Figure S1.

Prediction of the ssDNA structure surrounding eight original ISMex4 insertion sites in the *Methylobacterium* genome. To deduce the ssDNA structure of original sequences before ISMex4 insertions, ISMex4 and the 4-bp direct repeat generated by transposition were removed. Target sequences and insertion sites are indicated by bold text and arrows, respectively. The META1 numbers indicate the loci where these ISMex4 copies reside on chromosome 1.

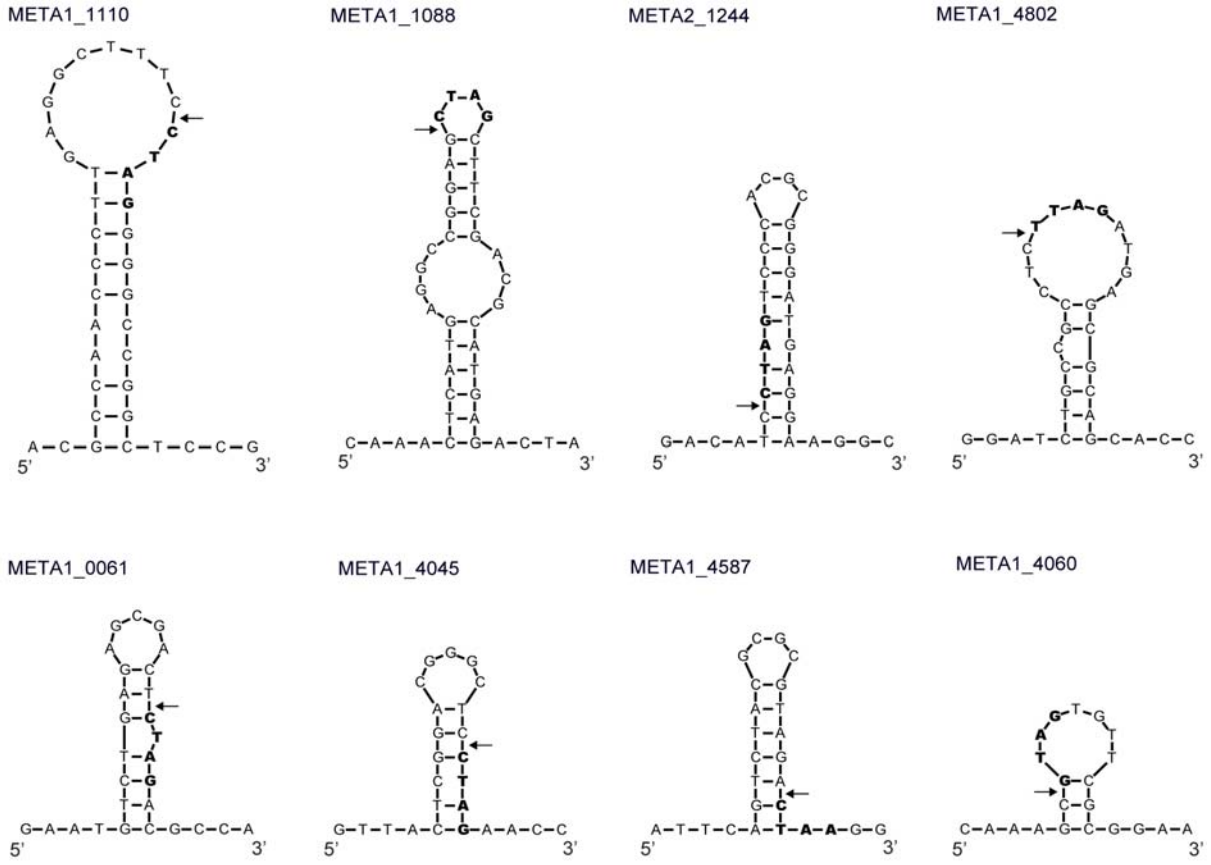

Figure S2.

The *icuAB*^{T1} mutant and the WT strain grow similarly in response to iron, manganese, and zinc titration. Growth rates of the *icuAB*^{T1} mutant (▲) and the WT strain (◯) in response to different concentrations of (A) iron, (B) manganese, and (C) zinc in EDTA-free media. Error bars are 95% confidence intervals. BG, undetermined background concentration.

