A Time-Course Transcriptome Analysis of *Escherichia coli* with Direct Electrocatalytic Behavior in Microbial Fuel Cells

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Methods

MFC set up and electrochemical measurement

A dual chamber MFC was used in this work. The capacity of each chamber is 120 mL and a nafion 117 membrane with diameter of 15 mm was clamped between the two chambers. Both of the anode and cathode are carbon cloth (2cm×2cm). The catholyte is $K_3[Fe(CN)_6]$ and anolyte is the cell culture containing glucose. For the current generation measurement, an external load of 2 kΩ was connected into the MFC and the Current and potential measurements on the MFC were carried out by using a benchtop digital multimeter (ESCORT 3146A) during discharge. For the cyclic voltammetry measurement, the reference electrode (saturated calomel electrode, SCE) was inserted into anodic chamber and the cyclic voltammograms were measured by Autolab PGSTAT30 electrochemical working station. The electrochemical impedance spectra were measured at open circuit potential in a frequency range of 100 kHz to 1 mHz with an ac signal of 10 mV amplitude.

Bacterial strains and growth conditions

E. coli K12 overnight cultures were grown from freezer stocks in 100 ml aliquots (500 ml shaker flasks) of Luria–Bertani (LB) broth (10 g of tryptone, 5 g of yeast extract, and 10 g of sodium chloride per liter) at 37°C, with shaking at 250 rpm for 16 h. The overnight cultures were diluted 1:100 in 100 ml prewarmed LB broth and incubated at 37°C, with shaking at 200 rpm until the optical density at 600 nm (OD600) reached early logarithmic phase (OD600~0.5). Then the cell suspension were transferred into the anodic chamber of the MFC and purged nitrogen for 30 min. Before current generation test , glucose was added into anodic chamber to final concentration of 55 mM. the cell growth was monitored by measuring OD600 with a Lambda 25 spectrophotometer (Perkin Elmer).

RNA isolation

E. coli cells were harvested by centrifugation (>8,000×g) at different time points, and then incubated in TE buffer with 1 mg/ml of lysozyme (Sigmaaldrich). RNA was isolated immediately using RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. Finally, samples were eluted with 50 µl of nuclease-free water (Ambion). RNA quality, purity, and integrity were determined using both a NanoDrop™ 1000 Spectrophotometer (Thermo Scientific) and an RNA 6000 Nano LabChips with an Agilent 2100 Bioanalyzer (Agilent Technologies).

cDNA synthesis and labeling

cDNA was synthesized, purified and labeled from 10 µg of RNA with FairPlay® III Microarray Labeling Kit (Stratagene) according to the protocol provided by the manufacturer. The fluorescent dye-labeled cDNA is purified using the DNA-binding solution and microspin cups provided in the kit.

Hybridization and scanning

Microarray hybridizations were carried out on Agilent *E. coli* oligo microarrays using 300ng Cy_3-labeled 'reference' sample and 300ng Cy_5-labeled 'experimental' sample. Hybridizations were carried out using the Agilent hybridization kit and a hybridization oven (Agilent) and then the microarray slides were washed with Gene

Expression Wash Buffer 1 & 2 (Agilent) containing 0.005% Triton X-102. The slides were scanned by Agilent G2565BA Microarray Scanner.

