SUPPORTING INFORMATION

Pei-Hsuan Chen, Chieh Lin, Kai-Hong Kuo, and Yi-Chun Yeh*
Department of Chemistry, National Taiwan Normal University

Materials and Methods

Bacterial strains and growth conditions

Details of plasmid construction including strains, plasmids, and primers were listed in Table S1, S2 and S3. *E. coli* DH5α cells were used as the host for molecular cloning. Cells were cultivated at 37℃ in LB broth with shaking at 250 rpm. *R. eutropha* and *C. metallidurans* cells were used as the hosts for whole-cell biosensor, and were incubated at 28℃ in LB with shaking at 200 rpm. Standard protocols for conjugation with *E. coli* strain S17-1 were used to generate *R. eutropha* H16 and *C. metallidurans* CH34 strains, as previously described.[1]

Metal ions induction

Single colonies were streaked from glycerol stock, and were inoculated in LB with 15 μg/mL gentamycin (Amresco), or 200 μg/mL kanamycin (Amresco) for overnight. Cells were diluted in LB at a ratio of 1:50 (v/v) and grown until reaching OD_{600} of 0.4~0.6. After that, cells were induced in triplicate with metal salt and grown for 24 hours in the case of RFP fluorescence measurement and for 4 hours in the case of betaxanthin fluorescence measurement. The metal salts were $CuCl_2$ (Acros Organics), $Fe(NO_3)_3 \cdot 9H_2O$ (Acros Organics), $CoCl_2 \cdot 6H_2O$ (Acros Organics), $NiSO_4 \cdot 6H_2O$ (Acros Organics), $ZnSO_4 \cdot 7H_2O$ (KISHIDA), and $PbNO_3$ (Acros Organics). All metal salts were analytical grade and the solutions are prepared using double distilled water that has been purified by a MilliQ purification system (Millipore).

Water sample detection

Overnight cultures were diluted in LB, and were grown to an OD_{600} of 0.4~0.6 as described above. After being centrifuged and resuspended in 1:1 (v/v) ratio of 2X LB to tap water or fresh pond water (pond at National Taiwan Normal University) in the presence of appropriate antibiotics, cells were induced with different concentrations of $CuCl_2$ and cells were grown for 24 hours before RFP fluorescence measurement.

RFP fluorescence intensity assay

After cells were induced with metal salt as described above, 300 μL of cell culture was transferred to 96-well microplate. RFP intensity (excitation/emission filters: 530 nm/590 nm) was measured using Synergy HT (BioTeK), and OD_{600} for normalization. Induction coefficient (IC) is the ratio between the normalized fluorescence intensity of the cells induced with metal ions and without metal ions.

Betaxanthin fluorescence intensity assay

After cells were grown with different concentrations of $CuCl_2$ for 4 hours, L-DOPA was added to the cells as the substrate of DOPA 4,5-Dioxygenase from *Mirabilis jalapa* (MjDOD) to a final concentration of 1 mM for 2 hours. Cell pellet was resuspended in PBS buffer for OD_{600} measurement. Betaxanthin fluorescence intensity (excitation/emission filters: 485 nm/528 nm) was measured using Synergy HT (BioTeK).

Determination of LOD (limit of detection)

The equation of LOD was used as below:

LOD = 3(STEYX/SLOPE)

Where STEYX is the standard error of the predicted fluorescence/OD_{600} value in the regression, and SLOPE is the slope of the calibration line.

