The sulfate-reducing bacterium *Desulfovibrio desulfuricans* ND132 as a model for understanding bacterial mercury methylation

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SUPPLEMENTAL MATERIAL

Table S1. Trace metal solution for SRM medium, 100X Stock

Trace metal	**µM**
$ZnCl_2$	500
$MnCl_2{\cdot}4H_20$	500
H_2BO_4	800
$CoCl{\cdot}6H_20$	1000
$CuCl_2{\cdot}2H_20$	100
$NiCl_2{\cdot}6H_20$	100
$Na_2MoO_4{\cdot}2H_20$	150

Table S2. Vitamin Stock solution for SRM medium, 100X Stock

Vitamin	**mg/L**
Biotin	2
Nicotinic acid	20
p-aminobenzoic acid	10
Thiamine	20
Pantothenic acid	10
Pyridoxine·2HCl	50
Cobalamine	10

Table S3. List *of Desulfovibrio* strains used in alignment, including the accession numbers for the 16S rRNA sequences used.

Strain	Sequence source	Accession #	DSM	ATCC
D. acrylicus	RDP	U32578	10141	
D. aerotolerans	RDP	AY746987	16695	
D. aespoeensis	RDP	X95230	10631	
D. africanus strain ADR13	RDP	AM419445.1		
D. africanus strain Benghazi	RDP	X99236	2603	
D. alaskensis	RDP	Y11984	16109	
D. alcholivorans	RDP	AF053751	5433	
D. alkalitolerans	RDP	AY649785	16529	
D. aminophilus	RDP	AF067964	12254	
D. bastinii	RDP	AY359868	16055	BAA-903
D. bizertensis	RDP	DQ422859	18034	
D. burkinensis	RDP	AF053752	6830	
D. caledoniensis	RDP	U53465		
D. carbinolicus	RDP	AY626035	3852	
D. carbinoliphilus	RDP	DQ186200	17524	
D. cuneatus	RDP	X99501	11391	
D. dechloroacetivorans	RDP	AF230530		
D. desulfuricans ND132	GenBank/SERC	HQ693571.1		
D. desulfuricans subs. aestuarii strain Styl 3	RDP/SERC	FJ655909	17919	29578
D. desulfuricans subsp. desulfuricans strain El Agheila	RDP/SERC	M37316.1	1926	none
D. desulfuricans subsp. desulfuricans strain Essex 6	RDP	AF192153.1	642	29577
D. desulfuricans subsp. desulfuricans strain MB	RDP	AF192154.1	6949	27774
D. desulfuricans strain G20	RDP	NC_007519.1		
D. desulfuricans strain LS	R. Devereux			
D. ferrireducens strain 61T	RDP	DQ148944	16995	
D. frigidus strain 18T	RDP	DQ148943	17176	
D. fructosivorans	RDP	AF050101	3604	
D. gabonensis	RDP	U31080	10636	
D. gigas	RDP	DQ447183	1382	19364
D. gracilis	RDP	U53464	16080	BA-904
D. halophilius	RDP	U48243	5663	
D. hydrothermalis	RDP	AF458778	14728	
D. indonesiensis	RDP	Y09504	15121	
D. inopinatus	RDP	AF177276	10711	
D. intestinalis	RDP	Y12254	11275	
D. littoralis	RDP	X99504	11393	
D. longreachensis	RDP	Z24450		
D. longus	RDP	AY359867	6739	51456
D. magneticus	RDP	D43944		
D. marinisediminis	RDP	AB353727	17456	
D. marinus	RDP	DQ365924	18311	
D. marrakechensis	RDP	AM947130	19337	
D. mexicanus	RDP	AF227984	13116	
D. oxamicus	RDP	DQ122124	1925	33405
D. oxyclinae	RDP	U33316	11498	
D. pasquesii	RDP	AY726757	16681	
D. piger	RDP	AF192152		29098
D. psychrotolerans	RDP	AM418397	19430	
D. putealis	RDP	AY574979	16065	
D. salexigens	RDP	M34401.1	2638	14822
D. senezii	RDP	AF050100	8436	
D. sp BerOc1	RDP	EU137840		
D. sp. T2	SERC/GenBank	HQ693572		
D. sp. X2	SERC/GenBank	HQ693573		
D. sulfodismutans	RDP	Y17764	3696	43913
D. termidus	RDP	X87409	5308	49858
D. tunisiensis	RDP	EF577029	19275	
D. vietnamensis	RDP	X93994	10520	
D. vulgaris strain Hildenborough	RDP	AF418179	644	29579
D. zosterae	RDP	Y18049	11974	
Desulfocurvus vexinensis	RDP	DQ841177	17965	

