LRSRLKWLIVFGLIHGIIWISGDILLTYISGFLALCYRDIITIAEQK
XM1 61 GLAAYPL-----LRSRLKWLIVFGLIHGIIWISGDILLTYISGFLALCYRDIITIAEQK
ANA-3 61 GLEAYSL-----LRSRLKWLIVFGLIHGIIWPGDILLTYISGFLALCYRDASIAELK
MR-4 61 GLAAYSL-----LRSRLKWLIVFGLIHGIIWPGDILLTYISGFLALCYRDASIAELK
MR-7 61 GLAAYSL-----LRSRLKWLIVFGLIHGIIWPGDILLTYISGFLALCYRDASIAELK
consensus 61 .. ..*.....*.....*.....*.....*.....*.....*.....*.....*.....*.....

Amazonensis 110 KRALQELGFSALVMLLLTQALPTEPLPSRLSPEYQASLAPWIGPYLGOVLDHLLAMMLMIF
Woodyi 115 KKSLEFI-AISVFTFTLESIFSPSEERIRGSELFESEBYLIWFSIYSEQLLMQEVFITEL
Piezotolerans 115 KKSIFIFI-TLPLFIFAITISIFSPSEAIIRGSAQFEDLSIWSGIYSGQLMMQLIIFTGML
Haliotis 115 RRALDFI-AIGTDAYGLMIFLPGDETIRGNVLFNEQYSAWTGSYGEQLLMHLEFMTLLML
Algae 115 RRALDFI-AIGTDAYGLMIFLPGDETIRGNVLFNEQYSAWTGSYGEQLLMHLEFMTLLML
Frigidimarina 120 RKAKWFI-FLSEFETALISLMPDQVYRDSAFFEQLAAWTGSYQQQLFLHLIMFAYML
W3-18-1 115 RKANIFI-FGALVITLVLVSLGSDPEFTRESSLFAEQYSAWTSSYADQLFLHLMQVGYMA
Putrefaciens 115 RKANIFI-LSALVITLVLVSLGSDPEFTRESSLFAEQYSAWTSSYADQLFLHLMQVGYMA
Baltica 115 RKANIFI-LSALVITLVLVSLGSDPEFTRESSLFAEQYSAWTSSYADQLFLHLMQVGYMA
MR-1 115 RKANIFI-FMSLVITCLLALSGSDELFRESAFFAEQYSAWTSSYENQLLHLIQVGYDA
XM1 115 RKANIFI-FMSLVITCLLALSGSDELFRESAFFAEQYSAWTSSYENQLLHLIQVGYDA
ANA-3 115 RKANIFI-FSSLVITCLISLNGSDELFRESPLFAEQYSAWTSSYANQLFLHVMQVGYMA
MR-4 115 RKANIFI-FSSLVITCLISLNGSDELFRESPLFAEQYSAWTSSYANQLFLHVMQVGYMA
MR-7 115 RKANIFI-FSSLVITCLISLNGSDELFRESPLFAEQYSAWTSSYENQLFLHVMQVGYMA
consensus 121 .....*.....*.....*.....*.....*.....*.....*.....*.....*.....*.....

Amazonensis 170 LVMLPITALLWYIDGMMLLGMVLYRSHFFNEGLTDAARIRLRLLEALLLGAVDLTLFYFSESER
Woodyi 174 FF-IPPTLMWFTAGLMLLGVLYRKGIFQGGFSKRHLIWFALASIFFSTLDSLLSFSENP
Piezotolerans 174 FI-IPPTLMWFTAGLMLLGVLYRKKVFEKGLTQRQLLQLLATIILLSALDSACTLATNP
Haliotis 174 IS-IPFTLLWFVSGVMLLGLISLYRRGFEDQGLPQGNLQKLLALSLLSLADTILSLQSRP
Algae 174 IS-IPFTLLWFVSGVMLLGLISLYRRGFEDQGLPQGNLQKLLALSLLSLADTILSLQTRP
Frigidimarina 179 AV-IPPTLMWFTAGLMLLGMALYKRNIFVDGLDNKTLMOCVFAWTLASLDSILSLSGNQ
W3-18-1 174 LV-IPPTLMWFTAGLMLLGMALYQGGFERGFSSVLVKLVLASVTLSSLDLTVLGLTKNF
Putrefaciens 174 LV-IPPTLMWFTAGLMLLGMALYQGGFERGFSSVLVKLVLASVTLSSLDLTVLGLTKNF
Baltica 174 LV-IPPTLMWFTAGLMLLGMALYQGGFERGFSSVLVKLVLASVTLSSLDLTVLGLTKNF
MR-1 174 LA-IPPTLMWFTAGLMLLGMALYRQGFEBEGFNNTLLKLLALASLVLSLMDTLGLTQNP
XM1 174 LA-IPPTLMWFTAGLMLLGMALYRQGFEBEGFNNTLLKLLALASLVLSLMDTLGLTQNP
ANA-3 174 LV-IPPTLMWFTAGLMLLGMALYRQGSFEBEGFNNTLLKLVLASLVLSALDITLLSLTONP
MR-4 174 LV-IPPTLMWFTAGLMLLGMALYRQGSFEBEGFNNTLLKLVLASLVLSALDITLLSLTONP
MR-7 174 LV-IPPTLMWFTAGLMLLGMALYRQGSFEBEGFNNTLLKLVLASLVLSALDITLLSLTONP
consensus 181 .....*.....*.....*.....*.....*.....*.....*.....*.....*.....*.....
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Fig. S6 (continued)



