

1 Table S1. Primers used in this study.

Primer	Sequence ^a	Added site(s)
HPT for	<u>GGCGCGCCGGATCCGGACGTCGACCTGAGGTAAT</u>	<i>Ascl</i> , <i>BamHI</i>
HPT rev	<u>GGCGCGCCGGATCCAGCTCACTGATCCCCAAAGA</u>	<i>Ascl</i> , <i>BamHI</i>
CB1L	<u>GGCGCGCCACTAGTCCGTAAGCCTCTTGATCTGG</u>	<i>Ascl</i> , <i>SpeI</i>
CB1R	<u>GGCGCGCCGGATCCGAAAAGCCGCTGATTTCAAG</u>	<i>Ascl</i> , <i>BamHI</i>
CB2L	<u>GGCGCGCCACTAGTGCAACTGGAAGACAGGAAGC</u>	<i>Ascl</i> , <i>SpeI</i>
CB2R	<u>GGCGCGCCGGATCCGCGTTTGCGGTTTTATCAAT</u>	<i>Ascl</i> , <i>BamHI</i>
CB3L	<u>GGCGCGCCACTAGTAGCAGTAACCTCTGGCAGGA</u>	<i>Ascl</i> , <i>SpeI</i>
CB3R	<u>GGCGCGCCGGATCCGCAGCACAGGGAGAAAGAAC</u>	<i>Ascl</i> , <i>BamHI</i>
mtaCB2for(AB)	GATCTAAATGGAGGTTTAGAAATATGTTGGATTTGACACTGGAAGACA	none
p-mtaC1+mtaC2rev(AB)	TGTCTTCCAGTGTCAAATCCAACATATTTCTAAACCTCCATTTAGATC	none
mtaCB3for(AB)	GATCTAAATGGAGGTTTAGAAATATGATAGATATAGACCCAGTGGTATTC	none
p-mtaC1+mtaC3rev(AB)	GAATACCACTGGGGTCTATATCTATCATATTTCTAAACCTCCATTTAGATC	none
mtaCB1(p2-1)for	CATTATTAATGGAGGTTAAATATGTTGGACTTTACAGAGGCA	none
p-mtaC2+mtaC1(p2-1)rev	TGCCTCTGTAAAGTCCAACATATTTAAACCTCCATTTAATAATG	none
mtaCB3(p2-3)for	ATTATTAATGGAGGTTAAATATGATAGATATAGACCCAGTGGTATTC	none
p-mtaC2+mtaC3(p2-3)rev	GAATACCACTGGGGTCTATATCTATCATATTTAAACCTCCATTTAATAAT	none

mtaCB1(p3-1)for	ATACTAAAATGGAGGTTAAATATATGTTGGACTTTACAGAGGCA	none
p-mtaC3+mtaC1(p3-1)rev	TGCCTCTGTAAAGTCCAACATATATTTAACCTCCATTTTAGTAT	none
mtaCB2(p3-2)for	TACTAAAATGGAGGTTAAATATATGTTGGATTTGACACTGGAAGAC	none
p-mtaC3+mtaC2(p3-2)rev	GTCTTCCAGTGTCAAATCCAACATATATTTAACCTCCATTTTAGTA	none
<i>mtaC1</i> art op fwd	GGCGCGCCCATATGTTGGACTTTACAGAGGC	<i>Ndel</i>
<i>mtaC1</i> art op rev	GGCGCGCCATGCATGGATCCTCAGTGCTTGGAATTTCTCTCT	<i>Nsil</i>
<i>mtaC2</i> art op fwd	GGCGCGCCCATATGTTGGATTTGACACTGGA	<i>Ndel</i>
<i>mtaC2</i> art op rev	GGCGCGCCATGCATGGATCCTTAGTGCTTGGAATTCCT	<i>Nsil</i>
<i>mtaC3</i> art op fwd	GGCGCGCCCATATGGAGGTTAAATATTTGATAG	<i>Ndel</i>
<i>mtaC3</i> art op rev	GGCGCGCCATGCATGGATCCTCAGTGTTTATGGAATTTCT	<i>Nsil</i>
<i>mtaB1</i> art op fwd	GGCGCGCCAGATCTAATGGAGGTTAAATATGGCAGCAAAAAGATACAC	<i>BgIII</i>
<i>mtaB1</i> fuse fwd	ATGTCCTTGCCTACGACAACGCATACAAGGTCGGCGAAGC	none
<i>mtaB1</i> fuse rev	GCTTCGCCGACCTTGTATGCGTTGTCGTAGGCAAGGACAT	none
<i>mtaB1</i> art op rev	GGCGCGCCATGCATGGATCCTTAGAAGCCGTAGTTCTCGGGGAG	<i>Nsil</i>
<i>mtaB2</i> art op fwd	GGCGCGCCAGATCTAATGGAGGTTAAATATGGTAAAGAAATACACTTC	<i>BgIII</i>
<i>mtaB2</i> fuse fwd	TTAAACTCGGAAAGGCCAAAGGATCTCAGAGACATCCTCAC	none
<i>mtaB2</i> fuse rev	GTGAGGATGTCTCTGAGATCCTTTGCCTTTCCGAGTTTAA	none
<i>mtaB2</i> art op rev	GGCGCGCCATGCATGGATCCTTAGAGCCCGTAGTTTTCTG	<i>Nsil</i>