Table S4. Compilation of literature reports of Hg methylation assays for *Desulfovibrio* species.

Strain	**Culture ID**			**MeHg[a] production**	**Reference**
	DSMZ	**ATCC**	**Other**		
D. desulfuricans LS				+	(1)
D. desulfuricans ND132				+	This study
Desulfovibrio T2				+	This study
Desulfovibrio X2				+	This study
D. africanus Benghazi	2603			+	(2, 6), this study
D. africanus			ADR 13	+	(6)
D. desulfuricans aestuarii Sylt3		29578	NCIB 9335	-	This study
D. desulfuricans subsp. desulfuricans	1924	13541	NCIB 9467	+	(3, 4)
D. desulfuricans subsp. desulfuricans MB	6949	27774	NCIB 12833	-	(6), this study
D. desulfuricans subsp. desulfuricans Essex 6	642	29577	NCIB 8307	-	This study
D. desulfuricans subsp. desulfuricans El Agheila Z	1926		NCIB 8380	-	This study
D. desulfuricans subsp. desulfuricans G20				-	This study
D. desulfuricans M8 and M9				+	(5)
D. gigas	1382	19364	NCIB 8332	-	This study
D. salexigens	2638	14822		-	This study
D. vulgaris Hildenborough	644	29579		-	(6), this study
*D. vulgaris subsp. vulgaris*Marburg	2119			-	(2)
D. strain BerOc1			BerOc 1	+	(6)

[a]Methylation is listed as positive if MeHg production was reported at levels significantly above abiotic or negative controls. Because rates were measured under different culture conditions, and over different amounts of time, a quantitative comparison of rates is not possible.

