

Supplementary Information

Electrical tension-triggered conversion of anaerobic to aerobic respiration of *Shewanella putrefaciens* CN32 while promoting biofilm growth in microbial fuel cells

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Experimental section:

Bacteria culture A single colony of *S. putrefaciens* CN32 (ATCC, BBA-1097) was cultured aerobically in 100 ml LB at 30 °C overnight¹ and the cells were harvested and inoculated into fresh LB. After grown at 30 °C for 6 hours, the cells were by centrifuging at 4 °C (6000 rpm, 5 min) for MFC half-cell operation². Before every test, nitrogen was purged into the suspension for 30 min to remove oxygen from the cell.

MFC half-cell operation For the anodic half-cell analysis, a piece of 1 cm*1cm a NiO nanowire decelerated carbon cloth was used as working electrode, saturated calomel electrode (SCE) was used as the reference electrode and titanium plate as the counter electrode³. The half-cell electrolyte is an anaerobic *S. putrefaciens* CN32 cell suspension supplemented with 18 mM lactate in M9 buffer. All the tests were conducted at room temperature and the CVs were recorded between -0.8 V and 0.6 V (vs. SCE) with a scan rate of 1 mV s⁻¹ after discharge. All electrochemical experiments were carried out with a potentiostat (CHI660E, Shanghai Chenhua, China). Field emission scanning electron microscopy (FESEM, JSM-7800F, Japan) was used to investigate the surface morphologies of electrode.

RNA extraction and transcriptome analysis.

For performing the real-time sampling, the bottom of the 1ml sterilization gun head is pulled down into a thin and small tube under an alcohol lamp and then autoclaved to use as a sampling pipette. When performing the electric tension, the sampling tip is gently positioned at the center of the anode but very close to the electrode surface to directly and slowly suction 1ml of bacterial solution as a sample for RNA extraction without interfering the conducting of electrochemical experiments. Seal the top with parafilm when not sampling (Figure 1d and Scheme S1). Total RNA from cultures was extracted using RNAiso Plus (Takara Co., Dalian, China) according to manufacturer's protocol. RNA purity was checked using the NanoPhotometer®

spectrophotometer (IMPLEN, CA, USA) and RNA concentration was measured using Qubit® RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA). To preparation of RNA-seq libraries were using RNA Library Prep Kit (Illumina). Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads and rRNA was removed. The obtained cleaved RNA fragments were reverse transcribed to first strand cDNA, followed by synthesized Second strand cDNA. The clustering of the index-coded samples using TruSeq PE Cluster Kit v3-cBot-HS (Illumina). Both building index of reference genome and aligning clean reads to reference genome were used Bowtie2-2.2.3. HTSeq v0.6.1 was used to count the reads numbers mapped to each gene⁴. And then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene⁵. Differential expression analysis was performed using the DESeq R package (1.18.0). DESeq provide statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution⁶. Genes with an adjusted P-value <0.05 found by DESeq were assigned as differentially expressed and differential expression analysis of two conditions was performed using the DEGSeq R package (1.20.0). Then the P values were adjusted using the Benjamini & Hochberg method. Corrected P-value of 0.05 and fold change ≥ 1 were set as the threshold for significantly differential expression.

Quantitative RT-PCR (Q-RT-PCR)

Q-RT-PCR was used to measure the mRNA expression levels of 11 differential up-regulated genes by the RNA-seq data. Gene-specific RT-qPCR primers were listed in Table S2. Samples for Q-RT-PCR were prepared and collected the same way that we did for RNA-seq analysis. The reaction was conducted for all primer pairs in a QuantStudio 3D Digital PCR System (Thermo Fisher Scientific, US). The expression levels were normalized using the recA Ct values⁷. Basal expression levels were represented as folds over the expression levels of recA. Fold inductions were calculated with the $2^{-\Delta\Delta C_t}$ method between treatment and control samples for each biological replicate. All data were presented as mean $\pm$ SD (standard deviation)⁸.