<i>mtaB3</i> art op fwd	GGCGCGCC <u>AGATCT</u> AATGGAGGTTAAAATATGGCAGTAACAAGATGTAC	<i>BgIII</i>
<i>mtaB3</i> art op rev	GGCGCGCC <u>ATGCAT</u> GGATCCTTAGAGGCCGTAGTTCTCCG	<i>Nsil</i>
<i>mtaA1</i> art op fwd	GGCGCGCC <u>AGATCT</u> AATGGAGGTTAAAATATGACCGATATGAGCGAATT	<i>BgIII</i>
<i>mtaA1</i> art op rev	GGCGCGCC <u>ATGCAT</u> TTAGGCGTAGAATTCGTTTCTTGC	<i>Nsil</i>
<i>mtaA2</i> art op fwd	GGCGCGCCAGATCTAATGGAGGTTAAAATATGAGTGACATGACACTTAA	<i>BgIII</i>
<i>mtaA2</i> art op rev	GGCGCGCC <u>ATGCAT</u> TCAAGCGTAATACTCGTCCC	<i>Nsil</i>
RT <i>mtaC1F</i>	ACTCCGAAACTGCCCCTGTA	none
RT <i>mtaC1R</i>	CGTCGCCTTCTGCAACGT	none
RT <i>mtaC2F</i>	GCAAGGTCGTCTGCCATGT	none
RT <i>mtaC2R</i>	CAGCAACAATATTCTTTCCGATGT	none
RT <i>mtaC3F</i>	CTGCCGACGCCATGCT	none
RT <i>mtaC3R</i>	CTTTCCCGGCTTTTTGCTT	none
RT <i>mtaB1F</i>	CCGGTGCTGACCTGCTTT	none
RT <i>mtaB1R</i>	TGCGTGGTCGAATACTTCCTT	none
RT <i>mtaB2F</i>	TTACAAGGTTCGAAACCAATGCT	none
RT <i>mtaB2R</i>	CATCGGTAAGGGCTTCAAGGT	none
RT <i>mtaB3F</i>	GGTCCGAGACTCTTGCATACG	none
RT <i>mtaB3R</i>	TTTCATTGCCTGAATCAAGAGCTA	none

RT <i>mtaA1F</i>	<u>GGCCCTGACGTGCCAAT</u>	none
RT <i>mtaA1R</i>	CGGATGCGACTGTGATAGGA	none
RT <i>mtaA2F</i>	CCTCCATGTCTGCGGGAAT	none
RT <i>mtaA2R</i>	AAGCCGCAATCTGCCATATAG	none
RT <i>uidAF</i>	TGGCCTGGCAGGAGAACT	none
RT <i>uidAR</i>	CGTATCCACGCCGTATTCG	none
RT <i>rpoA1F</i>	ATGCAGAAGCTCGGAAAAGC	none
RT <i>rpoA1R</i>	GTCCCATGTGGCTATCTGGAA	none

2 ^a Added restriction sites are underlined.

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5 Table S2. Plasmids used in this study.