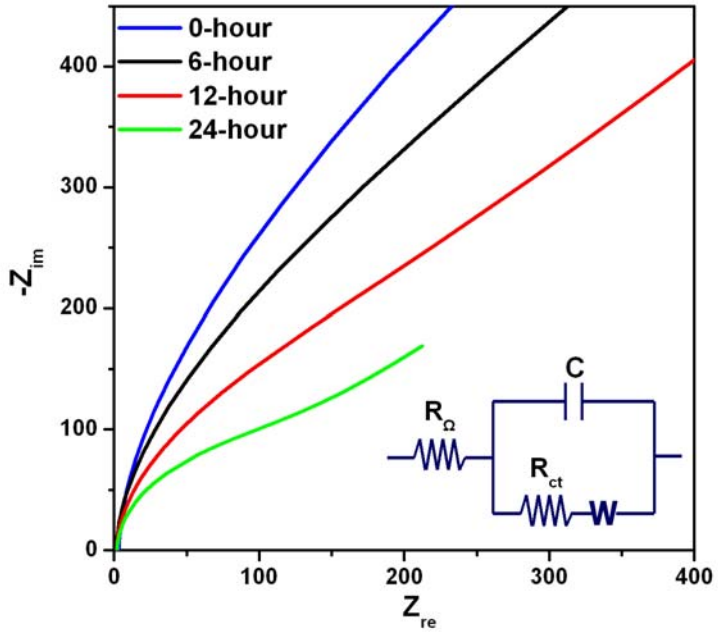
Data analysis

Data analysis was performed with the Feature Expression Software (Agilent Technologies) v. 10.5 and GeneSpring GX v. 10 (Agilent Technologies). Genes that received "present" and "marginal"calls from 50% or more of the replicates in all conditions were used for the analysis. Finally, gene expression changes with statistical significance were identified by an upper one-tailed t test (p cutoff value, 0.05). "Fold change" was calculated as the ratio between the signal averages of four untreated and four treated cultures. That is, results are from biological quadruplicate experiments. Genes with a twofold or more induction or repression in at least one condition were used in this analysis.

Supplementary Table S1

	0-hour	6-hour	12-hour	24-hour	
E_{pa} (mV)	-	-	257	-2	-268
Epa/2 (mV)	-	-	194	-36	-297
E_{pc} (mV)	-	123	118	-65	-317
ΔE_p (mV)	-	-	139	63	49
i_{pa} (μA)	-	-	-90.15	-153.9	-120
i_{pc} (μA)	-	68.6	201	91.91	354

Supplementary Figure S1 Simulated Nyquist Plots and equivalent circuit used for calculation

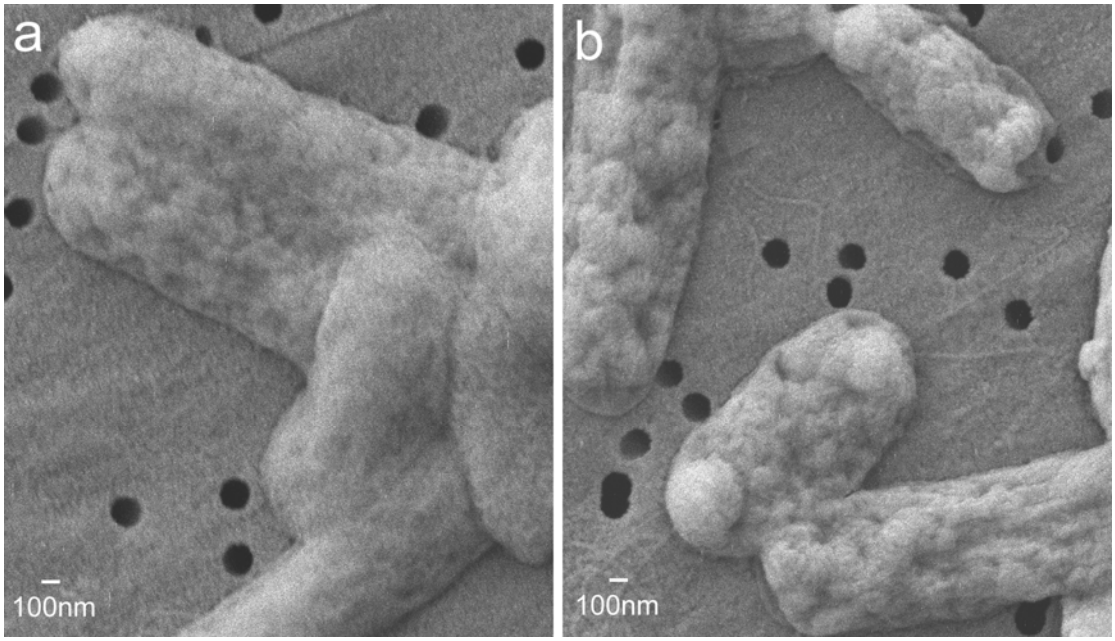


The parameters included in the equivalent circuit are omic resistance of electrolyte (R_Ω), capacitance of the electrode (C), charge transfer resistance (R_{ct}) and Warburg impedance (W).