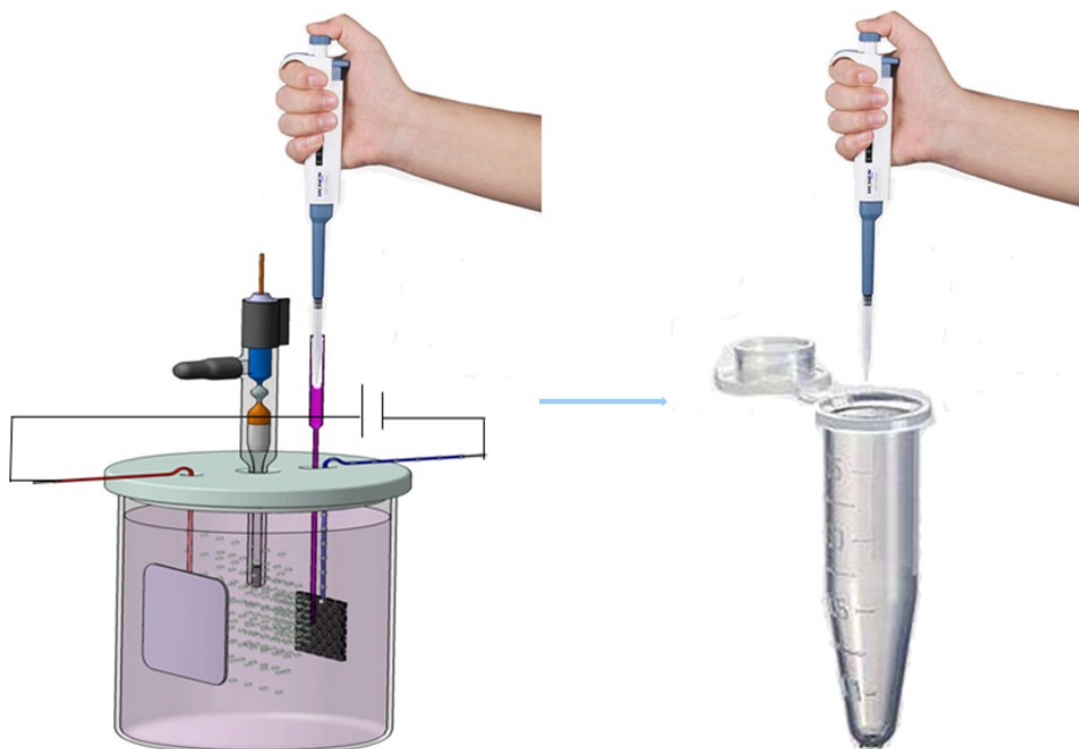
GO, KEGG and network enrichment analysis of differentially expressed genes

Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the Goseq R package. GO terms with corrected Pvalue less than 0.05 were considered significantly enriched by differential expressed genes⁹. We used KOBAS software to test the statistical enrichment of differential expression genes in KEGG pathways^{10, 11}.

RNA-SEQ results

Approximately 4,084 and 4079 unique genes were obtained for open circuit group and electrical-tension group, respectively. The differentially expressed genes were analyzed by pairwise comparisons of control group and the electrical-tension group in a range of the 4,075 identical unigenes (Figure S3a). The results show that 177 genes exhibit statistically significant up- or down-regulation. (Figure S3b, Table S1, 127 upregulated, 50 downregulated). With the aim to validate the RNA-seq data, a total 11 upward-expressed genes ($\log_2$ Fold Change ≥ 1) were randomly selected and quantified by quantitative RT-PCR (qRT-PCR). The 11 genes in RNA-seq data were upward expressed with the same trend of the data obtained from qRT-PCR

(Figure S4, Table S2). The description and functional classes of these 177 genes were listed in Table S1 with their fold changes in expression level.



Scheme S1 the real-time sampling (Figure. 1d)

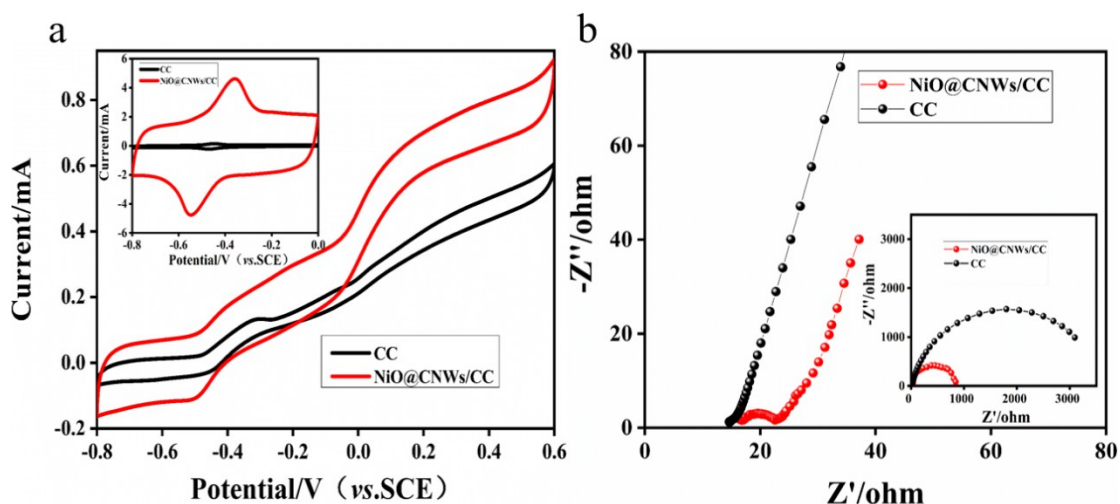


Figure S1 a) Steady-state current of nickel oxide @ carbon nanowire network decorated carbon cloth (NiO@CNWs/CC) and plain carbon cloth (CC) electrode measured in the *Shewanella putrefaciens* CN32 cell suspension at 1 mV s^{-1} after 48h discharging. (Inset: CVs of the NiO@CNWs/CC and CC in PBS buffer supplemented with riboflavin at 50 mV s^{-1}) b) The Nyquist plots of NiO@CNWs/CC and CC electrodes (after 48h discharging) measured in the *Shewanella putrefaciens* CN32 cell at -0.45 V vs. SCE.

