Supplementary Information

Rational design of direct and indirect electron transfer pathways to engineer efficient electroactive *Escherichia coli* for green bioelectrochemical system applications

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Table S1. Strains and plasmids used in this study

Strains	Feature	Source
E. coli Trans1-T1	Conventional clone host bacteria	Transgen
E. coli BA102	*E. coli* K12 (*Δldh, Δpfl, ΔptsG)*	Lab stock
S. oneidensis MR-1	Wils-type	Lab stock
P. aeruginosa PAO1	Wils-type	Lab stock
E. coli PCA	*E. coli* BA102 harboring pTrc99a-phz plasmid	This study
E. coli Mtr	*E. coli* BA102 harboring pBBR1MCS-mtr	This study
E. coli PCA+Mtr	*E. coli* BA102 harboring pTrc99a-phz and pBBR1MCS-mtr	This study
E. coli PCA+Mtr-TL	*E. coli* BA102 harboring pTrc99a-tac-phz and pBBR1MCS-mtr	This study
E. coli PCA+Mtr-L5L	*E. coli* BA102 harboring pTrc99a-lacUV5-phz and pBBR1MCS-mtr	This study
E. coli PCA+Mtr-LL	*E. coli* BA102 harboring pTrc99a-lac-phz and pBBR1MCS-mtr	This study
E. coli PCA+Mtr-LL5	*E. coli* BA102 harboring pTrc99a-lac-phz and pBBR1MCS-lacUV5-mtr	This study
E. coli MtrC	*E. coli* BA102 harboring pCWJ-mtrC	This study
E. coli MtrC-H500A	*E. coli* BA102 harboring pCWJ-mtrC-H500A	This study
E. coli BW25113	F-,DE(*ara*D-*ara*B)567, *lac*Z4787(*del*)::*rrn*B-3, LAM-, *rph*-1, DE*(rha*D-*rha*B)568, *hsd*R514	Datsenko and Wanner (2000)
E. coli BW25113-*ΔpdeH*	Single-gene *pdeH* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔpdeC*	Single-gene *pdeC* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔpdeD*	Single-gene *pdeD* deletion in *E. coli* BW25113	Lab stock

E. coli BW25113-*ΔpdeI*	Single-gene *pdeI* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔpdeL*	Single-gene *pdeL* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔpdeK*	Single-gene *pdeM* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔdgcM*	Single-gene *dgcM* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔdgcN*	Single-gene *dgcN* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔbssR*	Single-gene *bssR* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔbssS*	Single-gene *bssS* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔbdcA*	Single-gene *bdcA* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔlsrK*	Single-gene *lsrK* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔlsrR*	Single-gene *lsrR* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔlsrC*	Single-gene *lsrC* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔsdiA*	Single-gene *sdiA* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔcsgA*	Single-gene *csgA* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔcsgB*	Single-gene *csgB* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔdicB*	Single-gene *dicB* deletion in *E. coli* BW25113	Lab stock
E. coli BW25113-*ΔzapC*	Single-gene *zapC* deletion in *E. coli* BW25113	Lab stock
E. coli-ΔpdeH	Single-gene *pdeH* deletion in *E. coli* BA102	This study
E. coli-ΔlsrK	Single-gene *lsrK* deletion in *E. coli* BA102	This study
E. coli PCA+Mtr-LLΔpdeH	*E. coli*-ΔpdeH carrying pTrc99a-lac-phz and pBBR1MCS-mtr	This study
E. coli PCA+Mtr-LLΔpdeH-sqr	*E. coli*-ΔpdeH carrying pTrc99a-lac-phz and pBBR1MCS-mtr and integrating *sqr* from *Rhodobacter capsulatus*	This study
Plasmids		
pBBR1MCS-5	Broad-host-range cloning vector; mob^+, Gm^r	Kovach et al. (1995)

pTrc99a	Expression vector; Amp^r	TaKaRa Inc.
pBBR1MCS-mtr	pBBR1MCS-5 carrying the *mtrCAB* gene of *S. oneidensis* MR-1under Plac control	Feng et al. (2020)
pTrc99a-phz	pTrc99a carrying the *phzA1B1C1D1E1F1G1* gene cluster of *P.aeruginosa* PAO1 under Ptrc control	Feng et al. (2018)
pTrc99a-tac	The Ptrc promoter of pTrc99a is replaced with Ptac.	This study
pTrc99a-lacUV5	The Ptrc promoter of pTrc99a is replaced with PlacUV5.	This study
pTrc99a-lac	The Ptrc promoter of pTrc99a is replaced with Plac.	This study
pTrc99a-tac-phz	pTrc99a carrying the *phzA1B1C1D1E1F1G1* gene cluster of *P.aeruginosa* PAO1 under Ptac control	This study
pTrc99a-lacUV5-phz	pTrc99a carrying the *phzA1B1C1D1E1F1G1* gene cluster of *P.aeruginosa* PAO1 under PlacUV5 control	This study
pTrc99a-lac-phzAG	pTrc99a carrying the *phzA1B1C1D1E1F1G1* gene cluster of *P.aeruginosa* PAO1 under Plac control	This study
pBBR1MCS-lacUV5	The Plac promoter of pBBR1MCS-5 is replaced with PlacUV5.	This study
pBBR1MCS-lacUV5-mtr	pBBR1MCS-5 carrying the *mtrCAB* gene of *S. oneidensis* MR-1under PlacUV5 control	This study
pCWJ-mtrC-H500A	pCWJ carrying the mtrC-H500A	This study