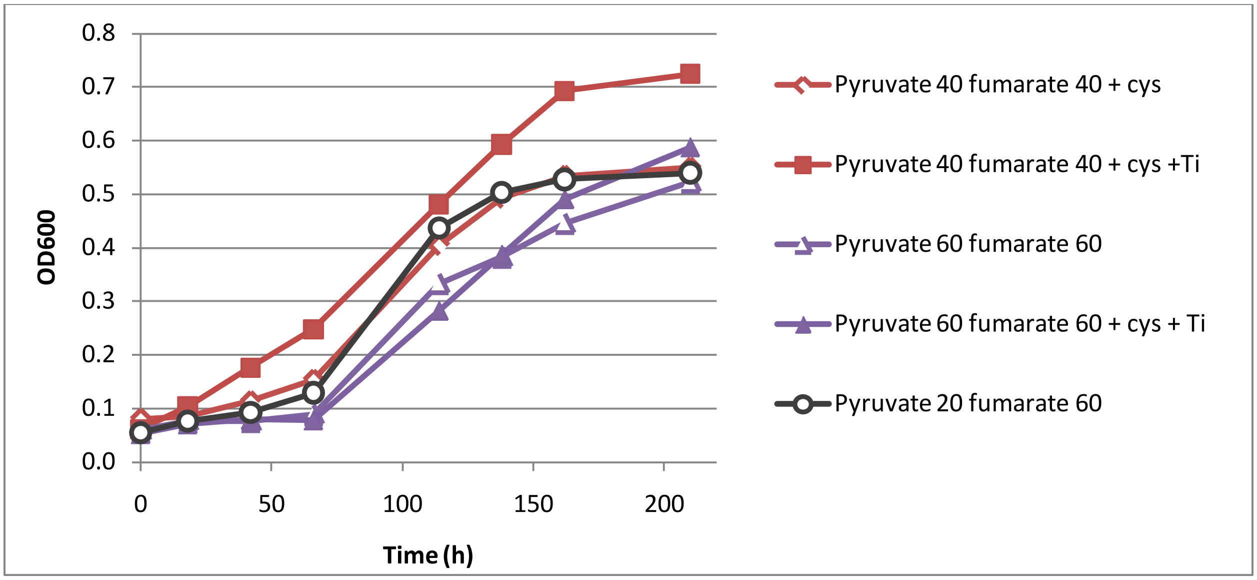

Figure S1. ND132 growth on pyruvate/fumarate medium (modified MOYPF) at 32^{o} C.Cysteine was added to 1mM; Ti-citrate to 0.38 mM. Data points are averages for triplicate tubes.