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Plasmid	Description or construction	Source
pJK200	Puromycin resistant plasmid with the ϕ C31 <i>attB</i> site s	(1)
pMP44	Vector used to construct UP and DOWN region cassettes to delete genes from the <i>M. acetivorans</i> C2A chromosome using the markerless exchange method.	(2)
pAMG63	Plasmid for insertion of ϕ C31 <i>int-attP</i> into <i>M. acetivorans hpt</i> locus	(1)
pAB5	Plasmid for insertion of ϕ C31 <i>int-attP</i> into <i>M. acetivorans hpt</i> locus of Δhpt strains. The <i>PmcrB-hpt</i> region was amplified (using HPTfor and HPTrev) from pMP44, digested with BamHI and cloned into BamHI-digested pAMG63.	This study
pAB7i	<i>HindIII/BamHI</i> -digested <i>mtaCB1</i> operon including a 1000 bp upstream region PCR product (using primers CB1L and CB1R) was cloned into <i>HindIII/BamHI</i> -digested pWM321	This study
pAB7	<i>HindIII/BamHI</i> -digested pAB7i was sub-cloned into <i>HindIII/BamHI</i> -digested pJK200	This study
pAB8i	<i>HindIII/BamHI</i> -digested <i>mtaCB2</i> operon including the 1000 bp upstream region PCR product (using primers CB2L and CB2R) was cloned into <i>HindIII/BamHI</i> -digested pWM321	This study
pAB8	<i>HindIII/BamHI</i> -digested pAB8i was sub-cloned into <i>HindIII/BamHI</i> -digested pJK200	This study
pAB9i	<i>HindIII/BamHI</i> -digested <i>mtaCB3</i> operon including the 1000 bp upstream region PCR product (using primers CB3L and CB3R) was cloned into <i>HindIII/BamHI</i> -digested pWM321	This study
pAB9	<i>HindIII/BamHI</i> -digested pAB9i was sub-cloned into <i>HindIII/BamHI</i> -digested pJK200	This study

pAB10i	<i>HindIII/BamHI</i> -digested fusion PCR product fusing the <i>mtaCB1</i> 1000 bp upstream region with the <i>mtaCB2</i> operon (using primers CB1L, p- <i>mtaC1+mtaC2rev</i> (AB), <i>mtaCB2for</i> (AB) and CB2R) was cloned into <i>HindIII/BamHI</i> -digested pWM321	This study
pAB10	<i>HindIII/BamHI</i> -digested pAB10 <i>i</i> was sub-cloned into <i>HindIII/BamHI</i> -digested pJK200	This study
pAB11i	<i>HindIII/BamHI</i> -digested fusion PCR product fusing the <i>mtaCB1</i> 1000 bp upstream region with the <i>mtaCB3</i> operon (using primers CB1L, p- <i>mtaC1+mtaC3rev</i> (AB), <i>mtaCB3for</i> (AB) and CB3R) was cloned into <i>HindIII/BamHI</i> -digested pWM321	This study
pAB11	<i>HindIII/BamHI</i> -digested pAB11 <i>i</i> was sub-cloned into <i>HindIII/BamHI</i> -digested pJK200	This study
pAB12i	<i>HindIII/BamHI</i> -digested fusion PCR product fusing the <i>mtaCB2</i> 1000 bp upstream region with the <i>mtaCB1</i> operon (using primers CB2L, p- <i>mtaC2+mtaC1rev</i> (AB), <i>mtaCB1(p2-1)for</i> and CB1R) was cloned into <i>HindIII/BamHI</i> -digested pWM321	This study
pAB12	<i>HindIII/BamHI</i> -digested pAB12 <i>i</i> was sub-cloned into <i>HindIII/BamHI</i> -digested pJK200	This study
pAB13i	<i>HindIII/BamHI</i> -digested fusion PCR product fusing the <i>mtaCB2</i> 1000 bp upstream region with the <i>mtaCB3</i> operon (using primers CB2L, p- <i>mtaC2+mtaC3rev</i> (AB), <i>mtaCB3(p2-3)for</i> and CB3R) was cloned into <i>HindIII/BamHI</i> -digested pWM321	This study
pAB13	<i>HindIII/BamHI</i> -digested pAB13 <i>i</i> was sub-cloned into <i>HindIII/BamHI</i> -digested pJK200	This study
pAB14i	<i>HindIII/BamHI</i> -digested fusion PCR product fusing the <i>mtaCB3</i> 1000 bp upstream region with the <i>mtaCB1</i> operon (using primers CB3L, p- <i>mtaC3+mtaC1rev</i> (AB), <i>mtaCB1(p3-1)for</i> and CB1R) was	This study