Table S1. Bacterial Strains Used in This Study

Strains	Relevant genotype	Source
***E. coli* DH5α**	F⁻ endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169 hsdR17(rK- mK+) λ⁻	Purchased from Protech Technology
***C. metallidurans* CH34**	Wild type	Purchased from BCRC (Bioresource Collection and Research Center, Taiwan).
***R. eutropha* H16**	Wild type	DSM 428 Purchased from BCRC (bioresource collection and research center, Taiwan).
YCY_337	*R. eutropha* H16 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopA-rfp*	pYCY_325 conjugated into *R. eutropha* H16. .
YCY_338	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopA-rfp*	pYCY_325 conjugated into *C. metallidurans* CH34.
YCY_423	*R. eutropha* H16 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopF-rfp*	pYCY_417 conjugated into *R. eutropha* H16.
YCY_424	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopF-rfp*	pYCY_417 conjugated into *C. metallidurans* CH34.
YCY_437	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopH-rfp*	pYCY_433 conjugated into *C. metallidurans* CH34.
YCY_448	*R. eutropha* H16 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopH-rfp*	pYCY_433 conjugated into *R. eutropha* H16.
YCY_490	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopE-rfp*	pYCY_475 conjugated into *C. metallidurans* CH34.
YCY_491	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopL-rfp*	pYCY_476 conjugated into *C. metallidurans* CH34.
YCY_492	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopM-rfp*	pYCY_477 conjugated into *C. metallidurans* CH34.
YCY_493	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopQ-rfp*	pYCY_478 conjugated into *C. metallidurans* CH34.
YCY_494	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R*	pYCY_479 conjugated into *C. metallidurans* CH34.

	(rev)-PcopT-rfp	
YCY_495	*R. eutropha* H16 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopE-rfp*	pYCY_475 conjugated into *R. eutropha* H16.
YCY_496	*R. eutropha* H16 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopL-rfp*	pYCY_476 conjugated into *R. eutropha* H16.
YCY_497	*R. eutropha* H16 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopM-rfp*	pYCY_477 conjugated into *R. eutropha* H16.
YCY_498	*R. eutropha* H16 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopQ-rfp*	pYCY_478 conjugated into *R. eutropha* H16.
YCY_499	*R. eutropha* H16 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopT-rfp*	pYCY_479 conjugated into *R. eutropha* H16.
YCY_547	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-PcopQ-MjDOD*	pYCY_543 conjugated into *C. metallidurans* CH34.
YCY_564	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-mPcopE(G)-rfp*	pYCY_562 conjugated into *C. metallidurans* CH34.
YCY_567	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-mPcopF(G)-rfp*	pYCY_565 conjugated into *C. metallidurans* CH34.
YCY_570	*C. metallidurans* CH34 *copS(rev)-copR(rev)-PcopS/R (rev)-mPcopL(G)-rfp*	pYCY_568 conjugated into *C. metallidurans* CH34.

Table S2. Plasmids Used in This Study

Plasmids	Relevant genotype	Construction and source
pYCY_037	pBBR1MCS plasmid with *pBAD-rfp, Kan*^{R}	[2]
pYCY_325	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-PcopA-rfp, Kan*^{R}	PCR fragments of *copS(rev)-copR(rev)-PcopS/R(rev)-PcopA* were amplified with primers 209 and 182, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with EcoRV and NdeI, and cloned into plasmid pYCY_037.
pYCY_414	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-rfp, Kan*^{R}	PCR fragments of *copS(rev)-copR(rev)-PcopS/R(rev)* were amplified with primers 209 and 286, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with EcoRV and NdeI, and cloned into plasmid pYCY_037.
pYCY_417	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-PcopF-rfp, Kan*^{R}	PCR fragments of P*copF* were amplified with primers 289 and 290, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.
pYCY_433	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-PcopH-rfp, Kan*^{R}	PCR fragments of P*copH* were amplified with primers 287 and 288, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.
pYCY_475	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-PcopE-rfp, Kan*^{R}	PCR fragments of P*copE* were amplified with primers 376 and 377, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.
pYCY_476	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-PcopL-rfp, Kan*^{R}	PCR fragments of P*copL* were amplified with primers 372 and 373, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.
pYCY_477	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/*	PCR fragments of P*copM* were amplified with primers 380 and 381,

