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Supplementary Materials

Heterologous Biosynthesis of Myxobacterial Antibiotic Miuraenamide A

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Table S1. Bacterial strains used in this study.

Strain	Description
"P. miuraensis" SMH-27-4	Original miuraenamide A producer (Ajinomoto)
E.coli HST08 premium	Cloning host for constructing genomic library (Takara bio)
E.coli DH5α	General cloning host (Toyobo)
E.coli SW105	Red/ET recombineering host (NCI)
M. xanthus ATCC 25232	Wild type, heterologous expression host (Ajinomoto)
M. xanthus::*miu*	Heterologous transformant expressing miuraenamide A biosynthetic gene cluster (*miu* cluster)
M. xanthus::*miu* Δ*miuD*	*miu*D gene-disrupted *M. xanthus*::*miu*
M. xanthus::*miu* Δ*miuE*	*miuE* gene-disrupted *M. xanthus*::*miu*
M. xanthus::*miu* Δ*miuF*	*miu*F gene-disrupted *M. xanthus*::*miu*
M. xanthus::*miu* Δ*miuG*	*miu*G gene-disrupted *M. xanthus*::*miu*
M. xanthus::*miu* Δ *orf25-29*	*orf25-29* genes-disrupted *M. xanthus*::*miu*
M. xanthus::*miu* Δ*orf25-29&1-10*	*orf25-29* and *orf1-10* genes-disrupted *M. xanthus*::*miu*
M. xanthus::*miu* Δ*orf25-29&14-16*	*orf25-29* and *orf14-16* genes-disrupted *M. xanthus*::*miu*
M. xanthus::*miu* Δ*orf25-29&19-23*	*orf25-29* and *orf19-23* genes-disrupted *M. xanthus*::*miu*

Table S2. Plasmids used in this study.

Plasmid	Description
pCCIBAC	BAC cloning vector, Chl^R (Epicentre)
p17-9A	pCCIBAC-derived, harbouring *miu* cluster, Chl^R
pTA-Kan^R	pGEM-T easy vector-derived, harbouring 1.8 kb *M. xanthus* homologous fragment, Amp^R, Kan^R [31]
p5TA-Kan^R	pTA-Kan^R-derived, harbouring 5 kb *M. xanthus* homologous fragment, Amp^R, Kan^R
miu BAC	p17-9A-derived, harbouring *miu* cluster, 5TA, Kan^R
miu BAC Δ*miuD*	*miu* BAC-derived, *miuD* gene was disrupted, Chl^R, Kan^R
miu BAC Δ*miuE*	*miu* BAC-derived, *miuE* gene was disrupted, Chl^R, Kan^R
miu BAC Δ*miuF*	*miu* BAC-derived, *miuF* gene was disrupted, Chl^R, Kan^R
miu BAC Δ*miuG*	*miu* BAC-derived, *miuG* gene was disrupted, Chl^R, Kan^R
miu BAC Δ*orf25-29*	p17-9A-derived, harbouring *orf25-29* genes-disrupted *miu* cluster, 5TA, Kan^R
miu BAC Δ*orf25-29&1-10*	*miu* BAC Δ*orf25-29*-derived, *orf1-10* genes were disrupted, Chl^R, Kan^R
miu BAC Δ*orf25-29&14-16*	*miu* BAC Δ*orf25-29*-derived, *orf14-16* genes were disrupted, Chl^R, Kan^R
miu BAC Δ*orf25-29&19-23*	*miu* BAC Δ*orf25-29*-derived, *orf19-23* genes were disrupted, Chl^R, Kan^R

Table S3. Primers used in this study.