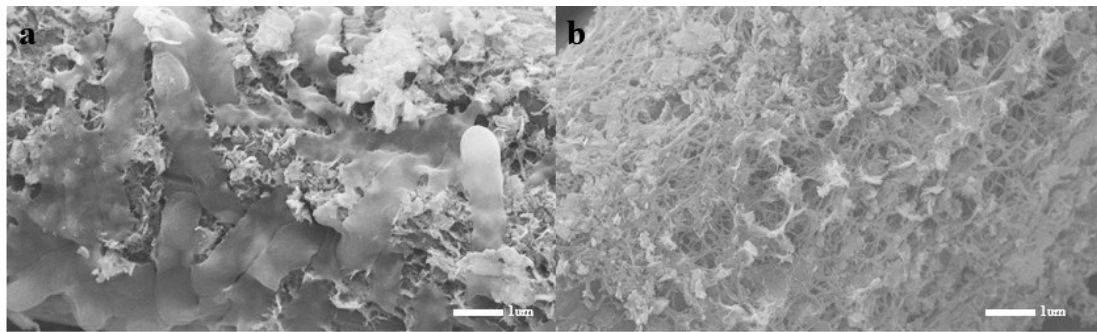


Figure S2 FE-SEM images of the anodes (a. Electrical tension, b. Open circuit) at 48h.

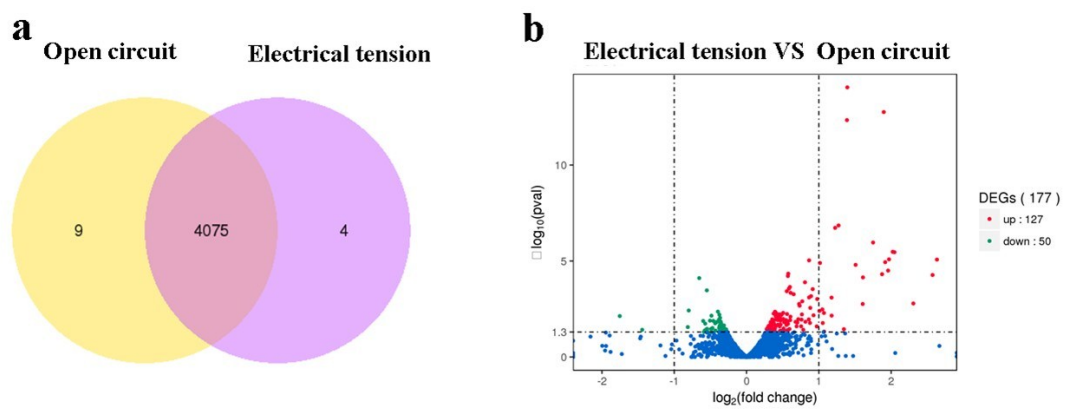


Figure S3 a) Venn diagram illustrates of expressed genes in *Shewanella putrefaciens* CN32 under electrical tension and open circuit condition, b) The number of differentially expressed genes electrical tension vs open circuit.

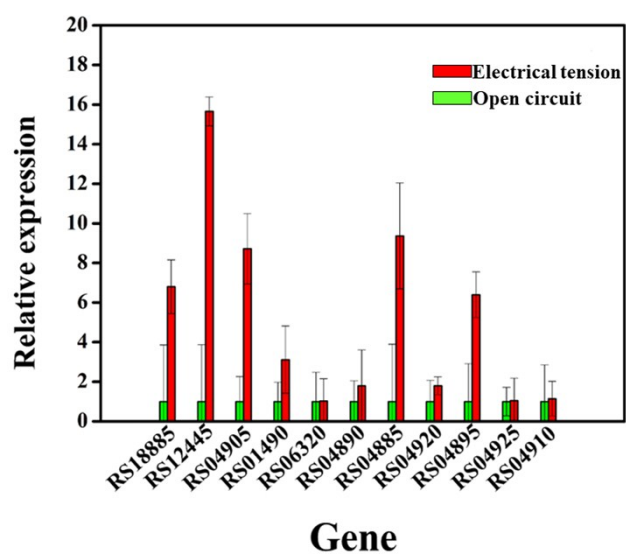


Figure S4 Electrical tension relatively open circuit 11 up-regulated genes by qRT-PCR.