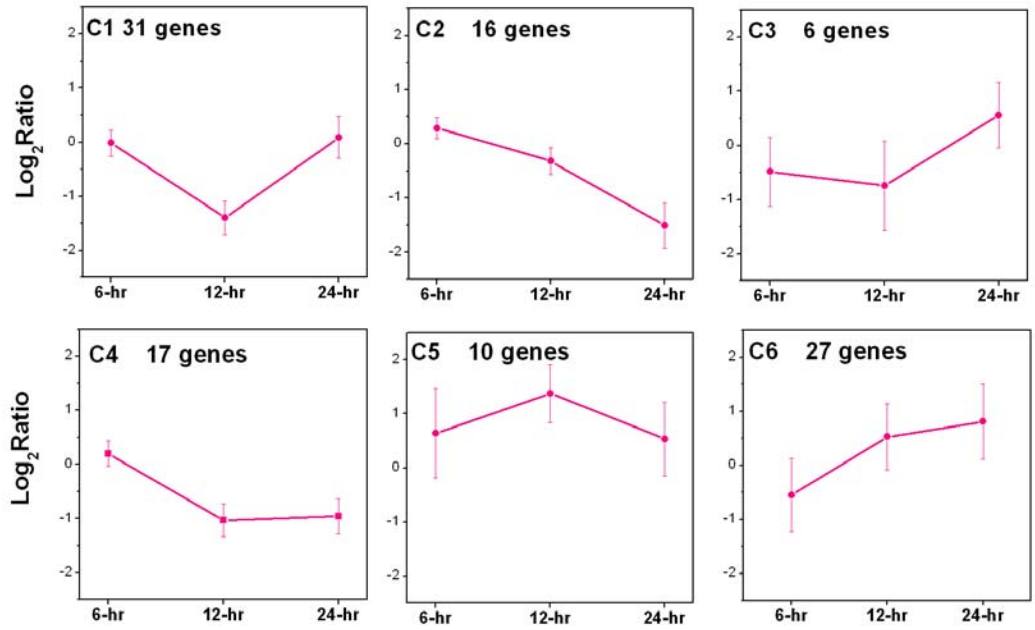
Supplementary Table S2 Calculated impedance values from the equivalent circuit

	0-hour	6-hour	12-hour	24-hour
R_{ct}	285 Ω	207 Ω	150.5 Ω	107.5 Ω

Supplementary Figure S2 SEM micrographs of *E. coli* cells surface morphology. a: Control cells at 24-hr; b: MFC cells at 24-hr.



Supplementary Figure S3 Cluster profiles shown as average log_2Ratio with standard deviation.



Supplementary Table S3 *E. coli* K12 genes that showed statistically significant mRNA level changes upon one of the three time points during discharge in MFC