Fig. S6. Multiple sequence alignments generated by ClustalW analysis of *S. oneidensis* MR-1 SO_1396 homologs identified in the genomes of 14 *Shewanella* strains. Black shades indicate identity and gray shades indicate similarity.

Table S1. Composition of SM medium

Compound	Concentration (mM)
Potassium Phosphate	10
NH ₄ SO ₄	15
MgSO ₄ ·7H ₂ O	1
CaCl ₂ ·2H ₂ O	0.48
EDTA, disodium salt	0.67
FeSO ₄ ·7H ₂ O	0.1
NaHCO ₃	0.2
FeCl ₃	0.1
Na ₂ SeO ₄	0.01
H ₃ BO ₃	0.05
ZnSO ₄ ·7H ₂ O	0.005
Na ₂ MoO ₄ ·2H ₂ O	0.007
CuSO ₄ ·5H ₂ O	0.001
MnSO ₄ ·H ₂ O	0.001
CoSO ₄ ·7H ₂ O	0.05
NiCl ₂ ·6H ₂ O	0.08
NaCl	0.1

Table S2. Blast analysis of SO_0900

	Protein/locus tag	Organism	Size (aa)	Identity (%)	Similarity (%)	e-value	Coverage (%)	Function
	SO_0900 (Template)	<i>S. oneidensis</i> MR-1	346					Aldo/keto reductase
Top 3 hits in <i>Shewanella sp</i>	SOHN41_00409	<i>Shewanella sp.</i> HN-41	346	97	98	0	100	Aldo/keto reductase
	Shewmr4_0747	<i>Shewanella sp.</i> MR-4	346	98	99	0	100	Aldo/keto reductase
	Shewmr7_3273	<i>Shewanella sp.</i> MR-7	346	99	99	0	100	Aldo/keto reductase
	AALB_3252	<i>Agarivorans albus</i> MKT 106	346	67	81	2.E-172	100	Oxidoreductase
Top 3 hits outside <i>Shewanella sp</i>	VIBNISFn118_7600 19	<i>Vibrio nigripulchritudo</i> SFn118	344	66	80	8.E-166	100	Aldo/keto reductase
	JV59_36730	<i>Vibrio coralliilyticus</i>	344	65	78	4.E-160	100	Aldo/keto reductase
Known D-xylose reductases	ECs0473	<i>Escherichia coli</i>	324	31	51	7.E-44	96	Xylose reductase
	XylR	<i>Candida tenuis</i>	322	33	60	4.E-10	32	Xylose reductase
	ZMO0976	<i>Zymomonas mobilis</i>	340	32	48	5.E-29	67	Aldo/keto reductase