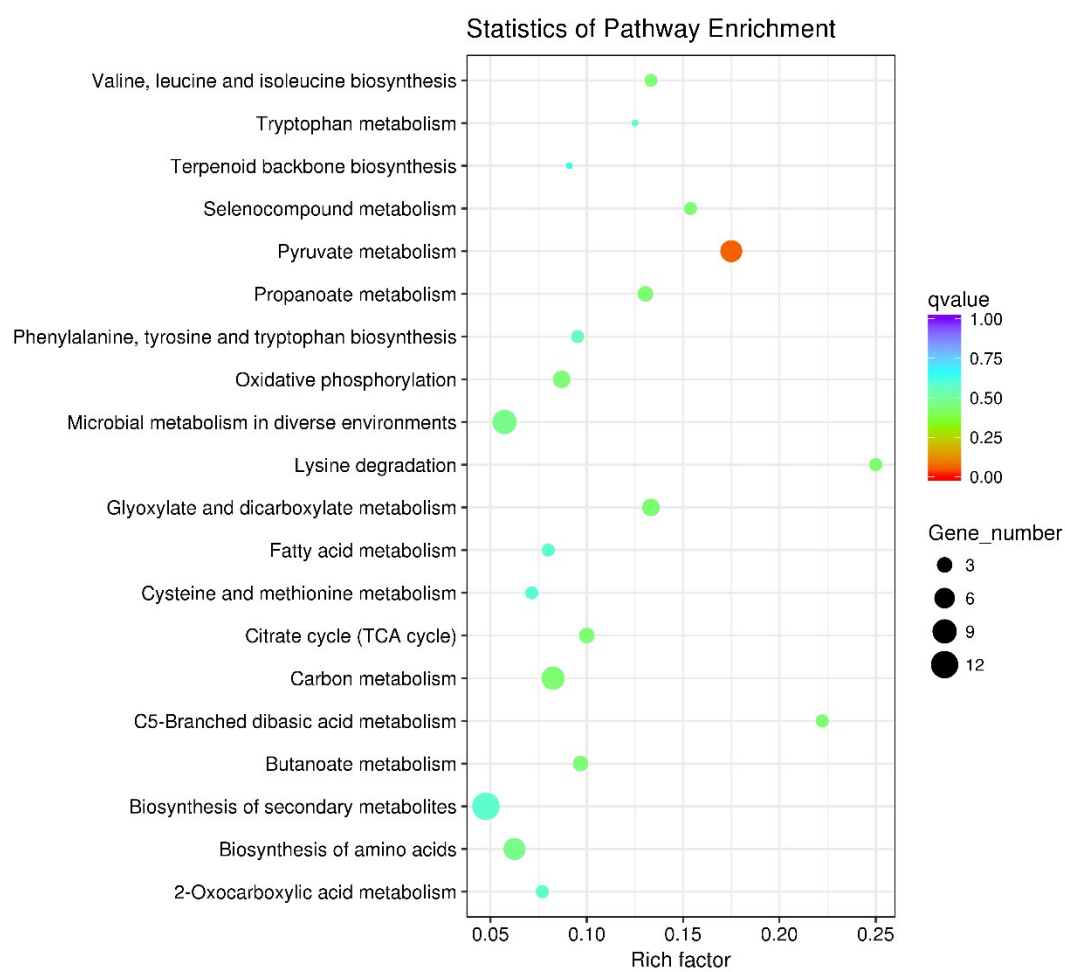


Figure S5 KEGG functional classification of differentially expressed genes electrical tension vs open circuit.

Table S1. Basic information of *Shewanella putrefaciens* CN32 different genes.

Genetic	readcount – Electrical tension	readcount _open circuit	log2FoldC hange	pval	Description
SPUTCN32_RS00035	398.0408	501.5028	-0.33334	0.035326	HDOD domain-containing protein
SPUTCN32_RS00240	2420.038	2961.959	-0.29152	0.035314	protein-export protein SecB
SPUTCN32_RS00510	274.4915	355.0114	-0.3711	0.022869	LemA family protein
SPUTCN32_RS00555	49.39809	30.57538	0.69208	0.025161	efflux RND transporter periplasmic adaptor subunit
SPUTCN32_RS00645	223.4149	155.808	0.51996	0.015376	membrane protein
SPUTCN32_RS01280	85.92841	60.82902	0.49838	0.047279	copper resistance protein CopC
SPUTCN32_RS01390	2145.71	2618.552	-0.28731	0.037322	hypothetical protein
SPUTCN32_RS01490	39.3543	15.47556	1.3465	0.035657	type 1 fimbrial protein
SPUTCN32_RS01540	322.7718	413.3961	-0.35701	0.023552	DNA-binding response regulator
SPUTCN32_RS01580	310.8667	109.2677	1.5084	1.59E-05	hypothetical protein
SPUTCN32_RS01585	78.63253	39.92703	0.97776	0.000957	nitrite reductase
SPUTCN32_RS01595	237.7329	90.78687	1.3888	4.55E-13	cytochrome c nitrite reductase subunit NrfD
SPUTCN32_RS01785	669.8637	506.7373	0.40263	0.007507	anion permease
SPUTCN32_RS01790	1619.4	1285.991	0.33258	0.015396	fumarate hydratase
SPUTCN32_RS01805	390.7423	301.3791	0.37464	0.017016	fumarate reductase subunit D
SPUTCN32_RS01820	1456.183	1108.371	0.39375	0.00439	fumarate reductase (quinol) flavoprotein subunit
SPUTCN32_RS02170	91.37222	60.14943	0.6032	0.012043	membrane protein
SPUTCN32_RS02405	355.5415	261.5993	0.44266	0.006515	3-isopropylmalate dehydrogenase
SPUTCN32_RS02415	364.8557	271.8657	0.42443	0.009558	3-isopropylmalate dehydratase small subunit
SPUTCN32_RS02625	251.1629	395.2921	-0.6543	7.76E-05	uroporphyrinogen decarboxylase
SPUTCN32_RS02935	190.747	248.2278	-0.38	0.03074	type II secretion system protein
SPUTCN32_RS02955	218.6562	165.6057	0.40091	0.026457	rod shape-determining protein MreC
SPUTCN32_RS03095	420.6854	277.5624	0.59993	0.000225	TonB-dependent siderophore receptor
SPUTCN32_RS03390	4.185081	11.3791	-1.4431	0.038401	hypothetical protein
SPUTCN32_RS03580	487.1166	628.2739	-0.36713	0.04807	LysR family transcriptional regulator