	R(rev)-PcopM-rfp, Kan^R	from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.
pYCY_478	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-PcopQ-rfp, Kan*^{R}	PCR fragments of P*copQ* were amplified with primers 374 and 375, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.
pYCY_479	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-PcopT-rfp, Kan*^{R}	PCR fragments of P*copT* were amplified with primers 378 and 379, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.
pYCY_543	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-PcopQ-MjDOD, Kan*^{R}	PCR fragments of *MjDOD* were amplified with primers 396 and 397, from pDEST15[3] as a template. The PCR products were treated with NdeI and XhoI, and cloned into plasmid pYCY_037 to replace *rfp* and get the plasmid *pBAD-MjDOD*. Plasmid *pBAD-MjDOD* was treated with NdeI and NotI to get *MjDOD-mob* as insert fragment. *MjDOD-mob* fragments were cloned into plasmid pYCY_478 to replace *rfp*.
pYCY_562	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-mPcopE(G)-rfp, Kan*^{R}	PCR fragments of mP*copE(G)* were amplified with primers 377 and 434, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.
pYCY_565	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-mPcopF(G)-rfp, Kan*^{R}	PCR fragments of mP*copF(G)* were amplified with primers 290 and 435, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.
pYCY_568	pBBR1MCS plasmid with *copS(rev)-copR(rev)-PcopS/ R(rev)-mPcopL(G)-rfp, Kan*^{R}	PCR fragments of mP*copL(G)* were amplified with primers 373 and 436, from *C. metallidurans* CH34 pMOL30 as the template. The PCR products were treated with XbaI and NdeI, and cloned into plasmid pYCY_414.

Table S3. Primers Used in This Study

Primers	**Sequence (5' → 3')**
0182_PcopA 3' NdeI+RBS	TTTTCATATGTATATCTCCTTCTTAAAgaaaatag tccttttcgcttgaatgg
0209_copS(rev) 5' EcoRV	TTTTGATATCtcaggtcagtccgaggcgac
0286_PcopR 3' XbaI+NdeI	TTTTCATATGTTTTTCTAGAggggtcacctcggtgcgta ctg
0287_PcopH 5' XbaI	TTTTTCTAGAgagctttccgagttgtaacgggtggg
0288_PcopH 3' NdeI+RBS	TTTTCATATGTATATCTCCTTCTTAAAgatgttctc cttataagtttcgggcctaacc
0289_PcopF 5' XbaI	TTTTTCTAGAcggcttgcatcgatgttgccttt
0290_PcopF 3' NdeI+RBS	TTTTCATATGTATATCTCCTTCTTAAAtgtgcgac tcctgtattccgacgc
0372_PcopL 5' XbaI	TTTTTCTAGAcgacccagcatcctgtactacgt
0373_PcopL 3' NdeI+RBS	TTTTCATATGTATATCTCCTTCTTAAAggcgtca ctttttgccggg
0374_PcopQ 5' XbaI	TTTTTCTAGAcccggcgacagtgccaaca
0375_PcopQ 3' NdeI+RBS	TTTTCATATGTATATCTCCTTCTTAAAcgcaatctcc aagaggttgggttcag
0376_PcopE 5' XbaI	TTTTTCTAGAcgcgaatagcgcaaccgagatt
0377_PcopE 3' NdeI+RBS	TTTTCATATGTATATCTCCTTCTTAAAcgtgacgcg aggctttggcgtaa
0378_PcopT 5' XbaI	TTTTTCTAGAcgcaagggagatctgtgcg
0379_PcopT 3' NdeI+RBS	TTTTCATATGTATATCTCCTTCTTAAATCGGAA Atgagtgtaattcgcattcg
0380_PcopM 5' XbaI	TTTTTCTAGAgcaggaaaagatttgtattcattaccgtatgaa
0381_PcopM 3' NdeI+RBS	TTTTCATATGTATATCTCCTTCTTAAAtttaatttta ttcccggacccgagcagc
0396_MjDOD 5' NdeI	TTTTCATATGaaaggaacatactatataaatcatggtgat
0397_MjDOD 3' XhoI	TTTTCTCGAGatcagtttttgagtggtgggagtg
0434_mPcopE(G) 5' XbaI	TTTTTCTAGAcgcgaatagcgcaaccgGgatt
0435_mPcopF(G) 5' XbaI	TTTTTCTAGAcggcttgcaGcgatgttgccttt
0436_mPcopL(G) 5' XbaI	TTTTTCTAGAcgacccagcGtcctgtactacgt