	cloned into <i>HindIII/BamHI</i> -digested pWM321	
pAB14	<i>HindIII/BamHI</i> -digested pAB14 <i>i</i> was sub-cloned into <i>HindIII/BamHI</i> -digested pJK200	This study
pAB15i	<i>HindIII/BamHI</i> -digested fusion PCR product fusing the <i>mtaCB3</i> 1000 bp upstream region with the <i>mtaCB2</i> operon (using primers CB3L, p- <i>mtaC3</i> + <i>mtaC2</i> rev (AB), <i>mtaCB2</i> (p3-2)for and CB2R) was cloned into <i>HindIII/BamHI</i> -digested pWM321	This study
pAB15	<i>HindIII/BamHI</i> -digested pAB15 <i>i</i> was sub-cloned into <i>HindIII/BamHI</i> -digested pJK200	This study
pJK024	<i>PmcrB-tetR-φC31int-attP</i>	Laboratory stock
pJK027A	cloning vector with <i>PmcrB(tetO1)</i> promoter and <i>φC31-attB</i>	(1)
pRO024	<i>mtaA1</i> (amplified by primers <i>mtaA1</i> art op fwd and <i>mtaA1</i> art op rev) was cloned into pCR®4-TOPO®	This study
pRO025	<i>mtaB2</i> (amplified by fusion product of primers <i>mtaB2</i> art op fwd, <i>mtaB2</i> fuse fwd, <i>mtaB2</i> fuse rev and <i>mtaB2</i> art op rev) was cloned into pCR®4-TOPO®	This study
pRO026	<i>mtaC2</i> (amplified by primers <i>mtaC2</i> art op fwd and <i>mtaC2</i> art op rev) was cloned into pCR®4-TOPO®	This study
pRO027	<i>mtaB1</i> (amplified by fusion product of primers <i>mtaB1</i> art op fwd, <i>mtaB1</i> fuse fwd, <i>mtaB1</i> fuse rev and <i>mtaB1</i> art op rev) was cloned into pCR®4-TOPO®	This study
pRO028	<i>mtaB3</i> (amplified by primers <i>mtaB3</i> art op fwd and <i>mtaB3</i> art op rev) was cloned into pCR®4-TOPO®	This study
pRO029	<i>mtaC1</i> (amplified by primers <i>mtaC1</i> art op fwd and <i>mtaC1</i> art op rev) was cloned into pCR®4-TOPO®	This study
pRO030	<i>mtaC3</i> (amplified by primers <i>mtaC3</i> art op fwd and <i>mtaC3</i> art op rev) was cloned into pCR®4-TOPO®	This study
pRO031	<i>mtaA2</i> (amplified by primers <i>mtaA2</i> art op fwd and <i>mtaA2</i> art op rev) was cloned into pCR®4-TOPO®	This study