SPUTCN32_RS03655	148.0821	97.24104	0.60676	0.007909	ribosome hibernation promoting factor
SPUTCN32_RS03950	500.5752	621.9535	-0.31322	0.038841	esterase
SPUTCN32_RS04050	563.1924	431.907	0.38291	0.011837	M13 family peptidase
SPUTCN32_RS04180	55.84257	81.6047	-0.54729	0.034207	teicoplanin resistance protein VanZ
SPUTCN32_RS04255	1940.507	1516.965	0.35525	0.010959	GlyGly-CTERM sorting domain-containing protein
SPUTCN32_RS04390	508.5615	392.1099	0.37516	0.017757	acylase
SPUTCN32_RS04570	191.8448	105.3179	0.86519	9.25E-06	alkyl hydroperoxide reductase subunit F
SPUTCN32_RS04575	302.7349	48.78942	2.6334	8.44E-06	peroxiredoxin
SPUTCN32_RS04815	2283.272	1870.455	0.28771	0.039431	NADH:ubiquinone reductase (Na(+)-transporting) subunit F
SPUTCN32_RS04885	55.63271	26.45472	1.0724	0.047756	heme ABC transporter ATP-binding protein
SPUTCN32_RS04890	75.91023	33.56919	1.1772	0.007008	iron ABC transporter permease
SPUTCN32_RS04895	134.1239	63.85981	1.0706	0.005094	hemin ABC transporter substrate-binding protein
SPUTCN32_RS04900	35.04738	11.48	1.6102	7.13E-05	biopolymer transporter ExbD
SPUTCN32_RS04905	93.45344	27.77619	1.7504	1.09E-06	flagellar motor protein MotA
SPUTCN32_RS04910	306.1874	78.81809	1.9578	3.15E-05	energy transducer TonB
SPUTCN32_RS04915	951.5536	251.8404	1.9178	1.15E-05	TonB-dependent hemoglobin/transferrin/lactoferrin family receptor
SPUTCN32_RS04920	130.0247	53.81317	1.2728	1.41E-07	heme utilization cytosolic carrier protein HutX
SPUTCN32_RS04925	541.562	133.3682	2.0217	3.32E-06	heme utilization protein HutZ
SPUTCN32_RS05205	1543.702	1875.295	-0.28072	0.036712	outer membrane protein assembly factor BamD
SPUTCN32_RS05660	7408.279	5209.576	0.50797	0.010463	GatB/YqeY domain-containing protein
SPUTCN32_RS05995	384.8867	232.557	0.72685	0.001721	3-deoxy-7-phosphoheptulonate synthase
SPUTCN32_RS06000	478.8184	345.9273	0.46901	0.012752	bifunctional chorismate mutase/prephenate dehydrogenase
SPUTCN32_RS06075	41.20766	18.23911	1.1759	0.000806	4Fe-4S dicluster domain-containing protein
SPUTCN32_RS06320	387.1772	147.3393	1.3939	8.98E-15	malate synthase A
SPUTCN32_RS06325	934.0436	461.9765	1.0157	1.26E-05	isocitrate lyase
SPUTCN32_RS06365	273.7756	352.5861	-0.36498	0.025992	4-alpha-glucanotransferase
SPUTCN32_RS06535	396.7845	580.665	-0.54935	0.000338	hypothetical protein