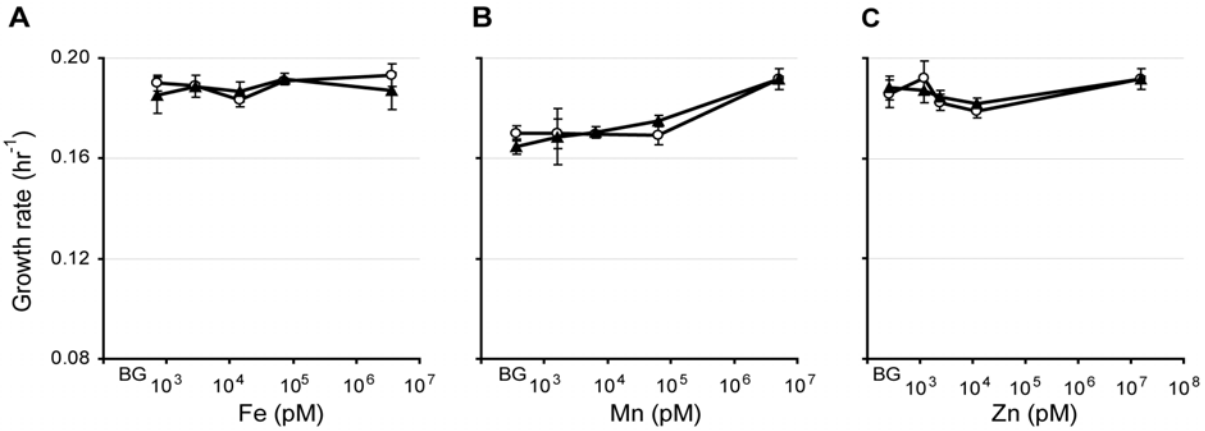

Figure S3.

Deletions of *icuA*, *icuB*, and *icuAB* exhibit minor fitness changes in growth on either methanol or succinate.

