The Cytochrome *bd* complex is essential for sulfide, sulfate and chromate resistance and is regulated by a GbsR-type regulator, CydE, in *Alishewanella* sp. WH16-1

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TABLE S1 Bacterial strains and plasmids used in this study

Strains or plasmids	Relevant characteristic(s)	Source or reference
Bacterial strains		
***Alishewanella* sp.**		
WH16-1	Wild type; Cr(VI)^r; Rif^r, Km^s, Tc^s , Cm^s	This study
△cyt*bd*	Mutant of WH16-1(Rif); Tn5 inserted in *cydB*; Rif^r, Km^r	This study
△cyt*bd*-C	Mutant of WH16-1(Rif) complemented with the Cytbd oxidase operon cloned in pCT-Zori vector; Rif^r, Km^r, Cm^r	This study
Escherichia coli		
E. coli S17-1(λ*pir*)	*Tpr Smr recA thi pro hsdR*$^-$ *hsdM*$^+$. RP4:2Tc:Mu:Km T7, λ*pir*	(Chen et al., 2015a)
E. coli DH5α	F$^-$ *endA1 hsdR17 supE44 thi-1 recA1 deoR gyrA96 relA1* Δ (*argF-lacZYA*)U169 Φ80d*lacZ*M15 λ$^-$	Promega
XL1-Blue	∆(mcrA)183∆(mcrCB-hsdSMR-mrr)173 endA1 supE44 thi-1 recA1 gyrA96 relA1lac [F' proAB lacIqZ∆M15 Tn*5* Km^r]	Stratagene
BL21	B F– *dcm ompT hsdS* ($r_B^-m_B^-$) *gal* λ(DE3)	Novagen
Plasmids		
pRL27	Tn*5*-based transposon vector, *ori*R6K , Km^r	(Larsen et al., 2002)
pCT-Zori	Cm^r	(Chen et al., 2015a)
pCT-Zori-*cytbd*	pCT-Zori vector cloned with the whole *cytbd* operon	This study
pLSP	*lacZ* reporter gene vector	(Li et al., 2015)
pLSP-Promoter	pLSP containing promoter region of *cytbd* operon	This study
pLSP-Promoter-*cydE*	pLSP containing promoter and *cydE* coding region of *cytbd* operon	This study
pTRG	Tet^r, for bacterial one-hybrid assay	Stratagene
pTRG-*cydE*	pTRG containing *cydE* coding region	This study
pBXcmT	Cm^r, for bacterial one-hybrid assay	(Guo et al., 2009)
pBXcmT-Promoter	pBXcmT containing *cytbd* operon promoter region	This study
pET28a	His6 Tag expression vector, Km^r	Novagen
pET28a-*cydE*	*cydE* in frame fusion to the multiple sites of pET-28a	This study

TABLE S2 Primers used in this study

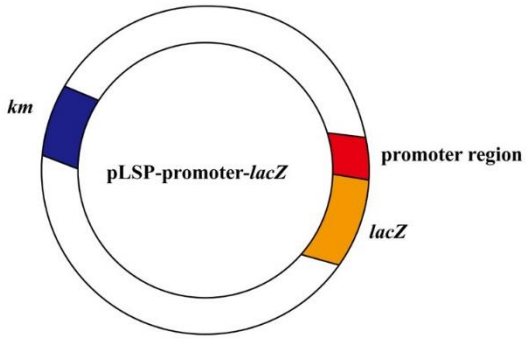
Primer	Sequence(5'-3')	Description
tpnRL17-1	AACAAGCCAGGGATGTAACG	Used for plasmid rescue
tpnRL13-2	CAGCAACACCTTCTTCACGA	
*cydE-cydA*F	TAGCCGCGGTAGTATCTG	Used to amplify *cydE-cydA*
*cydE-cydA*R	GATGGTAATGGTTGGGAA	
*cydA-cydB*F	GGTGTAGCCTGCGGTTGT	Used to amplify *cydA-cydB*
*cydA-cydB*R	TCCCAGAACGGACCAATAG	
*cytbd*F	AAAGAGCTCGGTTAAACCTTGCATTAAGT	Used for complementation of WH16-1-*cydB*(Tn*5*)
*cytbd*R	AAAAAGCTTTCTCATTGTCTTATTAGTAATACTT	
LacPF	AAAGAATTCGGTTAAACCTTGCATTAAGT	Used for construction the *lacZ* reporter gene verctor
LacPR	AAAGGATCCCATTGGAGTCATTTGCATA	
LacP-*cydE*R	AAAGGATCCACAGGTGGCAAAAACAG	
*cydE*F	AAAGGATCCATGCAAATGACTCCAATG	Used for construction CydE over-expression vector with pET28a
*cydE*R	AAAAAGCTTGGCTTGTTTTGCCGGATC	
BohpF	AAAGGATCCATGCAAATGACTCCAATG	Used for bacterial one-hybrid experiment of *cytbd* cluster regulatory region DNA
BohpR	AAAGAATTCAGAACAACAGGTGGCAAA	
BohsF	AAAGGATCCATGCAAATGACTCCAATG	Used for construction of CydE protein expression vector with pTRG
BohsR	AAAGAATTCAGAACAACAGGTGGCAAA	
PromoterF	GGTTAAACCTTGCATTAAGT	Used to amplify promoter region sequence of *cytbd* cluster
PrompterR	CATTGGAGTCATTTGCATA	
FAM-PrompterR	FAM-CATTGGAGTCATTTGCATA	Used to amplify FAM labeled promoter region of *cytbd* operon