Table S3. Blast analysis of SO_4673

	Protein/locus tag	Organism	Size (aa)	Identity (%)	Similarity (%)	e-value	Coverage (%)	Function
	SO_4673 (Template)	<i>S. oneidensis</i> MR-1	341					L-threonine 3-dehydrogenase
Top 3 hits in <i>Shewanella sp</i>	AEA42_15490	<i>Shewanella sp.</i> Sh95	341	99	99	0	100	L-threonine 3-dehydrogenase
	SOHN41_00043	<i>Shewanella sp.</i> HN-41	341	99	99	0	100	L-threonine 3-dehydrogenase
	Sputcn32_3902	<i>S. putrefaciens</i> CN-32	341	99	99	0	100	L-threonine 3-dehydrogenase
Top 3 hits outside <i>Shewanella sp</i>	VO69_19065	<i>Aeromonas salmonicida</i>	342	89	94	0	100	L-threonine 3-dehydrogenase
	ERIG_03807	<i>Escherichia fergusonii</i> B253	341	89	94	0	100	L-threonine 3-dehydrogenase
	SARI_03933	<i>Salmonella enterica</i>	341	88	94	0	100	L-threonine 3-dehydrogenase
Known D-xylitol dehydrogenases	AGROH133_13378	<i>Agrobacterium sp.</i> H13-3	345	28	46	4.E-35	89	Xylitol dehydrogenase
	L230_07742	<i>Staphylococcus aureus</i> CBD-635	341	22	40	7.E-06	92%	Xylitol dehydrogenase
	N42_2157	<i>Lactococcus lactis</i>	341	20	42	6.E-07	76%	Xylitol dehydrogenase

Table S4. Blast analysis of SO_2452

	Protein/locus tag	Organism	Size (aa)	Identity	Similarity	e-value	Coverage	Function
	SO_2452 (Template)	<i>S. oneidensis</i> MR-1	314					alcohol dehydrogenase (zinc containing)
Top 3 hits in <i>Shewanella sp</i>	AWJ07_20235	<i>S. frigidimarina</i>	335	29%	47%	9.E-23	71%	Zinc-dependent oxidoreductase
	Swoo_2967	<i>S. woodyi</i>	313	28%	46%	3.E-16	71%	alcohol dehydrogenase (zinc containing)
	Sden_2242	<i>S. denitrificans</i> OS217	314	27%	41%	1.E-15	92%	alcohol dehydrogenase
Top 3 hits outside <i>Shewanella sp</i>	Y702_01970	<i>Vibrio vulnificus</i> BAA87	313	71%	84%	1.E-168	99%	alcohol dehydrogenase
	AAY54_06065	<i>Vibrio metoecus</i>	313	70%	85%	1.E-167	99%	alcohol dehydrogenase
	VEJY3_09035	<i>Vibrio sp.</i> EJY3	313	70%	84%	3.E-166	99%	alcohol dehydrogenase
Known D-xylitol dehydrogenases	Shal_2008	<i>S. halifaxis</i>	344	22%	40%	8.E-07	68%	alcohol dehydrogenase/putative xylitol dehydrogenase (1)
	Spea_2286	<i>S. pealeana</i>	344	22%	36%	1.E-05	61%	alcohol dehydrogenase/putative xylitol dehydrogenase (1)
	AGROH133_13378	<i>Agrobacterium sp.</i> H13-3	345	21%	36%	1.E-05	49%	Xylitol dehydrogenase

Table S5. Blast analysis of SO_4230

	Protein/locus tag	Organism	Size (aa)	Identity (%)	Similarity (%)	e-value	Coverage (%)	Function
	SO_4230 (Template)	<i>S. oneidensis</i> MR-1	494					Glycerol kinase
Top 3 hits in <i>Shewanella sp</i>	SHD_0561	<i>S. decolorationis</i> S12	500	96	97	0	100	Glycerol kinase
	Shewana3_3756	<i>Shewanella sp.</i> ANA-3	494	96	97	0	100	Glycerol kinase
	Shewmr4_3583	<i>Shewanella sp.</i> MR-4	494	96	97	0	100	Glycerol kinase
Top 3 hits outside <i>Shewanella sp</i>	Tola_0068	<i>Tolomonas auensis</i> DSM 9187	497	75	87	0	99	Glycerol kinase
	WL1483_4080	<i>Aeromonas shubertii</i>	504	75	86	0	99	Glycerol kinase
	AH4AK4_1492	<i>Aeromonas hydrophilia</i> 4AK4	500	75	86	0	99	Glycerol kinase
Known D-xylulokinases	M943_03795	<i>Mycobacterium tuberculosis</i> EAI5	480	28	40	1.E-14	95	Xylulokinase
	STM3660	<i>Salmonella enterica</i>	484	24	42	4.E-26	89	Xylulokinase
	ECs4447	<i>Typhimurium Escherichia coli</i>	484	24	41	2.E-26	97	Xylulokinase
	GlpK	<i>Escherichia coli</i>	502	72	84	0	99	Glycerol kinase

Table S6. Comparison of protein characteristics of SO_0900 and SO_4230 with xylose reductase and xylulokinase from other organisms respectively.