b number	Gene Name	Description	Functional class	Fold Change		
				6-hr	12-hr	24-hr
Cluster 1						
b0660	*ybeZ*	putative ATP-binding protein in pho regulon	Cellular processes	-1.30	-2.01	-1.13
b2669	*stpA*	DNA-binding protein; H-NS-like protein; chaperone activity; RNA splicing?	DNA metabolism	1.16	-2.30	-1.02
b3635	*mutM*	formamidopyrimidine DNA glycosylase	DNA metabolism	1.27	-2.98	1.59
b0629	*ybeF*	putative transcriptional regulator LYSR-type	Energy metabolism	1.04	-2.27	-1.20
b0492	*ybbN*	putative thioredoxin-like protein	Hypothetical proteins	1.04	-2.59	1.43
b0631	*ybeD*	orf, hypothetical protein	Hypothetical proteins	1.05	-3.52	1.19
b0659	*ybeY*	orf, hypothetical protein	Hypothetical proteins	-1.11	-2.03	-1.03
b1321	*ycjX*	putative EC 2.1 enzymes	Hypothetical proteins	-1.14	-2.49	1.23
b1322	*ycjF*	orf, hypothetical protein	Hypothetical proteins	1.06	-2.38	1.34
b1521	*uxaB*	altronate oxidoreductase	Hypothetical proteins	-1.01	-3.72	-2.17
b2726	*hypA*	pleiotrophic effects on 3 hydrogenase isozymes	Hypothetical proteins	1.24	-2.74	-1.08
b2731	*fhlA*	formate hydrogen-lyase transcriptional activator for fdhF, hyc and hyp operons	Hypothetical proteins	1.09	-2.01	-1.49
b3400	*hslR*	orf, hypothetical protein	Hypothetical proteins	-1.11	-3.98	1.35
b3401	*hslO*	orf, hypothetical protein	Hypothetical proteins	1.05	-3.79	1.41
b4173	*hflX*	GTP - binding subunit of protease specific for phage lambda cII repressor	Hypothetical proteins	1.22	-2.26	-1.24
b0015	*dnaJ*	chaperone with DnaK; heat shock protein	Protein fate	-1.11	-3.00	-1.07
b0439	*lon*	DNA-binding, ATP-dependent protease La; heat shock K-protein	Protein fate	-1.13	-2.63	1.20
b0473	*htpG*	chaperone Hsp90, heat shock protein C 62.5	Protein fate	-1.30	-3.47	1.39
b0630	*lipB*	protein of lipoate biosynthesis	Protein fate	-1.01	-2.86	1.17
b2592	*clpB*	heat shock protein	Protein fate	-1.16	-2.96	1.20
b2614	*grpE*	phage lambda replication; host DNA synthesis; heat shock protein; protein repair	Protein fate	-1.24	-3.67	1.42
b3931	*hslU*	heat shock protein hslVU, ATPase subunit, homologous to chaperones	Protein fate	-1.05	-2.23	1.21
b3932	*hslV*	heat shock protein hslVU, proteasome-related peptidase subunit	Protein fate	-1.02	-2.72	-1.05
b4143	*groL*	GroEL, chaperone Hsp60, peptide-dependent ATPase, heat shock protein	Protein fate	-1.24	-2.24	1.57
b4174	*hflK*	protease specific for phage lambda cII repressor	Protein fate	1.08	-2.56	-1.14
b4175	*hflC*	protease specific for phage lambda cII repressor	Protein fate	1.17	-2.11	-1.20
b4142	*groS*	GroES, 10 Kd chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activity	Protein fate	-1.61	-2.49	1.17
b4171	*miaA*	delta	Protein synthesis	1.11	-2.17	1.11

b4237	*nrdG*	anaerobic ribonucleotide reductase activating protein	Purines, pyrimidines, nucleosides, and nucleotides	1.11	-2.47	-1.45
b3292	*zntR*	putative transcriptional regulator	Regulatory functions	-1.02	-2.01	1.19
b3071	*yqjI*	orf, hypothetical protein	Transcription	1.03	-2.59	-1.70
Cluster 2						
b0273	*argF*	ornithine carbamoyltransferase 2, chain F	Amino acid biosynthesis	1.39	-1.55	-4.41
b2019	*hisG*	ATP phosphoribosyltransferase	Amino acid biosynthesis	1.22	-1.53	-2.71
b2020	*hisD*	L-histidinal:NAD+ oxidoreductase; L-histidinol:NAD+ oxidoreductase	Amino acid biosynthesis	1.14	-1.53	-3.36
b2818	*argA*	N-acetylglutamate synthase; amino acid acetyltransferase	Amino acid biosynthesis	1.14	-1.15	-2.43
b3172	*argG*	argininosuccinate synthetase	Amino acid biosynthesis	1.45	-1.13	-2.75
b3359	*argD*	acetylornithine delta-aminotransferase	Amino acid biosynthesis	1.35	-1.13	-2.06
b3958	*argC*	N-acetyl-gamma-glutamylphosphate reductase	Amino acid biosynthesis	1.26	-1.34	-3.38
b3959	*argB*	acetylglutamate kinase	Amino acid biosynthesis	1.30	-1.39	-3.79
b3960	*argH*	argininosuccinate lyase	Amino acid biosynthesis	1.50	1.03	-2.39
b4254	*argI*	ornithine carbamoyltransferase 1	Amino acid biosynthesis; Energy metabolism	1.23	-1.23	-2.96
b0272	*yagI*	putative regulator	Regulatory functions	1.38	-1.18	-5.30
b3405	*ompR*	response regulator	Regulatory functions	1.01	-1.54	-2.12
b0860	*artJ*	arginine 3rd transport system periplasmic binding protein	Transport and binding proteins	1.23	-1.35	-3.26
b0862	*artQ*	arginine 3rd transport system permease protein	Transport and binding proteins	1.08	1.10	-2.08
b2309	*hisJ*	histidine-binding periplasmic protein of high-affinity histidine transport system	Transport and binding proteins	-1.07	-1.32	-2.13
b3957	*argE*	acetylornithine deacetylase	Unclassified	1.09	1.01	-2.28
Cluster 3						
b0606	*ahpF*	alkyl hydroperoxide reductase, F52a subunit; detoxification of hydroperoxides	Cellular processes	-2.06	-1.76	1.13
b2582	*trxC*	putative thioredoxin-like protein	Energy metabolism	-2.35	-2.97	-1.14
b0802	*ybiJ*	orf, hypothetical protein	Hypothetical proteins	-1.09	-1.19	2.51
b4104	*phnE*	membrane channel protein component of Pn transporter	Unclassified	1.59	1.12	2.04
b4367	*fhuF*	orf, hypothetical protein	Unknown function	-2.13	-1.88	1.04
b3913		orf, hypothetical protein	Unknown function	1.41	1.57	2.23