pCWJ-mtrC	pCWJ carrying the *mtrC*	This study
pTargetF-C	pTarget series harboring sgRNAs, Cm^r	Feng et al. (2020)
pTargetF-C-pdeH	PTargetF-C carrying pdeH N20 sites	This study
pTargetF-C-lsrK	PTargetF-C carrying lsrK N20 sites	This study
pCas	repA101(Ts) kan Pcas-cas9 ParaB-Red lacIq Ptrc-sgRNA-pMB1	Jiang et al.(2015)

Table S2. Primers in this study

Primers	Oligonucleotides
Lac-F	ttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataacaatttcacacaggaa
Lac-R	caacatacgagccggaagcataaagtgtaaacagctcatttcagaatatttgccagaaccgttatgatgtc
Tac-F	ttgacaattaatcatcggctcgtataatgtgtggaattgtgagcggataacaatttcaca
Tac-R	cattatacgagccgatgattaattgtcaacagctcatttcagaatatttgccagaacc
LacUV5-F	tttacactttatgcttccggctcgtataatgtgtggaattgtgagcg
LacUV5-R	cattatacgagccggaagcataaagtgtaaacagctcatttcagaatatttgccagaaccgttat
LacUV5-pBB-F	tcgtataatgtgtggaattgtgagcggataacaa
LacUV5-pBB-R	cacacattatacgagccggaagcataaagtg
phz-F	ggggtaccatgaacggtcagcggtacagg
phz-R	tgctctagatcacggttgcaggtagcggtg
Mtr-F	ccgcgaattcatgaacgcacaaaaatcaaaaatcgc
Mtr-R	cgcggatccttagagtttgtaactcatgctc
pdeH-sgRNA-up	cgatctatcaaacatgcggggttttagagctagaaatagcaagttaaaataaggc
pdeH-sgRNA-down	cccgcatgtttgatagatcgactagtattatacctaggactgagctagctgtc
pdeH-donor-1-up	ctcatattcttcctgtgccagtcctaaag
pdeH-donor-1-down	gagagcttgcaagccatcggatggaggttg
pdeH-donor-2-up	tccgatggcttgcaagctctcgatgcttgc
pdeH-donor-2-down	agactgctcactctccagcc
lsrK-sgRNA-up	cttcggcacagaaagcatcggttttagagctagaaatagcaagttaaaataaggc
lsrK-sgRNA-down	cgatgctttctgtgccgaagactagtattatacctaggactgagctagctgtc
lsrK-donor-1-up	atatcgtactggtgatggaacgatgaat
lsrK-donor-1-down	cgcgttaatcccgtaatgccgatcttctccgac
lsrK-donor-2-up	cggcattacgggattaacgcgcacgttcatttc

lsrK-donor-2-down	tggatgccagagcggc
sqr-donor-up	ctgaacggcagcggct
sqr-donor-down	cgccgtggagctattaacgg

Table S3. Energy terms of binding free energy between PCA and MtrC protein

System	MtrC-PCA
Item	Energy(kJ/mol)
ΔG_{vdw}(kJ/mol)	-113.668
ΔG_{ele}(kJ/mol)	1.281
ΔG_{PB}(kJ/mol)	25.328
ΔG_{np}(kJ/mol)	-10.918
ΔG_{bind}(kJ/mol)	-97.978

Table S4. The dissociation constant (K_d) in the interactions between PCA and *E. coli* MtrC or *E. coli* MtrC-H500A

System	MtrC-PCA	Fold
MtrC-PCA	2.53	1.00
MtrC-H500A-PCA	19.61	7.75

*The calculation of K_d was based on Eqs. (1–4) reported by Okamoto et al.

PCA- MtrC(PL) ⇋PCA(L)+MtrC(P) (1)

$$K_d = \frac{[P][L]}{[PL]} \quad (2)$$

[P], [L], and [PL] are the concentrations of MtrC, soluble PCA, and the MtrC-PCA complex, respectively. Under different PCA concentrations of $[L]_1$ and $[L]_2$, the relationship of peak currents (I_{p1} and I_{p2}) of the bound PCA in DPV measurements between the concentration of MtrC-PCA complex ($[PL]_1$ and $[PL]_{2)}$ was shown as Eq. 3.

$$\frac{I_{p2}}{I_{p1}} = \frac{[PL]_2}{[PL]_1} = \alpha \quad (3)$$

By using these equations, K_d could be described as Eq. 4.

$$K_d = \frac{(\alpha - 1)[L]_1}{1 - \alpha\frac{[L]_1}{[L]_2}} \quad (4)$$

In short, PCA was initially added to the three-electrode test reactor with the Ag/AgCl as the reference electrode, the carbon cloth (1 cm × 1 cm) fixed by the strains MtrC or MtrC-H500A as the working electrode and Pt (1 cm × 1 cm) as the counter electrode at a final concentration of 4 μM ($[L]_1$). The DPV measurements were carried out to determine the peak current I_{p1} of the bound PCA. The PCA concentration was subsequently further increased to 10 μM ($[L]_2$). Then the I_{p2} was determined by the DPV measurement.