Primer	Sequence
pCC-BlnI 2 F	atgcctaggatcctctagagtcgacctg
pCC-BlnI 2 R	atgcctaggatccccgggtaccgagctc
miuBGC p F	caaccaagacggtcagagca
miuBGC p R	ctgaacccgaacgaactcac
loTA Gib F	aacagtgagaggttcgggtgg
loTA Gib R	cacggcgaacatccggaagaac
ploTA F	ccacccgaacctctcactgtt
ploTA R	tcttccggatgttcgccgtgccttttcaattcagaagaactcgtcaag
ploTA check F	ttctccgccgagtagacgaa
ploTA check R	cattaggcaccccaggcttt
ploTA-Kan red F	agcagtgacaggccgtagaggcttccgatcgccgagatcggaatgagcataacagtgagaggttcgggtg
ploTA-Kan red R	cctgttgataccgggaagccctgggccaacttttggcgaaaatgagacgtggcgcaagggctgctaaagg
T1PKS1 cF	ggcgaaggcgtggaggaatt
T1PKS1 cR	cagttggcggcggcatagtt
T1PKS2 cF	gacttcttcgtgctgttctcctctt
T1PKS2 cR	gaagcgacaaccgataccgatgat
NRPS cF	gctggctgttcgttctggtcatc
NRPS cR	tggcggtggtgaatcggaagt
kanf	cgtcaagaaggcgatagaagg
pCC1BAC R	accgttctgtccgtcacttc
loTA cF	agccgaagcgataggtgag
loTA cR	cactgccgttgttgaggag
loupF	aagtgctggttcggctcggagtagttc
loupR	ttcagcatcgcaaccgcatcagactcc
0lodownF	gcatcgagctggtggaatttctggaacagc
2lodownR	agcagcaccacaccttcttcaccatcc
Cm-miuD-F	ccacctgcggcgaatcctccggaccaacgtgcgcaactatccaaaggcggtattcaggcgtagcaaccaggcg
Cm-miuD-R	ggtcacgaacggcaccacgttcgtgttctcgcccgctcgcacccggactcgttgatcggcacgtaagaggttccaa
Cm-miuE-F	cctgataggagacgatctcgaagccggatgtctcgaccgcattgcggtagtattcaggcgtagcaaccaggcg
Cm-miuE-R	gggattccaccccgtgcatcggtacagcgtcgcgttcatcgaattcctctgttgatcggcacgtaagaggttccaa
Cm-miuF-F	agacgaggctgcgcgagcccccactcaccgagatgagcgcgctcgtgagttattcaggcgtagcaaccaggcg
Cm-miuF-R	catggtgccgaaccacgcggcgatcggtacttcgagcggtgcctcggcaagttgatcggcacgtaagaggttccaa
Cm-miuG-F	gttcgaggctctgctcggcgagttcgggcgcggagtctggaccggtgaggtattcaggcgtagcaaccaggcg
Cm-miuG-R	atccgcagccggatccacctagagcatcgggtcgagcaggcgcacttcgagttgatcggcacgtaagaggttccaa
5TA-orf25-F	agtagatcttcacgcccgcacccaggtgcatcgcggcatcgacgtcgttgaacagtgagaggttcgggtggt
Cm-orf1-F	tcgaggacgaggtactgcatgcgctcttcgaacagttcgtggtcgagcattattcaggcgtagcaaccaggcg
Cm-orf10-R	acgaccgaccacacgtttggccacacgtttggcccgcccactggccaaatgttgatcggcacgtaagaggttccaa
Cm-orf14-F	gacgaatgcctcgagcagcaggcggttgagcgcattcttgatctcgaagttattcaggcgtagcaaccaggcg
Cm-orf16-R	gccgagatcgaccagcagttcgatctgtcgctcgtgtgcctgggcatcaagttgatcggcacgtaagaggttccaa
Cm-orf19-F	atcactcttgtcgtcgctcaatcggtccaagaggtcaccggtgaagggcctattcaggcgtagcaaccaggcg
Cm-orf23-R	tgagctggtggaccgagctgcgggattttggactcgagctcatccaggatgttgatcggcacgtaagaggttccaa
Cm cF	ggtattcactccagagcgat

Cm cR	agacggtgagctggtgatatgg
dmiuD cF	cgtgaagatgccgctgtggaa
dmiuD cR	cctgccacgattctcatcg
dmiuE cR	accagacgacaccgacaa
miuEF	gctcttcaagtacgcctcca
dmiuF cF	cagacacaacgaacgacgaac
dmiuF cR	agcaagccgagcgagatt
dmiuG cF	atggtgacgagtggcggtag
miuGR	cgctcaaggagttcactgc
orf24 cF	cgaactatgccgatgaggaga
dorf1 cF	agtggtgaggtgcttccga
dorf10 cR	ggctggcaacctacactt
dorf14 cF	cctcgaactcgatgcccttc
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dorf19 cF	tcggcttgtagtcaacggatg
dorf23 cR	accatgacggcgatgaac