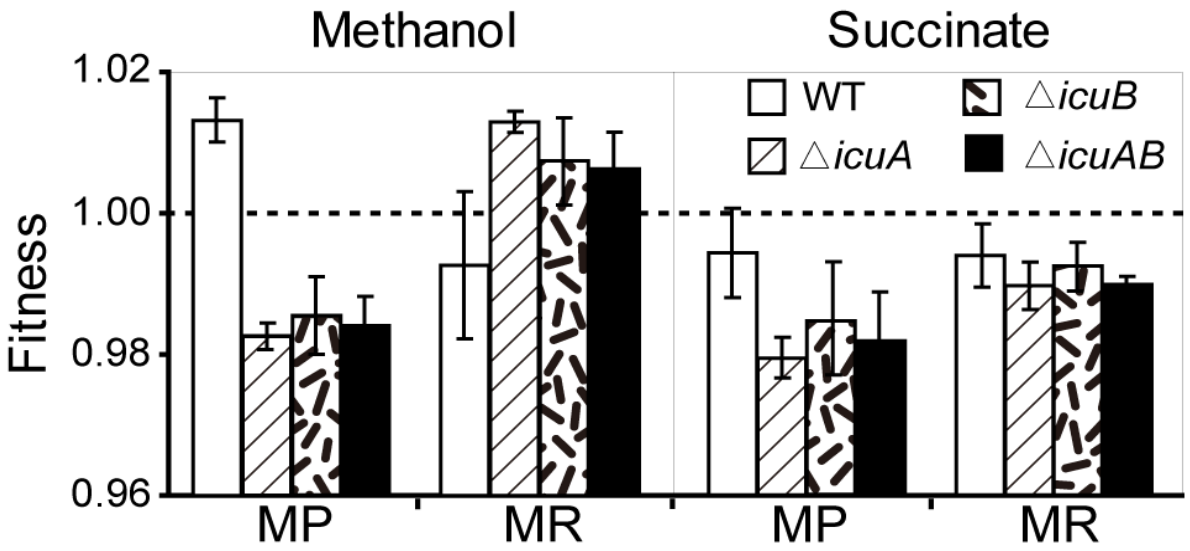

Table S1. Bacterial strains and plasmids

Strain or plasmid	Description	GenBank accession no.	[a]Source or reference
Strains			
WT	Wild-type *Methylobacterium extorquens* AM1 (CM502)		72
EM	Engineered *Methylobacterium* strain (CM702, Δ*mptG*, with pCM410)		HC & CJM, unpublished
CM1145	Evolved isolate from the F4 population at generation 600		
CM1180	WT expressing yellow fluorescent protein Venus		35
CM1275	EM, $fghA^{CM1145}$		
CM1304	WT, $icuAB^{T1}$		
CM1312	EM, $pntAB^{CM1145}$		
CM1316	EM, $gshA^{CM1145}$		
CM1319	CM1145, $icuAB^{WT}$		
CM1320	EM, $icuAB^{T1}$		
CM1321	EM, $fghA^{CM1145}$, $icuAB^{T1}$		
CM1792	EM, $pntAB^{CM1145}$, $icuAB^{T1}$		
CM1794	EM, $gshA^{CM1145}$, $icuAB^{T1}$		
CM1861	WT, $icuAB^{T2}$		
CM1846	WT, Δ*icuA*		
CM1857	WT, Δ*icuB*		
CM1849	WT, Δ*icuAB*		
Plasmids			
pCM132	LacZ-based promoter probe plasmid; [b]Km^r	AF327720	51
pCM160	P_{mxaF} expression plasmid; Km^r	AF327717	51
pCM433	*sacB*-based allelic exchange plasmid; [c]Tc^r	EU118176	72
pHC36	pCM433 with $pntAB^{1145}$; Tc^r		HC & CJM, unpublished
pHC38	pCM433 with $gshA^{1145}$; Tc^r		HC & CJM, unpublished
pHC40	pCM433 with $icuAB^{1145}$; Tc^r	FJ389183	
pHC41	pCM132; *lacZ* replaced by a 33-bp polylinker fragment; Km^r	FJ389165	
pHC42	GFPuv-based Promoter-probe plasmid; Km^r	EU679506	
pHC44	968-bp 5` upstream region of $icuAB^{WT}$ in pHC42; Km^r	FJ389166	
pHC46	282-bp 5` upstream region of $icuAB^{T1}$ in pHC42; Km^r	FJ389167	
pHC47	Full-length ISMex4 in pHC42; Km^r	FJ389168	

pHC51	1737-bp 5` upstream region of *icuAB*^{T1} in pHC42; Km^r	FJ389169
pHC55	113-bp 5`upstream region of *icuAB*^{WT} in pHC42; Km^r	FJ389170
pHC60	P_{tac} in pHC41; P_{tac} expression plasmid; Km^r	EU679507
pHC62	P_{tac} in pHC42; Km^r	FJ389171
pHC65	pCM433 with *icuA* upstream and downstream flanks; Tc^r	FJ389184
pHC67	pCM433 with *icuB* upstream and downstream flanks; Tc^r	FJ389185
pHC68	pCM433 with *icuAB* upstream and downstream flank; Tc^r	FJ389186
pHC69	pHC60 with RBS$_{fae}$-*icuA* ; Km^r	FJ389172
pHC70	pHC60 with RBS$_{fae}$-*icuB* ; Km^r	FJ389173
pHC71	pHC60 with RBS$_{fae}$-*icuAB* ; Km^r	FJ389174
pHC82	pCM433 with *pntAB*^{CM1059}; Tc^r	FJ389187
pHC91	P_{lac} in pHC41; P_{lac} expression plasmid; Km^r	FJ389176
pHC92	pHC91 with RBS$_{fae}$-*icuA* ; Km^r	FJ389177
pHC93	pHC91 with RBS$_{fae}$-*icuB* ; Km^r	FJ389178
pHC94	pHC91 with RBS$_{fae}$-*icuAB* ; Km^r	FJ389179

[a]All strains and plasmid from this study unless noted otherwise.

[b]Km^r, kanamycin resistance.

[c]Tc^r, tetracycline resistance.