SPUTCN32_RS06590	443.6236	567.0465	-0.35413	0.023385	hypothetical protein
SPUTCN32_RS07125	142.9416	81.64474	0.80799	0.000128	acetyl-CoA C-acyltransferase
SPUTCN32_RS07270	45.55903	66.31456	-0.54159	0.047094	hypothetical protein
SPUTCN32_RS07285	219.7472	148.3553	0.56679	0.044076	extradiol dioxygenase
SPUTCN32_RS07290	217.0409	288.2433	-0.40932	0.015014	hypothetical protein
SPUTCN32_RS07610	2835.526	2286.983	0.31017	0.023195	DNA-binding protein HU-beta
SPUTCN32_RS07730	35705.33	25133.02	0.50656	0.005031	TonB-dependent receptor
SPUTCN32_RS07890	86.83508	120.3818	-0.47127	0.030985	two-component system response regulator YehT
SPUTCN32_RS08065	1544.412	1202.999	0.36042	0.021312	acyl carrier protein
SPUTCN32_RS08100	290.5865	195.2465	0.57367	0.01858	TonB-dependent siderophore receptor
SPUTCN32_RS08190	1147.193	867.1383	0.40377	0.004903	peptidoglycan-associated lipoprotein Pal
SPUTCN32_RS08395	537.7337	418.2279	0.3626	0.021009	beta-hydroxydecanoyl-ACP dehydratase
SPUTCN32_RS08620	1509.469	1011.912	0.57696	4.57E-05	TonB-dependent receptor
SPUTCN32_RS08625	156.6676	219.2932	-0.48515	0.007065	hypothetical protein
SPUTCN32_RS08645	814.1342	634.6319	0.35935	0.039289	AMP-binding protein
SPUTCN32_RS08650	553.9975	685.4019	-0.30707	0.040086	PAS domain S-box protein
SPUTCN32_RS08850	63.01451	41.74759	0.59399	0.034984	MerR family DNA-binding transcriptional regulator
SPUTCN32_RS08855	758.771	541.3314	0.48715	0.006231	propionyl-CoA synthetase
SPUTCN32_RS09105	395.4732	310.3194	0.34983	0.032419	DUF885 domain-containing protein
SPUTCN32_RS09115	191.0714	129.6008	0.56004	0.005809	ribonuclease E inhibitor RraA
SPUTCN32_RS09390	89.55085	48.61643	0.88126	0.002765	cytochrome c
SPUTCN32_RS09470	210.439	143.9917	0.54742	0.011952	TonB-dependent siderophore receptor
SPUTCN32_RS09475	511.4519	246.2837	1.0543	0.017032	PepSY domain-containing protein
SPUTCN32_RS09480	154.1401	90.65819	0.76573	0.006066	hypothetical protein
SPUTCN32_RS09800	244.4261	316.2947	-0.37187	0.02692	hypothetical protein
SPUTCN32_RS09900	64.86242	96.442	-0.57228	0.01827	AraC family transcriptional regulator
SPUTCN32_RS10410	4989.568	3847.323	0.37506	0.005491	50S ribosomal protein L20
SPUTCN32_RS10555	3337.71	2224.846	0.58515	0.000276	integration host factor subunit beta
SPUTCN32_RS10975	40.89975	60.93035	-0.57507	0.040498	alpha/beta hydrolase
SPUTCN32_RS11045	4070.045	4916.489	-0.27258	0.047578	heat-shock protein IbpA
SPUTCN32_RS11225	442.4591	354.0472	0.3216	0.038088	FAD-binding oxidoreductase
SPUTCN32_RS11275	152.5952	104.0751	0.55209	0.021473	tyrosine transporter TyrP
SPUTCN32_RS11340	1342.796	1055.827	0.34687	0.016476	DUF1302 domain-containing

					protein
SPUTCN32_RS11425	454.9508	587.2985	-0.36838	0.013553	aminodeoxychorismate lyase
SPUTCN32_RS11490	1796.418	944.9192	0.92686	0.006756	cold shock domain protein CspD
SPUTCN32_RS11545	552.1945	704.7255	-0.35188	0.01743	MarR family transcriptional regulator
SPUTCN32_RS11605	758.4276	612.7304	0.30776	0.037626	hypothetical protein
SPUTCN32_RS12095	1349.479	907.645	0.5722	6.06E-05	acetyl-coenzyme A synthetase cyclic nucleotide-
SPUTCN32_RS12155	364.875	279.0967	0.38664	0.020597	binding/CBS domain- containing protein
SPUTCN32_RS12160	1522.46	898.4883	0.76083	0.011313	cation acetate symporter
SPUTCN32_RS12165	100.9423	48.80698	1.0484	0.003327	DUF4212 domain-containing protein
SPUTCN32_RS12255	301.0811	217.9251	0.46632	0.006085	formate transporter FocA
SPUTCN32_RS12260	1760.077	2268.557	-0.36614	0.009135	DUF3360 domain-containing protein
SPUTCN32_RS12350	1725.766	1394.178	0.30782	0.036629	lipase
SPUTCN32_RS12430	154.7151	39.47737	1.9705	8.19E-06	L-lysine 6-monooxygenase
SPUTCN32_RS12435	35.95244	6.042442	2.5729	5.35E-05	N-acetyltransferase
SPUTCN32_RS12440	109.8952	29.48701	1.898	1.74E-13	IucA/IucC family siderophore biosynthesis protein
SPUTCN32_RS12445	108.2045	35.52879	1.6067	0.001733	TonB-dependent siderophore receptor
SPUTCN32_RS12450	40.44421	20.11245	1.0078	0.004006	siderophore-iron reductase FhuF
SPUTCN32_RS12540	794.1209	1016.955	-0.35682	0.014421	sigma-54-dependent Fis family transcriptional regulator
SPUTCN32_RS12555	104.5279	21.12363	2.307	0.001628	hypothetical protein
SPUTCN32_RS12625	145.1732	110.0584	0.39951	0.048492	MFS transporter
SPUTCN32_RS12660	92.50097	55.6156	0.73398	0.002132	hypothetical protein
SPUTCN32_RS12875	61.85106	38.65334	0.67821	0.01485	tRNA-Glu
SPUTCN32_RS12925	4.197787	14.15116	-1.7532	0.007373	tRNA-Val
SPUTCN32_RS13015	277.5262	198.9196	0.48044	0.046206	electron transfer flavoprotein subunit beta/FixA family protein
SPUTCN32_RS13030	100.7054	68.16239	0.56309	0.015165	amidohydrolase
SPUTCN32_RS13085	57.0878	79.71295	-0.48163	0.048525	endonuclease
SPUTCN32_RS13135	231.033	294.5948	-0.35063	0.036232	glycosyltransferase family 2 protein
SPUTCN32_RS13140	530.755	691.636	-0.38197	0.011337	hypothetical protein
SPUTCN32_RS13405	785.2828	627.9793	0.3225	0.029377	flagellar hook-associated protein 3