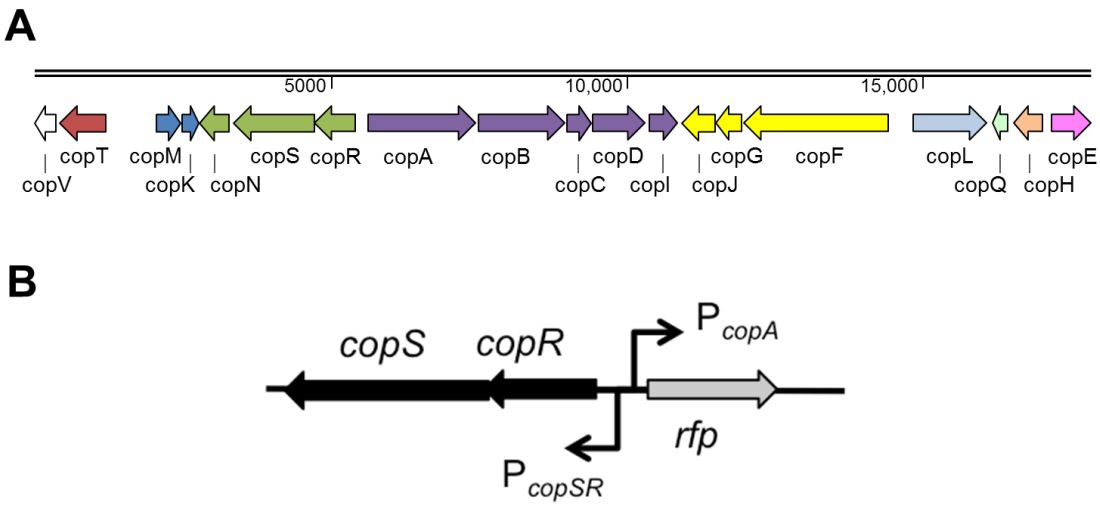

Figure S1 (A) The *cop* cluster of *C. metallidurans* CH34 pMOL30. Genes belonging to the same operon are shown in the same color. (B) Schematic representation of the biosensor construction in this work. The promoter P*copA* was replaced by other *cop* promoters, including P*copH*, P*copT*, P*copM*, P*copF*, P*copL*, P*copQ*, and P*copE*. (C) The sequences of *cop* promoters used in this work. They are aligned with *E. coli pco* promoter consensus sequence[4] which is indicated in boldface. The identical sequences between eight promoters were highlighted in grey.