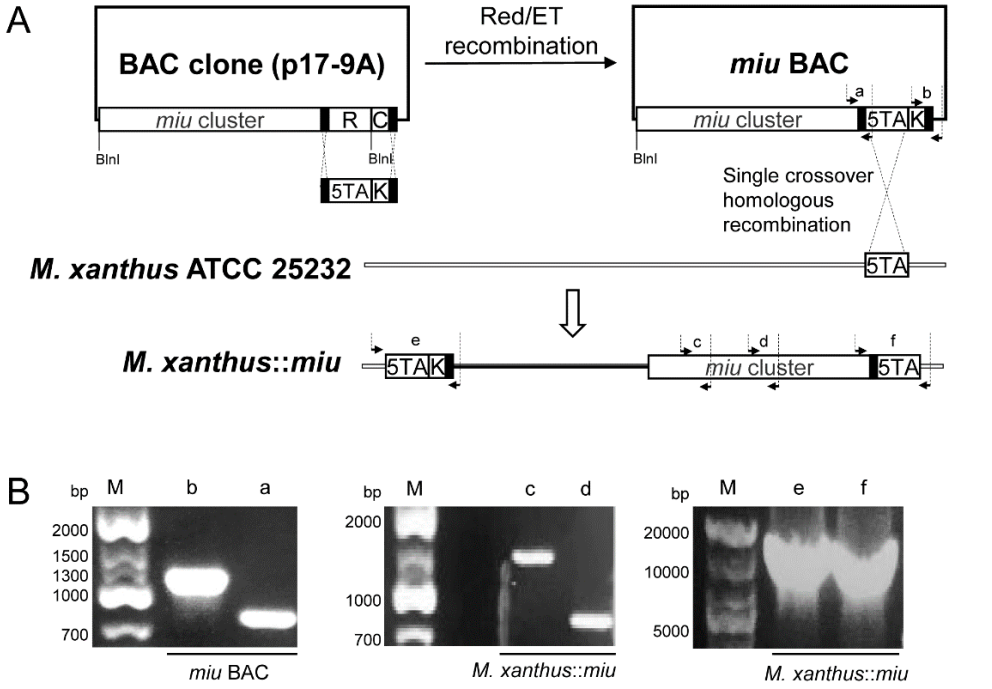

Figure S1. Construction of *M. xanthus*::*miu*. (A) The redundant fragment (genomic fragment outside the *miu* cluster) and chloramphenicol resistance gene (R|C) in the BAC clone p17-9A was replaced with the cassette 5TA-Kan^R (5TA|K, amplified from p5TA-Kan^R with primer pair ploTA-Kan red F/R) by Red/ET recombination. The generated *miu* BAC was integrated into the genome of *M. xanthus* ATCC 25232 by homologous recombination via the 5TA region. (B) PCR verification of the constructed vector and mutant by the following primer pairs: a (loTA cF/loTA cR, 886 bp), b (kanf/pCC1BAC R, 1299 bp), c (T1PKS1 cF/T1PKS1 cR, 1274 bp), d (T1PKS2 cF/T1PKS2 cR, 783 bp), e (loupF/loupR, 7957 bp), and f (0lodownF/2lodownR, 7488). M: DNA marker (Gene Ladder Wide 1, Nippon Gene).