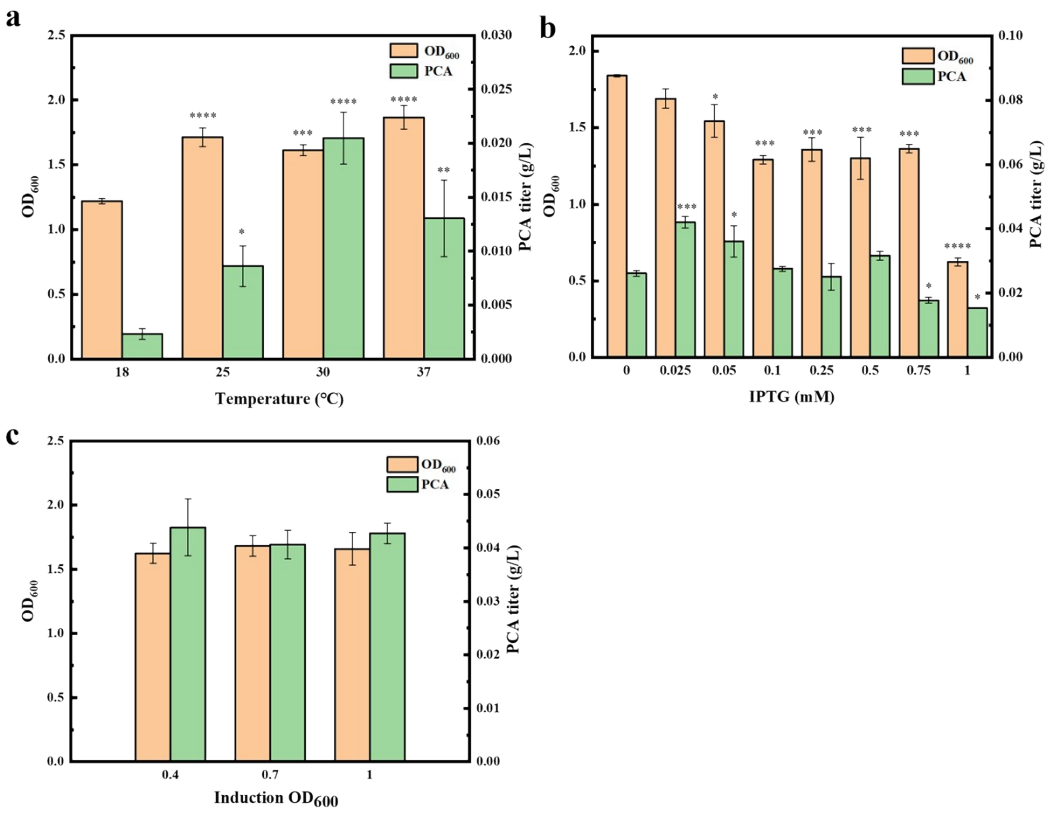

Figure S1. Effects of different induction conditions on PCA production. (a) Induction temperature, (b) Inducer IPTG concentration, and (c) Induction OD_{600}.

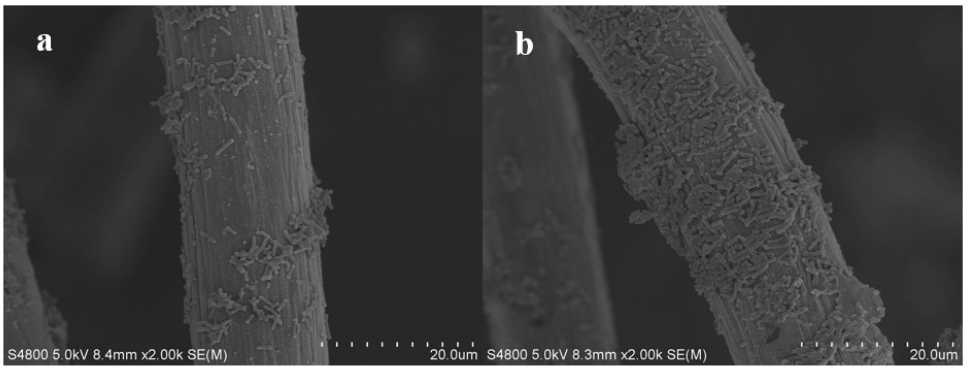

Figure S2. SEM images of biofilms. (a) The control strain and (b) engineered electroactive strain.

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