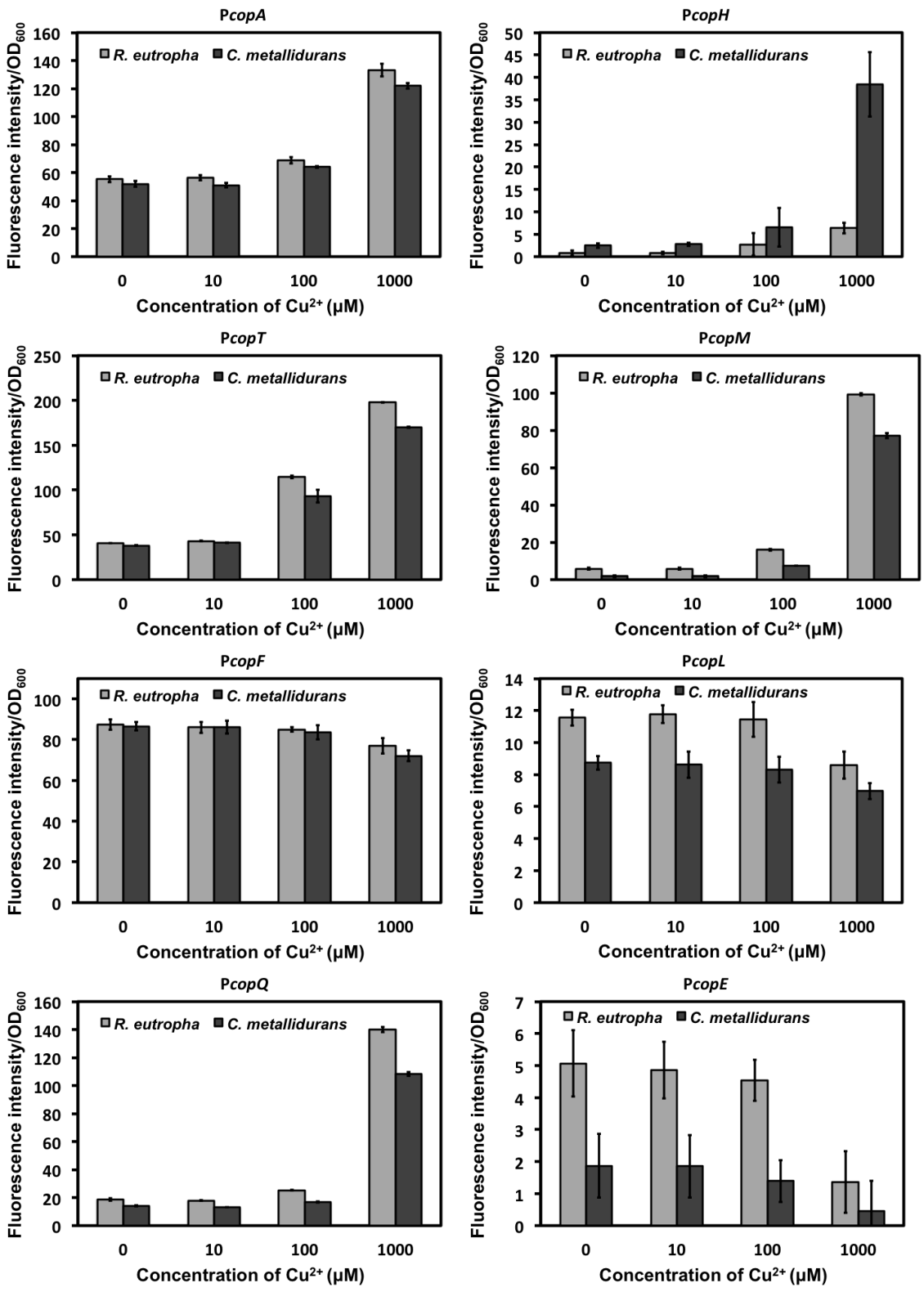

Figure S2 Normalized RFP fluorescence intensities of *R. eutropha* and *C. metallidurans* harboring *copS-copR-PcopSR-cop promoter-rfp*. Error bars represent the standard deviation from triplicate measurements.

Table S4 Comparison between linear ranges obtained with several whole-cell based biosensors for the detection of copper ions.

	regulator	reporter gene	host strain	range of calibration curve	reference
copper	*Pseudomonas putida* **CueR**	*gfp*	*Pseudomonas putida* ATCC 17485	15~1000 μM	Li, P. S., Z. W. Peng, J. Su and H. C. Tao (2014)[5]
copper	*Escherichia coli* **CusSR**	*gfp*	*Escherichia coli* XL1-Blue	1~100 μM	Ravikumar, S., V. D. Pham, S. H. Lee, I. K. Yoo and S. H. Hong (2012)[6]
copper	*Cupriavidus metallidurans* CH34 **CopSR**	*rfp*	*Cupriavidus metallidurans* CH34 and *Ralstonia eutropha* H16	0~1000 μM	This study

Arabinose	–	+	+	–
L-DOPA	–	–	+	+

Figure S3 Images of *C. metallidurans* cells harboring *pBAD-MjDOD*. Arabinose and L-DOPA were used at final concentrations of 0.2% and 1mM, respectively.

Reference

1. R. Simon, U. Priefer and A. Puhler, *Bio-Technol*, 1983, **1**, 784-791.
2. Y. C. Yeh, J. Muller, C. Bi, N. J. Hillson, H. R. Beller, S. R. Chhabra and S. W. Singer, *Chem Commun (Camb)*, 2013, **49**, 910-912.
3. N. Sasaki, Y. Abe, Y. Goda, T. Adachi, K. Kasahara and Y. Ozeki, *Plant & cell physiology*, 2009, **50**, 1012-1016.
4. D. A. Rouch and N. L. Brown, *Microbiology*, 1997, **143**, 1191-1202.
5. P.-S. Li, Z.-W. Peng, J. Su and H.-C. Tao, *Biotechnology letters*, 2014, **36**, 761-766.
6. S. Ravikumar, I. K. Yoo, S. Y. Lee and S. H. Hong, *Applied biochemistry and biotechnology*, 2011, **165**, 1674-1681.