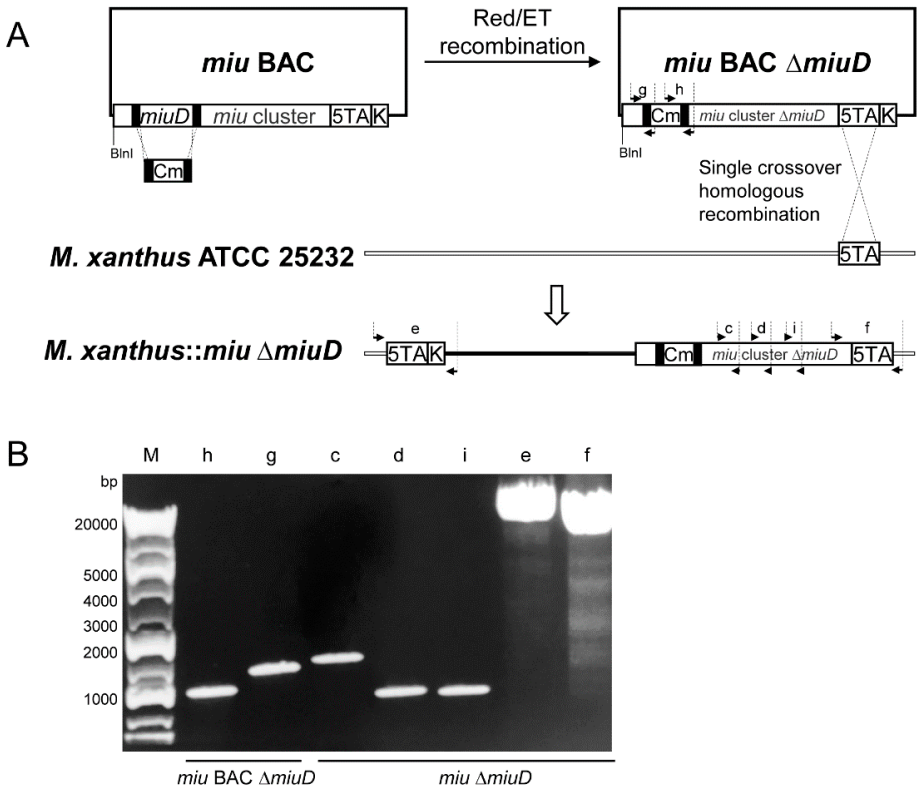

Figure S2. Construction of *M. xanthus*::*miu* Δ*miuD*. (A) The gene *miuD* in *miu* BAC was replaced with the chloramphenicol resistance gene (Cm, amplified from pCCIBAC with primer pair Cm-miuD-F/R) by Red/ET recombination. The generated *miu* BAC Δ*miuD* was integrated into the genome of *M. xanthus* ATCC 25232 by homologous recombination via the 5TA region. (B) PCR verification of the constructed mutants by the following primer pairs: g (dmiuD cF/Cm cR, 1186 bp), h (Cm cF/dmiuD cR, 949 bp), c (T1PKS1 cF/T1PKS1 cR, 1274 bp), d (T1PKS2 cF/T1PKS2 cR, 783 bp), I (NRPS cF/NRPS cR, 756 bp), e (loupF/loupR, 7957 bp), and f (0lodownF/2lodownR, 7488 bp). M: DNA marker.