Metabolic function	Protein	Putative function	Organism	Substrate	Specific activity (U/mg)	K_m (mM)	V_{max} (U/mg)	Reference
Xylose reductase	SO_0900	Aldo/keto reductase	<i>S. oneidensis</i>	Xylose	2.93 ± 0.17	93 ± 7	3.41 ± 0.21	This study
	XR	Aldo/keto reductase	<i>Zymomonas mobilis</i>	Xylose	3.4 ± 0.2	258 ± 43	6.9 ± 1.2	(2)
	XR	Xylose reductase	<i>Pichia stipitis</i>	Xylose	16.7	42	16.7	(3)
	XR	Xylose reductase	<i>S. cerevisiae</i>	Xylose	2	142	NA*	(4)
	XR	Xylose reductase	<i>Neospora crassa</i>	Xylose	72.5	34	NA*	(5, 6)
Xylulokinase	SO_4230	Glycerol kinase	<i>S. oneidensis</i>	Xylulose	0.2 ± 0.013	1.7 ± 0.12	0.52 ± 0.02	This study
	XK	Xylulokinase	<i>Escherichia coli</i>	Xylulose	1.6	0.3	NA*	(7, 8)
	XK	Xylulokinase	<i>S. cerevisiae</i>	Xylulose	3	0.31	38.4	(9)
	XK	Xylulokinase	<i>Mucor circinelloides</i>	Xylulose	10.8	0.29	32	(10, 11)
	XK	Xylulokinase	<i>Pichia stipitis</i> NCYC 1541	Xylulose	21.4	0.52	NA*	(12)

Error values represent range of errors in duplicate samples. Substrate concentrations are given in brackets for respective specific activities

*Not available

Table S7. Percentage pairwise identity matrix of SO_1396^{Q207H} amino acid sequence identified in the genomes of 15 *Shewanella* species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1: Amazonensis	100.00	44.29	42.94	40.11	39.58	44.57	41.55	41.73	42.43	41.76	43.45	43.45	43.80	44.59	44.06
2: Haliotis	44.29	100.00	94.77	43.72	44.81	46.46	50.00	50.99	51.97	51.38	53.97	53.70	51.64	51.37	51.91
3: Algae	42.94	94.77	100.00	44.38	44.67	45.24	49.28	50.58	51.16	51.45	53.85	53.54	51.87	51.87	52.16
4: Woodyi	40.11	43.72	44.38	100.00	62.47	47.75	52.06	52.33	54.03	53.96	53.97	53.70	55.14	54.64	53.88
5: Piezotolerans	39.58	44.81	44.67	62.47	100.00	50.27	52.07	53.39	54.31	53.47	53.97	53.97	53.77	53.77	54.02
6: Frigidimarina	44.57	46.46	45.24	47.75	50.27	100.00	54.64	54.26	54.79	54.79	54.11	54.11	55.44	55.17	55.17
7: W3-18-1	41.55	50.00	49.28	52.06	52.07	54.64	100.00	85.79	86.58	86.40	75.98	75.98	72.11	71.61	72.61
8: Putrefaciens	41.73	50.99	50.58	52.33	53.39	54.26	85.79	100.00	91.62	91.62	76.06	76.06	71.93	71.93	71.68
9: Baltica	42.43	51.97	51.16	54.03	54.31	54.79	86.58	91.62	100.00	98.99	77.25	77.25	74.94	74.18	74.94
10: OS678	41.76	51.38	51.45	53.96	53.47	54.79	86.40	91.62	98.99	100.00	77.35	77.35	74.31	73.57	74.31
11: MR-1	43.45	53.97	53.85	53.97	53.97	54.11	75.98	76.06	77.25	77.35	100.00	99.73	80.82	81.37	81.64
12: XM1	43.45	53.70	53.54	53.70	53.97	54.11	75.98	76.06	77.25	77.35	99.73	100.00	80.55	81.10	81.64
13: ANA-3	43.80	51.64	51.87	55.14	53.77	55.44	72.11	71.93	74.94	74.31	80.82	80.55	100.00	94.93	95.17
14: MR-4	44.59	51.37	51.87	54.64	53.77	55.17	71.61	71.93	74.18	73.57	81.37	81.10	94.93	100.00	97.34
15: MR-7	44.06	51.91	52.16	53.88	54.02	55.17	72.61	71.68	74.94	74.31	81.64	81.64	95.17	97.34	100.00

Supplementary References.

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