SPUTCN32_RS13550	31.46247	18.26169	0.78481	0.0382	hypothetical protein
SPUTCN32_RS13850	390.5758	281.8135	0.47086	0.020428	mechanosensitive ion channel protein
SPUTCN32_RS14040	186.9344	138.3136	0.43459	0.020649	class I SAM-dependent methyltransferase
SPUTCN32_RS14080	189.1989	145.8733	0.37519	0.04959	hypothetical protein
SPUTCN32_RS14120	1685.238	2086.613	-0.30821	0.034481	ribosomal subunit interface protein
SPUTCN32_RS14135	308.1993	240.5487	0.35753	0.035996	PepSY domain-containing protein
SPUTCN32_RS14140	107.1223	63.4761	0.75497	0.025205	DUF3325 domain-containing protein
SPUTCN32_RS14275	1536.869	1130.688	0.44279	0.006042	DNA recombination/repair protein RecA
SPUTCN32_RS14285	2062.585	1571.463	0.39235	0.032567	RNA polymerase sigma factor RpoS
SPUTCN32_RS14290	1097.441	719.0285	0.61002	0.000455	LysM peptidoglycan-binding domain-containing protein
SPUTCN32_RS14350	837.0728	475.9902	0.81442	0.014586	methionine synthase
SPUTCN32_RS14355	771.5591	393.5783	0.97113	0.025494	DUF1852 domain-containing protein
SPUTCN32_RS14490	239.0246	309.6726	-0.37358	0.025375	AraC family transcriptional regulator
SPUTCN32_RS14795	543.096	369.3004	0.55641	0.000376	DUF4198 domain-containing protein
SPUTCN32_RS14800	43.20827	23.79129	0.86087	0.01105	DUF2271 domain-containing protein
SPUTCN32_RS14890	69.88791	98.3461	-0.49282	0.034697	type II secretion system protein
SPUTCN32_RS14985	92.97671	22.52578	2.0453	3.48E-06	DNA starvation/stationary phase protection protein
SPUTCN32_RS15055	545.5862	701.9353	-0.36353	0.015237	hypothetical protein
SPUTCN32_RS15370	161.6013	222.2866	-0.45998	0.013143	hypothetical protein
SPUTCN32_RS15480	95.98695	129.3366	-0.43022	0.041954	EamA/RhaT family transporter
SPUTCN32_RS15540	200.4575	127.6331	0.65129	0.000538	transporter
SPUTCN32_RS15550	108.1302	57.34912	0.91493	0.000292	curli production assembly protein CsgE
SPUTCN32_RS15680	64.71054	98.02141	-0.5991	0.013272	protein SlyX
SPUTCN32_RS15730	1504.708	1140.697	0.39957	0.00483	carbon starvation protein A
SPUTCN32_RS16070	2117.276	2859.607	-0.43361	0.017602	DUF2170 domain-containing protein
SPUTCN32_RS16120	1053.533	1406.326	-0.4167	0.015144	hypothetical protein
SPUTCN32_RS16300	308.0555	169.1381	0.86499	0.000788	curlin