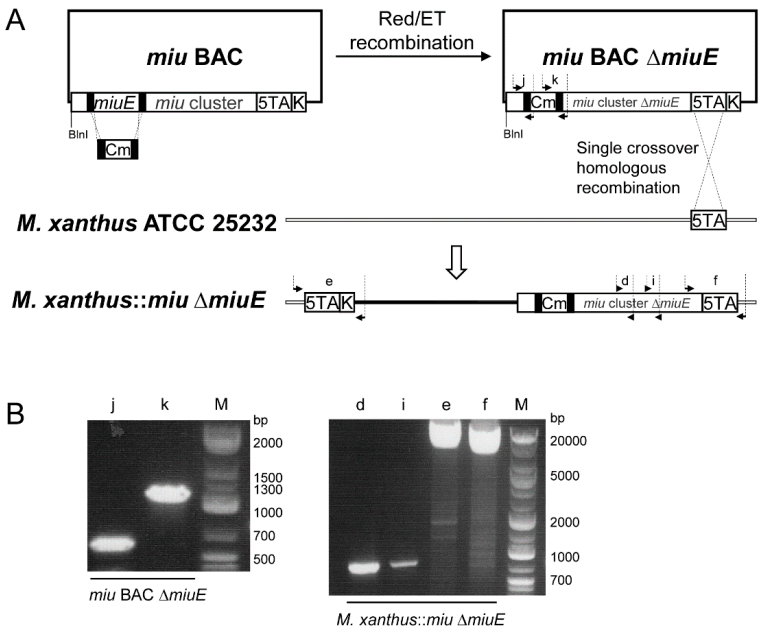

Figure S3. Construction of *M. xanthus*::*miu* Δ*miuE*. (A) The gene *miuE* in *miu* BAC was replaced with the chloramphenicol resistance gene (Cm, amplified from pCCIBAC with primer pair Cm-miuE-F/R) by Red/ET recombination. The generated *miu* BAC Δ*miuE* was integrated into the genome of *M. xanthus* ATCC 25232 by homologous recombination via the 5TA region. (B) PCR verification of the constructed mutants by the following primer pairs: j (miuEF/Cm cR, 599 bp), k (Cm cF/dmiuE cR, 1037 bp), d (T1PKS2 cF/T1PKS2 cR, 783 bp), i (NRPS cF/NRPS cR, 756 bp), e (loupF/loupR, 7957 bp), and f(0lodownF/2lodownR, 7488 bp). M: DNA marker.

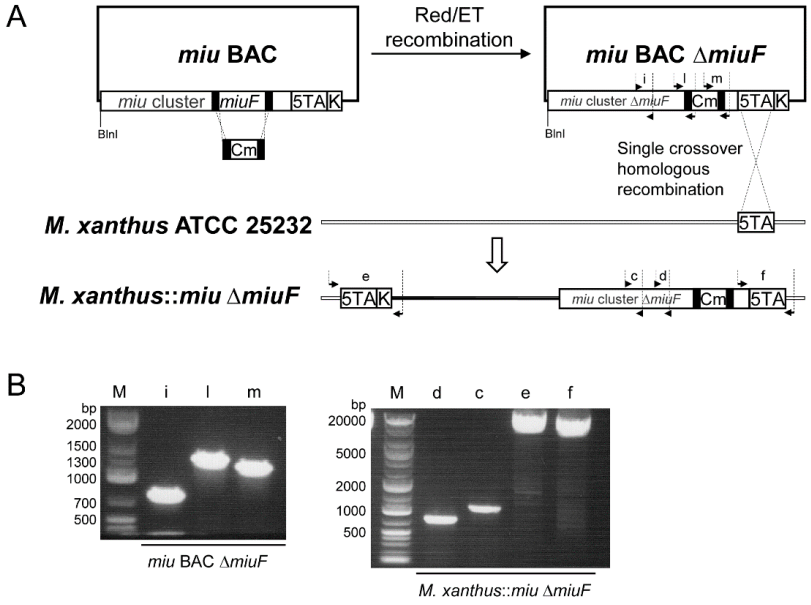

Figure S4. Construction of *M. xanthus*::*miu* Δ*miuF*. (A) The gene *miuF* in *miu* BAC was replaced with the chloramphenicol resistance gene (Cm, amplified from pCCIBAC with primer pair Cm-miuF-F/R) by Red/ET recombination. The generated *miu* BAC Δ*miuF* was integrated into the genome of *M. xanthus* ATCC 25232 by homologous recombination via the 5TA region. (B) PCR verification of the constructed mutants by the following primer pairs: i (NRPS cF/NRPS cR, 756 bp), l (dmiuF cF/Cm cR, 1201 bp), m (Cm cF/dmiuF cR, 1121 bp), c (T1PKS1 cF/T1PKS1 cR, 1274 bp), d (T1PKS2 cF/T1PKS2 cR, 783 bp), e (loupF/loupR, 7957 bp), and f (0lodownF/2lodownR, 7488 bp). M: DNA marker.