* The underlined sequence denotes the restriction enzyme sites.

A

GGTTAAACCTTGCATTAAGTTAACGGCGTAACTAACTTGATCCGTAATGAATGTTTCGCGGGTGAGTTTCAC
TACTTTAGCAGTTCTATCAAGCCTTAACAGTCCTGTCGATAGAAAAGCCCAACTTTTTTTCTGAATCGTGCGC
TCAACGCCCACTAAATTCTTTTAATTAGTTCGCAGCCATACCTGCAAATTATGTTGATCTGGATCAAGCAACA
AGCGTATTATTATTTCAGAAATTTCTGAAAGTTCAGTAACGGGATGCGCATATGCAAATGACTCCAATGATTG
AATCCTTTGTTTACCACTTTGGCGAAATGGGCAGCCGTTGGGGCTTTAACCGTACCGTTGGCCAAATTTACG
CCCTGCTGGTGATTAGCGAGCAACCGCTCTGTGCCAATGAAATTGGTGAGCTGCTGGGGATTTCGCGCGG
CAATGTCAGTATGGGGTTAAAAGAGTTGGGTAGCTGGCAGCTGGTGCAGGTTAGCCATAAACCCGGCGAG
CGGCGCGAGTTTTATCATAGCCGCGGTAGTATCTGGGATATGGCCAACCGGGTGTTTGAAGAGCGGCGCA
AACGGGAAATGGACCCGACGCTTAGCTTACTGCGTGACATTATGATGGACGAAGCGGCTAATCCGGAAGA
AGCCTTTGCCCAAAAGCGCATCGCCGAAATTCACGATCTGCTGGAGAACATTACTGAATGGACGCAAAGCC
TGCAATCCTTGTCGCCGGAGAAGCTGAATACCTTAATGAAGTTAGGCTCCGGCGTTGGCAAGGTACTGGAA
TTTACCGATAAGTTTTTAGGTAAAAAAGATCCGGCAAAACAAGCCTAGCTGTTTTTGCCACCTGT

B



C

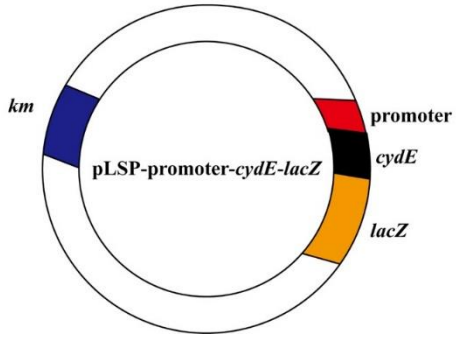


FIG S1 Sequence of *cydE* and its promoter and the construction of related vectors. (A) The red nucleotides are the *cydE* promoter region and 18 nucleotides of *cydE*, and the underlined nucleotides are *cydE* coding sequence; (B) The *cydE* promoter sequence (red) was cloned into the pLSP-kt2*lacZ* plasmid to generate the pLSP-promoter-*lacZ* vector; (C) All of the sequence shown in "A" including *cydE* promoter and *cydE* was cloned into the pLSP-kt2*lacZ* plasmid to generate the pLSP-promoter-*cydE*-*lacZ* vector. For the construction of the pBX-promoter vector in the bacterial one-hybrid assay (FIG 5), the promoter sequence of *cydE* (red) was cloned into the pBXcmT plasmid.