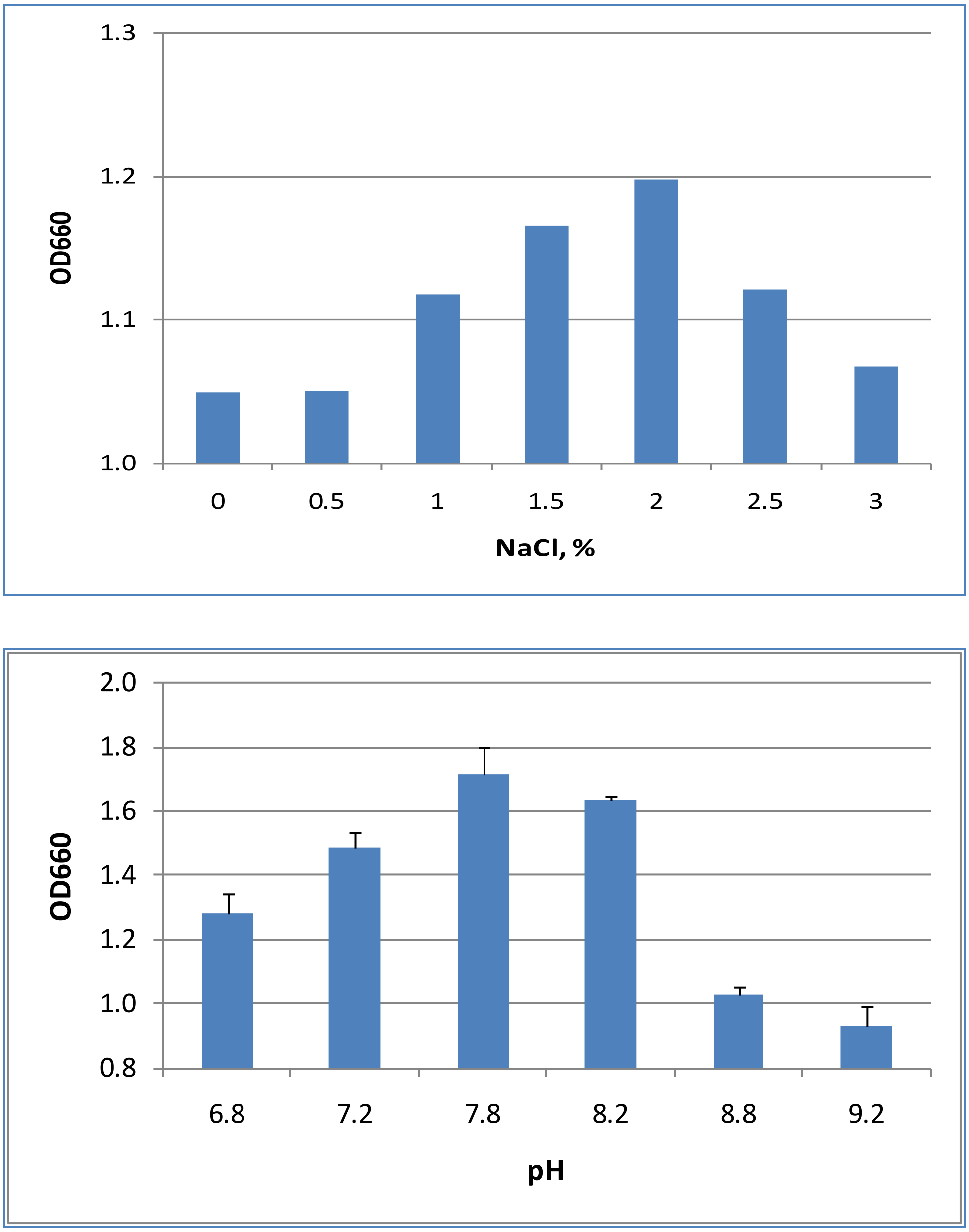

Figure S2. Optimal growth parameters for ND132 on lactate/sulfate medium (MOYLS4) at 32^{o} C. Top, salt tolerance, bottom pH. Bars represent the average maximal optical density after growth to stationary phase (29 h) for duplicate cultures.

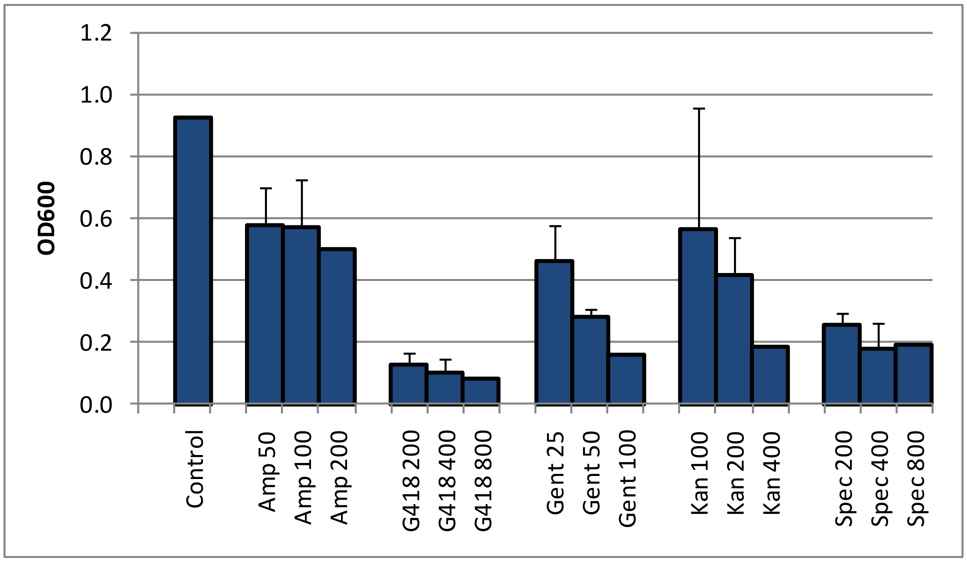

Figure S3: Antibiotic sensitivity of *D. desulfuricans* ND132, tested on lactate/sulfate (MOYLS4) medium. Growth is shown as optical density after 48 h growth at 32^{o}C, minus the OD measured immediately after inoculation. Amp = ampicillin; Gen = gentamicin; Kan = kanamycin; Spec = spectinomycin. Antibiotic concentration values in μg/mL.