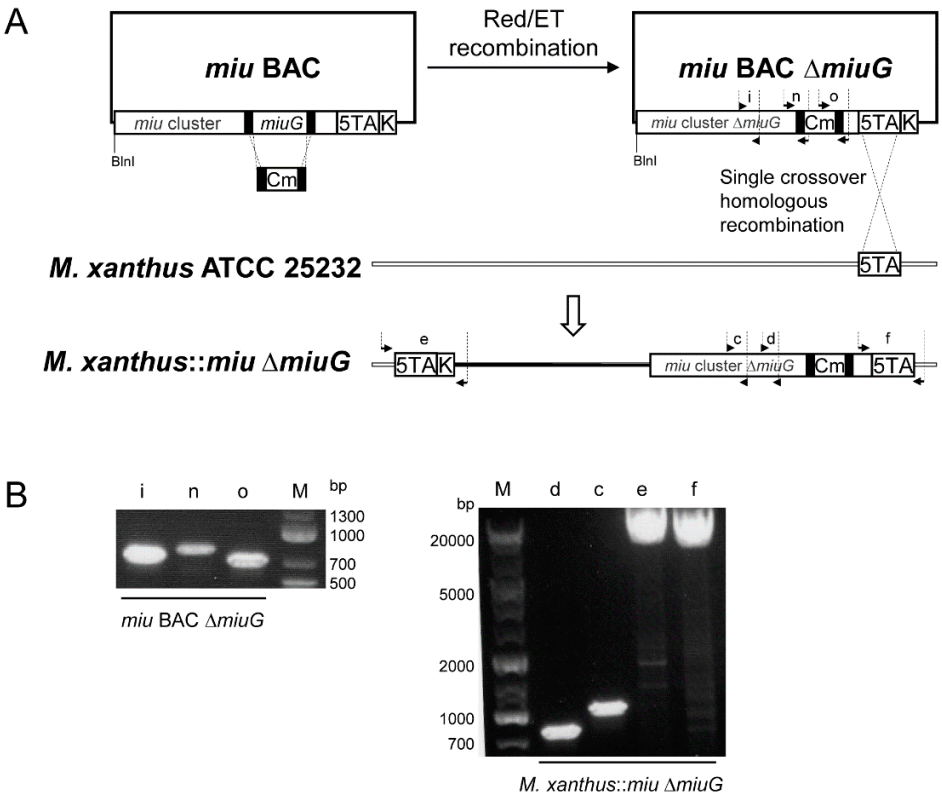

Figure S5. Construction of *M. xanthus*::*miu* Δ*miuG*. (A) The gene *miuG* in *miu* BAC was replaced with the chloramphenicol resistance gene (Cm, amplified from pCCIBAC with primer pair Cm-miuG-F/R) by Red/ET recombination. The generated *miu* BAC Δ*miuG* was integrated into the genome of *M. xanthus* ATCC 25232 by homologous recombination via the 5TA region. (B) PCR verification of the constructed mutants by the following primer pairs: i (NRPS cF/NRPS cR, 756 bp), n (dmiuG cF/Cm cR, 778 bp), o (Cm cF/miuGR, 701 bp), c (T1PKS1 cF/T1PKS1 cR, 1274 bp), d (T1PKS2 cF/T1PKS2 cR, 783 bp), e (loupF/loupR, 7957 bp), and f (0lodownF/2lodownR, 7488 bp). M: DNA marker.