pRO032	<i>NdeI/Nsil</i> -digested <i>mtaC1</i> from pRO029 was subcloned into <i>NdeI/Nsil</i> -digested pJK027A	This study
pRO033	<i>NdeI/Nsil</i> -digested <i>mtaC2</i> from pRO026 was subcloned into <i>NdeI/Nsil</i> -digested pJK027A	This study
pRO034	<i>NdeI/Nsil</i> -digested <i>mtaC3</i> from pRO030 was subcloned into <i>NdeI/Nsil</i> -digested pJK027A	This study
pRO035	<i>BglIII/Nsil</i> -digested <i>mtaB1</i> from pRO027 was subcloned into <i>BamHI/Nsil</i> -digested pRO032	This study
pRO036	<i>BglIII/Nsil</i> -digested <i>mtaB2</i> from pRO025 was subcloned into <i>BamHI/Nsil</i> -digested pRO032	This study
pRO037	<i>BglIII/Nsil</i> -digested <i>mtaB1</i> from pRO027 was subcloned into <i>BamHI/Nsil</i> -digested pRO033	This study
pRO038	<i>BglIII/Nsil</i> -digested <i>mtaB2</i> from pRO025 was subcloned into <i>BamHI/Nsil</i> -digested pRO033	This study
pRO039	<i>BglIII/Nsil</i> -digested <i>mtaB3</i> from pRO028 was subcloned into <i>BamHI/Nsil</i> -digested pRO033	This study
pRO040	<i>BglIII/Nsil</i> -digested <i>mtaB1</i> from pRO027 was subcloned into <i>BamHI/Nsil</i> -digested pRO034	This study
pRO041	<i>BglIII/Nsil</i> -digested <i>mtaB2</i> from pRO025 was subcloned into <i>BamHI/Nsil</i> -digested pRO034	This study
pRO042	<i>BglIII/Nsil</i> -digested <i>mtaB3</i> from pRO028 was subcloned into <i>BamHI/Nsil</i> -digested pRO032	This study
pRO043	<i>BglIII/Nsil</i> -digested <i>mtaB3</i> from pRO028 was subcloned into <i>BamHI/Nsil</i> -digested pRO034	This study
pRO044	<i>BglIII/Nsil</i> -digested <i>mtaA1</i> from pRO024 was subcloned into <i>BamHI/Nsil</i> -digested pRO036	This study
pRO045	<i>BglIII/Nsil</i> -digested <i>mtaA2</i> from pRO031 was subcloned into <i>BamHI/Nsil</i> -digested pRO042	This study
pRO046	<i>BglIII/Nsil</i> -digested <i>mtaA1</i> from pRO024 was subcloned into <i>BamHI/Nsil</i> -digested pRO038	This study
pRO047	<i>BglIII/Nsil</i> -digested <i>mtaA2</i> from pRO031 was subcloned into <i>BamHI/Nsil</i> -digested pRO038	This study
pRO048	<i>BglIII/Nsil</i> -digested <i>mtaA1</i> from pRO024 was subcloned into <i>BamHI/Nsil</i> -digested pRO039	This study
pRO049	<i>BglIII/Nsil</i> -digested <i>mtaA2</i> from pRO031 was subcloned into <i>BamHI/Nsil</i> -digested pRO039	This study

pRO050	<i>BglIII/Nsil</i> -digested <i>mtaA1</i> from pRO024 was subcloned into <i>BamHI/Nsil</i> -digested pRO041	This study
pRO051	<i>BglIII/Nsil</i> -digested <i>mtaA2</i> from pRO031 was subcloned into <i>BamHI/Nsil</i> -digested pRO041	This study
pRO052	<i>BglIII/Nsil</i> -digested <i>mtaA1</i> from pRO024 was subcloned into <i>BamHI/Nsil</i> -digested pRO035	This study
pRO053	<i>BglIII/Nsil</i> -digested <i>mtaA2</i> from pRO031 was subcloned into <i>BamHI/Nsil</i> -digested pRO036	This study
pRO054	<i>BglIII/Nsil</i> -digested <i>mtaA2</i> from pRO031 was subcloned into <i>BamHI/Nsil</i> -digested pRO037	This study
pRO055	<i>BglIII/Nsil</i> -digested <i>mtaA1</i> from pRO024 was subcloned into <i>BamHI/Nsil</i> -digested pRO040	This study
pRO056	<i>BglIII/Nsil</i> -digested <i>mtaA2</i> from pRO031 was subcloned into <i>BamHI/Nsil</i> -digested pRO040	This study
pRO057	<i>BglIII/Nsil</i> -digested <i>mtaA2</i> from pRO031 was subcloned into <i>BamHI/Nsil</i> -digested pRO035	This study
pRO058	<i>BglIII/Nsil</i> -digested <i>mtaA1</i> from pRO024 was subcloned into <i>BamHI/Nsil</i> -digested pRO037	This study
pRO059	<i>BglIII/Nsil</i> -digested <i>mtaA2</i> from pRO031 was subcloned into <i>BamHI/Nsil</i> -digested pRO043	This study
pRO060	<i>BglIII/Nsil</i> -digested <i>mtaA1</i> from pRO024 was subcloned into <i>BamHI/Nsil</i> -digested pRO043	This study
pRO061	<i>BglIII/Nsil</i> -digested <i>mtaA1</i> from pRO024 was subcloned into <i>BamHI/Nsil</i> -digested pRO042	This study

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Supplementary References

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