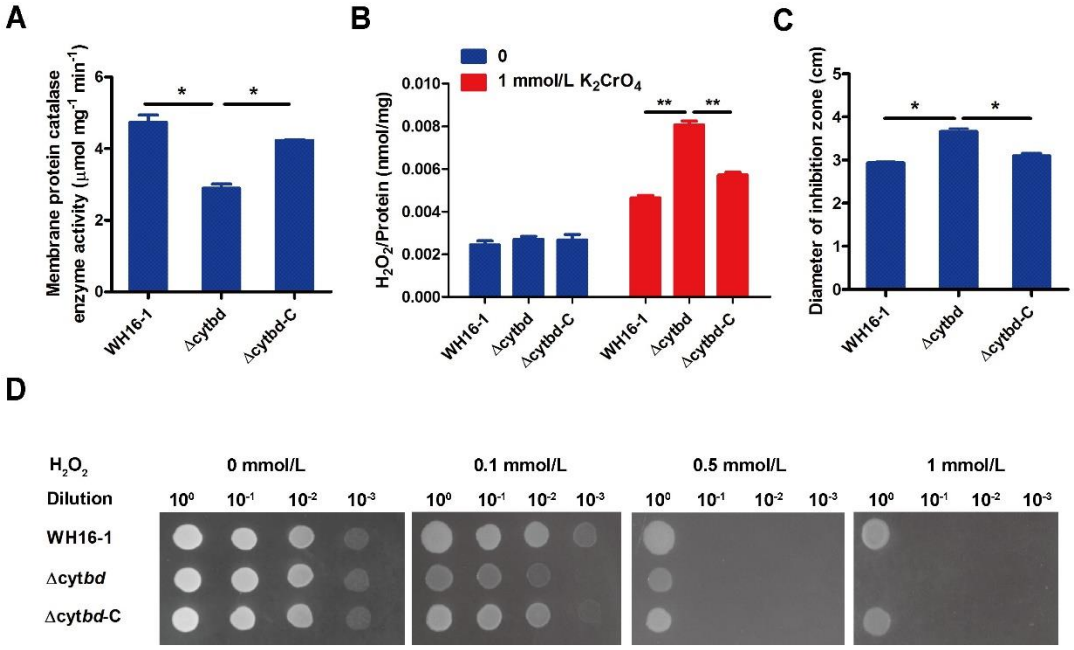


FIG S2 Effects of Cytbd on the reduction of cellular oxidative stress in strain WH16-1, mutant Δ*cytbd* and complementary strain Δ*cytbd*-C. (A) The H_2O_2 decomposition ability of membrane proteins. (B) Cellular H_2O_2 contents with 0 and 1 mmol/L K_2CrO_4, respectively. (C) The inhibition zones when exposed to 3% H_2O_2. (D) H_2O_2 sensitive tests on LB plates. Every sample was prepared in triplicates and the results are presented as mean values. *, remarkable difference; **, extremely remarkable difference.

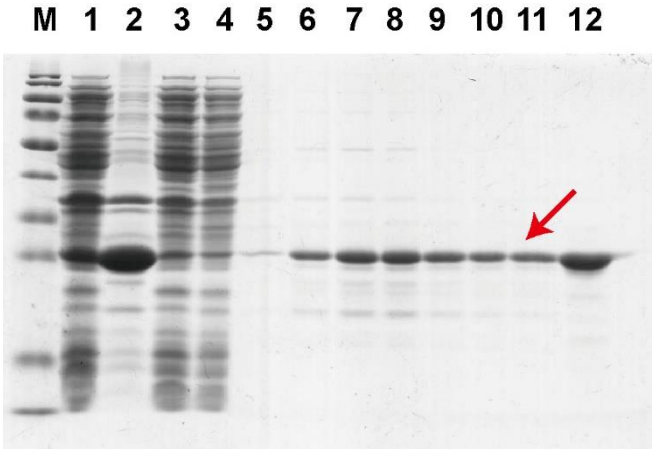


FIG S3 Purification of CydE. Lanes 1 and 2 show cell lysis product suspensions and precipitates after centrifuging, respectively. Lanes 3-5 and 6-12 show proteins eluted from a nickel-ion affinity chromatography column by 200 and 500 mM imidazole, respectively. The red arrow marks proteins that were used in a subsequent EMSA test.