Cluster 4						
b3767	*ilvG*	acetolactate synthase II, large subunit, cryptic, interrupted	Amino acid biosynthesis	1.15	-1.50	-2.01
b2727	*hypB*	guanine-nucleotide binding protein, functions as nickel donor for large subunit of hydrogenase 3	Central intermediary metabolism;Energy metabolism	1.08	-2.07	-2.45
b2724	*hycB*	probable small subunit of hydrogenase-3, iron-sulfur protein	Energy metabolism	1.15	-2.17	-1.59
b2719	*hycG*	hydrogenase activity	Energy metabolism	1.15	-1.77	-2.12
b2729	*hypD*	pleiotrophic effects on 3 hydrogenase isozymes	Energy metabolism	1.09	-2.15	-2.23
b4079	*fdhF*	selenopolypeptide subunit of formate dehydrogenase H	Energy metabolism	1.09	-2.23	-2.23
b2997	*hybO*	putative hydrogenase subunit	Energy metabolism	1.04	-2.06	-1.78
b3673	*emrD*	2-module integral membrane pump; multidrug resistance	Hypothetical proteins	2.02	-1.23	-1.10
b4218	*ytfL*	putative transport protein	Hypothetical proteins	1.22	-2.10	-1.55
b4212	*ytfH*	orf, hypothetical protein	Hypothetical proteins	1.14	-2.19	-1.96
b2728	*hypC*	pleiotrophic effects on 3 hydrogenase isozymes	Hypothetical proteins	1.02	-2.45	-2.41
b3010	*yqhC*	putative ARAC-type regulatory protein	Regulatory functions	1.12	-1.68	-2.18
b4063	*soxR*	redox-sensing activator of soxS	Unclassified	1.22	-2.33	-1.65
b0674	*asnB*	asparagine synthetase B	Unclassified	1.09	-2.08	-1.69
b2730	*hypE*	plays structural role in maturation of all 3 hydrogenases	Unclassified	-1.08	-1.96	-2.06
b3154	*yhbP*	orf, hypothetical protein	Unknown function	1.17	-3.20	-2.97
b3596	*yibG*	orf, hypothetical protein	Unknown function	1.14	-2.24	-1.88
Cluster 5						
b0003	*thrB*	homoserine kinase	Amino acid biosynthesis	1.06	2.40	-1.36
b0002	*thrA*	aspartokinase I, homoserine dehydrogenase I	Amino acid biosynthesis	1.12	2.32	-1.24
b0775	*bioB*	biotin synthesis, sulfur insertion?	Biosynthesis of cofactors, prosthetic groups, and carriers	2.37	2.70	1.58
b2867	*xdhB*	putative dehydrogenase	Energy metabolism	1.04	2.00	1.36
b0734	*cydB*	cytochrome d terminal oxidase polypeptide subunit II	Energy metabolism	2.47	2.99	2.08
b2870	*ygeW*	putative carbamoyl transferase	Energy metabolism	-1.71	2.73	1.76
b0733	*cydA*	cytochrome d terminal oxidase, polypeptide subunit I	Energy metabolism	2.80	2.19	1.47
b2579	*yfiD*	putative formate acetyltransferase	Hypothetical proteins	3.35	5.09	2.81
b0953	*rmf*	ribosome modulation factor	Unknown function	1.06	3.83	2.47
b1823	*cspC*	cold shock protein	Unknown function	2.04	1.29	-1.18
Cluster 6						