SPUTCN32_RS16305	4791.478	2581.25	0.8924	0.000678	curlin
SPUTCN32_RS16490	602.6229	353.2853	0.77042	0.001264	5-methyltetrahydropteroyltriglutamate--homocysteine S-methyltransferase
SPUTCN32_RS16770	179.0742	137.39	0.38228	0.045279	hypothetical protein
SPUTCN32_RS16840	332.1617	224.433	0.5656	0.039919	hydrolase
SPUTCN32_RS16955	77.27566	54.04764	0.51578	0.040832	DUF1566 domain-containing protein
SPUTCN32_RS17060	2161.061	2683.681	-0.31247	0.022792	hypothetical protein
SPUTCN32_RS17200	388.6245	310.5955	0.32334	0.046995	zinc ABC transporter substrate-binding protein
SPUTCN32_RS17315	439.5672	353.0621	0.31616	0.043099	sodium:alanine symporter family protein
SPUTCN32_RS17560	338.1525	246.654	0.45519	0.049459	C4-dicarboxylate ABC transporter
SPUTCN32_RS17630	214.0371	165.0329	0.37511	0.042671	PepSY domain-containing protein
SPUTCN32_RS17645	276.8116	201.2631	0.45982	0.00915	hypothetical protein
SPUTCN32_RS17715	764.1181	606.0192	0.33443	0.022839	M13 family peptidase
SPUTCN32_RS18160	278.7605	202.4183	0.46169	0.007876	ligand-gated channel protein
SPUTCN32_RS18240	36.48836	54.88496	-0.58897	0.049559	type II toxin-antitoxin system RelE/ParE family toxin
SPUTCN32_RS18425	1371.824	1114.643	0.29951	0.034149	tandem-95 repeat protein
SPUTCN32_RS18430	90.17016	55.41483	0.70238	0.015931	phage tail collar domain-containing protein
SPUTCN32_RS18885	926.8223	396.418	1.2253	1.87E-07	TonB-dependent receptor
SPUTCN32_RS19190	199.5245	145.2813	0.45772	0.02534	EamA/RhaT family transporter
SPUTCN32_RS19250	61.05251	37.184	0.71537	0.010815	pilus assembly protein PilP
SPUTCN32_RS19275	926.1955	730.1845	0.34306	0.015148	argininosuccinate lyase
SPUTCN32_RS19385	1959.861	1597.816	0.29465	0.029986	50S ribosomal protein L17
SPUTCN32_RS19400	2060.613	1488.151	0.46955	0.042402	30S ribosomal protein S11
SPUTCN32_RS19415	8197.447	6527.938	0.32855	0.034973	preprotein translocase subunit SecY
SPUTCN32_RS19555	6692.152	5516.281	0.27877	0.035206	50S ribosomal protein L7/L12
SPUTCN32_RS19605	19.79111	34.75876	-0.81252	0.027636	tRNA-Thr
SPUTCN32_RS19640	277.7108	205.9826	0.43106	0.011866	LexA repressor
SPUTCN32_RS19655	383.2322	278.2551	0.46181	0.044321	cytochrome c oxidase subunit I
SPUTCN32_RS20035	41.81923	72.79699	-0.79971	0.00379	hypothetical protein
SPUTCN32_RS20065	856.7246	466.4093	0.87723	0.01684	TonB-dependent siderophore receptor
SPUTCN32_RS20085	259.2628	70.71687	1.8743	4.85E-05	malate synthase

SPUTCN 32_RS20395	1029.629	1355.133	-0.39631	0.004342	XRE family transcriptional regulator
SPUTCN32_RS20540	5679.218	4611.182	0.30056	0.026254	ATP synthase subunit beta
SPUTCN32_RS20610	94.44491	66.24749	0.51161	0.03252	multidrug efflux RND transporter permease subunit
SPUTCN32_RS20630	141.9629	184.2547	-0.37619	0.045903	hypothetical protein
SPUTCN32_RS20825	962.2463	1199.632	-0.31811	0.025638	hypothetical protein
SPUTCN32_RS20980	629.9054	894.2298	-0.50551	0.012825	hypothetical protein
SPUTCN32_RS21030	1259.943	1634.426	-0.37542	0.006273	signal recognition particle sRNA small type

Table S2 Primers used in RT-qPCR verification for 11 genes.

Gene_id	Forward primer (5'-3')	Reverse primer (5'-3')
SPUTCN32_RS01490	GTCGCTGCATCTGAGTTAGTAGCC	CCAACATGCAATGCTCCATCAGTG
SPUTCN32_RS04905	GTTCAACCTCAACGGCAACAACAG	TGTAGGTCACGCTGCTGCTTATTG
SPUTCN32_RS18885	GCGAGTATCCTCTTGGCACAGTTG	CATTCTCCACCACTGGCAAGGC
SPUTCN32_RS12445	CTCGGCGGCCATTAACCTTATCC	GCTGCTGCTCGTACCTGTCTTG
SPUTCN32_RS06320	GGTGAGCGTACTGAGCAAGGTATG	ACACAGCCATTGCCACTAATCCAC
SPUTCN32_RS04890	CGGCATTATCGGCGTATCATCTGG	GCAATAGGAGCACGACTGAGGTTC
SPUTCN32_RS04895	CGTGAGCGCCGAGCAAGATATC	GCGGAGAGCATTCTGTGGTAGC
SPUTCN32_RS04920	AGCTGCATTACCGCCAGAAC	CCCACTCTGGCAGCATTTGT
SPUTCN32_RS04925	TGTCGGATAATCTTGCGGCCTTG	TCGCGCTTCCAATCCACATCTAAG
SPUTCN32_RS04885	ATCAGGCGCAGTTACGGCATTAC	GTGTTGTGGCAGTACCGCTAAGG
SPUTCN32_RS04910	GAACCGAGGTTAGCCGATGC	CCGCCATTTTCAGTCTTGGCT

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