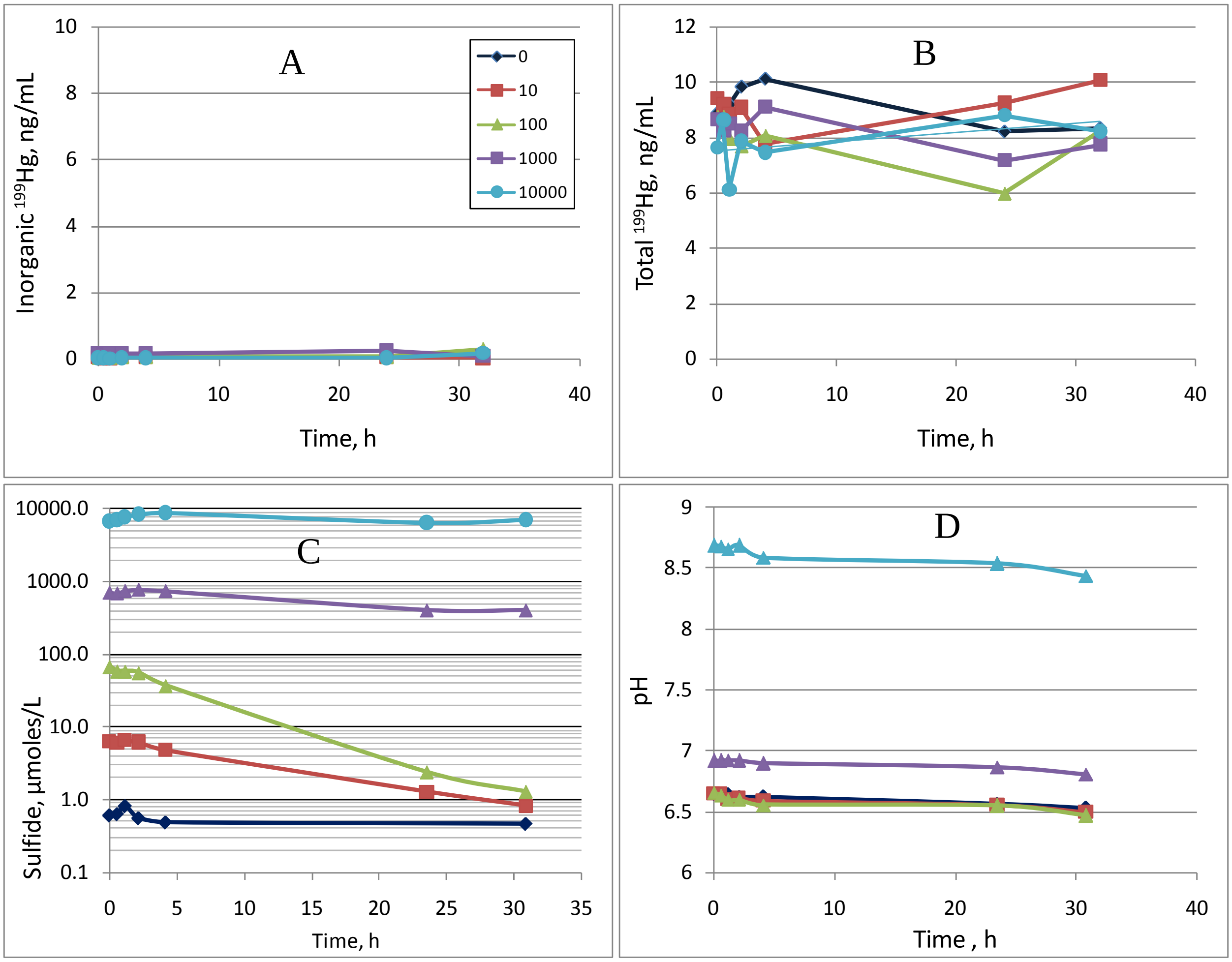

Figure S4.Lack of abiotic degradation of Me^{199}Hg by sulfide over time in un-inoculated culture medium. Me^{199}Hg was added at 10 ng/ml to un-inoculated SRM culture medium containing sulfide concentrations ranging from 10 to 1000 µM (plus a control without added sulfide), and the concentrations of and inorganic ^{199}Hg (A), total ^{199}Hg (B), sulfide (C) and pH (D)were followed over 32 h. Media were reduced with 100 µM TiNTA.

Inorganic ^{199}Hg was measured directly by $SnCl_2$ reduction of samples; total ^{199}Hg was measured after the addition of BrCl. Bottom panels show sulfide and pH in the bottles over time. Additionally, on average, 98% of the total ^{199}Hg measured at the first time point was recovered from the bottles at the end of the experiment (after addition of BrCl to the bottles), demonstrating that MeHg was not degraded to elemental Hg.

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