b2871	*ygeX*	putative dehydratase	Amino acid biosynthesis; Energy metabolism	-1.20	2.59	1.00
b1925	*fliS*	flagellar biosynthesis; repressor of class 3a and 3b operons	Cellular processes	-2.25	-1.04	1.30
b1924	*fliD*	flagellar biosynthesis; filament capping protein; enables filament assembly	Cellular processes	-2.20	-1.05	-1.25
b1887	*cheW*	positive regulator of CheA protein activity	Cellular processes	-2.39	-1.07	1.18
b1070	*flgN*	protein of flagellar biosynthesis	Cellular processes	-2.25	-1.08	-1.38
b1082	*flgK*	flagellar biosynthesis, hook-filament junction protein 1	Cellular processes	-2.33	-1.11	-1.13
b1923	*fliC*	flagellar biosynthesis; flagellin, filament structural protein	Cellular processes	-2.53	-1.11	-1.04
b1882	*cheY*	chemotaxis regulator transmits chemoreceptor signals to flagelllar motor components	Cellular processes	-2.01	-1.23	-1.05
b1922	*fliA*	flagellar biosynthesis; alternative sigma factor 28; regulation of flagellar operons	Cellular processes; Transcription	-3.11	-1.05	-1.04
b1397	*paaJ*	putative acyltransferase	Central intermediary metabolism	-1.19	1.39	2.25
b1676	*pykF*	pyruvate kinase I	Energy metabolism	-1.37	1.38	2.30
b0849	*grxA*	glutaredoxin1 redox coenzyme for glutathione-dependent ribonucleotide reductase	Energy metabolism	-3.09	-1.64	1.05
b1789	*yeaL*	orf, hypothetical protein	Hypothetical proteins	1.07	1.74	2.07
b2151	*galS*	mgl repressor, galactose operon inducer	Regulatory functions	-1.12	1.83	2.22
b1818	*manY*	PTS enzyme IIC, mannose-specific	Transport and binding proteins	-1.14	2.53	2.51
b1819	*manZ*	PTS enzyme IID, mannose-specific	Transport and binding proteins	-1.20	2.19	2.88
b1817	*manX*	PTS enzyme IIAB, mannose-specific	Transport and binding proteins	-1.28	2.18	2.23
b3528	*dctA*	uptake of C4-dicarboxylic acids	Transport and binding proteins	1.04	1.87	2.18
b3751	*rbsB*	D-ribose periplasmic binding protein	Transport and binding proteins	-1.06	1.83	2.47
b2215	*ompC*	outer membrane protein 1b	Transport and binding proteins	1.30	1.81	2.62
b3748	*rbsD*	D-ribose high-affinity transport system; membrane-associated protein	Transport and binding proteins	-1.69	1.47	2.61
b2150	*mglB*	galactose-binding transport protein; receptor for galactose taxis	Transport and binding proteins	-1.36	1.43	3.75
b2149	*mglA*	ATP-binding component of methyl-galactoside transport and galactose taxis	Unclassified	-1.18	1.71	2.23
b3914		orf, hypothetical protein	Unclassified	1.44	1.69	2.21
b1921	*fliZ*	orf, hypothetical protein	Unclassified	-2.04	1.10	1.16

b2872	*ygeY*	putative deacetylase	Unclassified	-1.86	2.83	2.65
b0735	*ybgE*	orf, hypothetical protein	Unknown function	2.04	2.83	3.05