

**Supplementary Table S1.** Primers used in this study.

primers	Sequence	
pAWP89-Tac-For	TAGTTGTCGGGAAGATGCGT	For amplifying a pAWP89 backbone containing Ptac promoter
pAWP89-Tac-Rev	AGCTGTTTCCTGTGTGAATA	
ldcC-For	tattcacacaggaacagctATGAACATCATTGCCATTATGGGAC	For amplifying a <i>ldcC</i> and <i>cadA</i> genes from genomic DNA of <i>E. coli</i> MG1655, the PCR product was ligated into a pAWP89 backbone to construct pAWP89-ldcC or pAWP89- <i>cadA</i> vector For amplifying a <i>lysA</i> and <i>lysC</i> genes from genomic DNA of <i>M. trichosporium</i> OB3b, the PCR product was ligated into the <i>KpnI</i> site of digested pAWP89-ptac-lacZ For amplifying a <i>pyc</i> gene from genomic DNA of <i>Methylobacter</i> sp. DH-1, the PCR product was ligated into the <i>EcoRI</i> site of digested pAWP89-lysA For amplifying a <i>ptac-ldc</i> gene from pAWP89-ldcC, the PCR product was ligated into the <i>BamHI</i> site of digested pAWP89-lysA, pAWP89-lysC, pAWP89-lysA-pyc For amplifying a <i>cadB</i> gene from genomic DNA of <i>E. coli</i> MG1655, the PCR product was ligated into the <i>XbaI</i> site of digested pAWP89-lysA-pyc-ldc
ldcC-Rev	gcactttcccgacaactaTTATCCCGCCATTTTAGGACTC	
cadA-K12-For	tattcacacaggaacagctATGAACGTTATTGCAATATT	
cadA-K12-Rev	gcactttcccgacaactaTTATTTTTTGCTTTCTT	
lysA-For	gggaacaaaagctgggtacATGCATCATTTTCTGACTATCG	
LysA-Rev	cgagggggggcccggtacTCACAGCCAATCGGGAACGC	
lysC-For	gggaacaaaagctgggtacATGGCTCGTCTGGTGATGAA	
LysC-Rev	cgagggggggcccggtacTCAGGCCGCTCCAGCCCAT	
Pyc-For	ggtatcgataagcttgatcgATGTTACGCAAAATCCTGAT	
Pyc-Rev	gatccccgggctgcaggTCAATTTATTTCCACCAACA	
P <sub>tac</sub> -ldc-BamHI-For	ttgacctgccagccggggTTGACAATTAATCATCGGCT	
P <sub>tac</sub> -ldc-BamHI-Rev	gccgctctagaactagtTATCCCGCCATTTTAGGA	
cadB-XbaI-For	ggcgggataacactagttTTAACCAACAGTTTTTCTACCTA	
cadB-XbaI-Rev	accgcggtggcggccgctTTAATGTGCGTTAGACGCGG	

Homology regions used for Gibson Assembly are in lowercase.

**Supplementary Table S2.** Plasmids and strains used in this study

Name	Relevant characteristics	Reference
<b>Plasmids</b>		
pAWP89-lacZ	pAWP89 without dTomato, carrying lacZ reason from pBBR1MCS-2	19
pAWP89-ldcC	pAWP89-lacZ containing <i>ldcC</i> gene from <i>E.coli</i> driven by $P_{tac}$ promoter	This study
pAWP89-cadA	pAWP89-lacZ containing <i>cadA</i> gene from <i>E.coli</i> driven by $P_{tac}$ promoter	This study
pAWP89-lysC	pAWP89-lacZ containing <i>lysC</i> gene from <i>M. trichosporium</i> OB3b driven by $P_{tac}$ promoter	This study
pAWP89-lysA	pAWP89-lacZ containing <i>lysA</i> gene from <i>M. trichosporium</i> OB3b driven by $P_{tac}$ promoter	This study
pAWP89-lysA-pyc	pAWP89-lacZ containing <i>lysA</i> gene from <i>M. trichosporium</i> OB3b and <i>pyc</i> gene from <i>Methylomonas</i> sp. DH-1 driven by $P_{tac}$ promoter	This study
pAWP89-lysC-ldcC	pAWP89-lacZ containing <i>lysC</i> gene from <i>M. trichosporium</i> OB3b, <i>ldcC</i> gene from <i>E.coli</i> driven by $P_{tac}$ promoter	This study
pAWP89-lysA-ldcC	pAWP89-lacZ containing <i>lysA</i> gene from <i>M. trichosporium</i> OB3b, <i>ldcC</i> gene from <i>E.coli</i> driven by $P_{tac}$ promoter	This study
pAWP89-lysA-pyc-ldcC	pAWP89-lacZ containing <i>lysA</i> gene from <i>M. trichosporium</i> OB3b and <i>pyc</i> gene from <i>Methylomonas</i> sp. DH-1, <i>ldcC</i> gene from <i>E.coli</i> driven by $P_{tac}$ promoter	This study
pAWP89-lysA-pyc-ldcC-cadB	pAWP89-lacZ containing <i>lysA</i> gene from <i>M. trichosporium</i> OB3b and <i>pyc</i> gene from <i>Methylomonas</i> sp. DH-1, <i>ldcC</i> gene from <i>E.coli</i> driven by $P_{tac}$ promoter, <i>cadB</i> gene from <i>E.coli</i> driven by $P_{tac}$ promoter	This study
<b>Strains</b>		
<i>Escherichia coli</i>		
MG1655	Cloning host	Invitrogen
Methanotrophs		
<i>M. trichosporium</i> OB3b	Wild type strain	19
<i>Methylomonas</i> sp. DH-1	A novel type I, isolated from brewery waste sludge	36
OB3b/ <i>ldcC</i>	<i>M. trichosporium</i> OB3b harboring pAWP89-ldcC	This study
OB3b/ <i>cadA</i>	<i>M. trichosporium</i> OB3b harboring pAWP89-cadA	This study
Ob3b/ <i>cad1</i>	<i>M. trichosporium</i> OB3b harboring pAWP89-lysC-ldcC	This study
Ob3b/ <i>cad2</i>	<i>M. trichosporium</i> OB3b harboring pAWP89-lysA-ldcC	This study
Ob3b/ <i>cad3</i>	<i>M. trichosporium</i> OB3b harboring pAWP89-lysA-pyc-ldcC	This study
Ob3b/ <i>cad4</i>	<i>M. trichosporium</i> OB3b harboring pAWP89-lysA-pyc-ldcC-cadB	This study