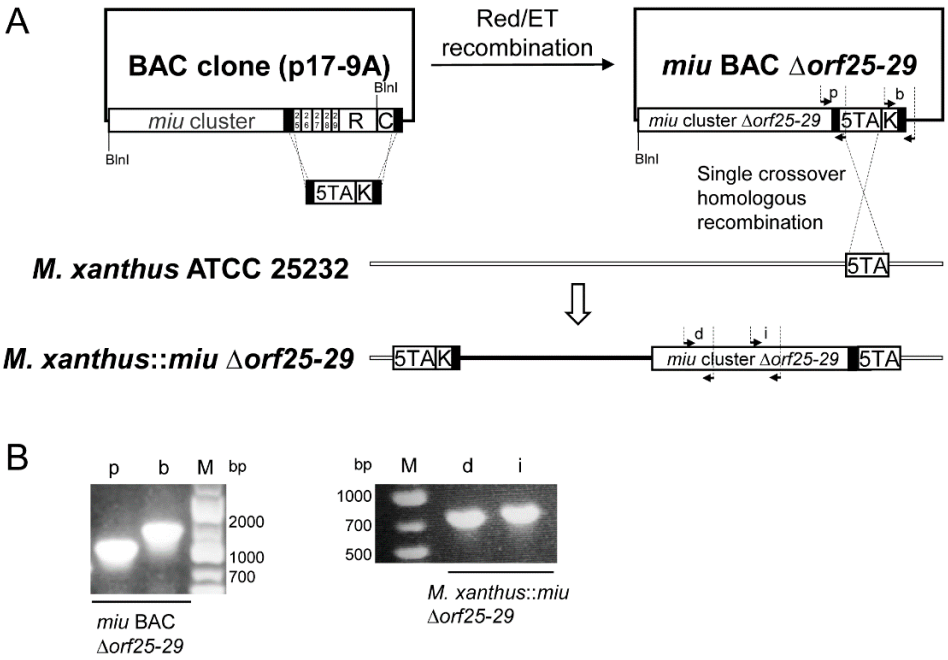

Figure S6. Construction of *M. xanthus*::*miu* Δ*orf25-29*. (A) The gene region *orf25-29,* the redundant fragment and chloramphenicol resistance gene (*25*|*26*|*27*|*28*|*29*|R|C), in the BAC clone p17-9A was replaced with the cassette 5TA-Kan^R (5TA|K, amplified from p5TA-Kan^R with primer pair 5TA-orf25-F/ploTA-Kan red R) by Red/ET recombination. The generated *miu* BAC Δ*orf25-29* was integrated into the genome of *M. xanthus* ATCC 25232 by homologous recombination via the 5TA region. (B) PCR verification of the constructed mutants by the following primer pairs: p (orf24 cF/loTA cR, 1020 bp), b (kanf/pCC1BAC R, 1299 bp), d (T1PKS2 cF/T1PKS2 cR, 783 bp), and i (NPRS cF/NRPS cR, 756 bp). M: DNA marker.

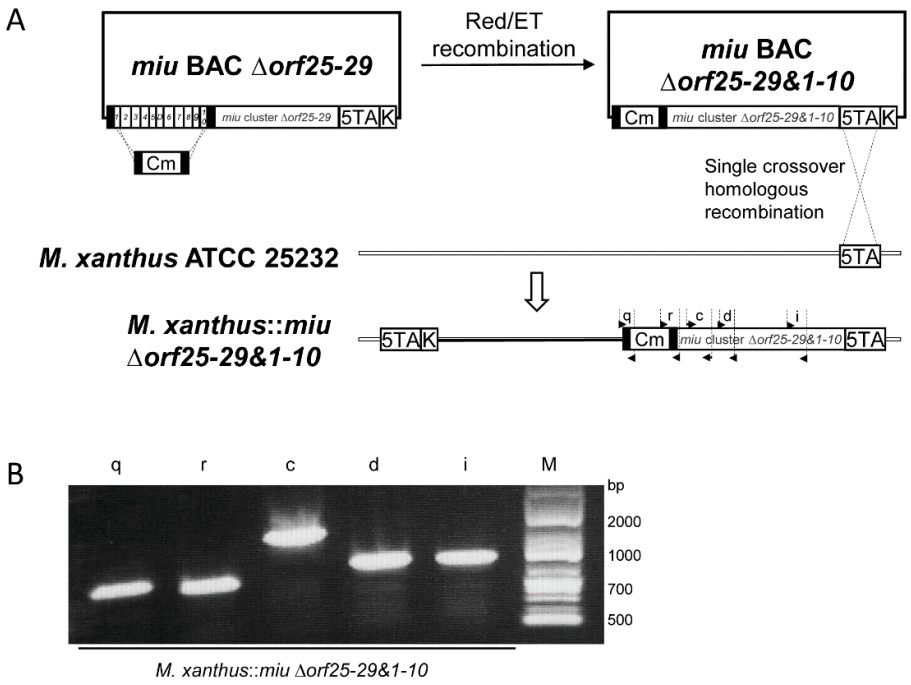

Figure S7. Construction of *M. xanthus*::*miu* Δ*orf25-29&1-10*. (A) The gene region *orf1-10* (*1*|*2*|*3*|*4*|*5*|*D*|*6*|*7*|*8*|*9*|*10*) in *miu* BAC Δ*orf25-29* was replaced with the chloramphenicol resistance gene (Cm, amplified from pCCIBAC with primer pair Cm-orf1-F/ Cm-orf10-R) by Red/ET recombination. The generated *miu* BAC Δ*orf25-29&1-10* was integrated into the genome of *M. xanthus* ATCC 25232 by homologous recombination via the 5TA region. (B) PCR verification of *M. xanthus*::*miu* Δ *orf25-29&1-10* by the following primer pairs: q (dorf1 cF/Cm cR, 694 bp), r (Cm cF/dorf10 cR, 621 bp), c (T1PKS1 cF/T1PKS2 cR, 1274 bp), d (T1PKS2 cF/T1PKS2 cR, 783 bp), and i (NPRS cF/NRPS cR, 756 bp). M: DNA marker.

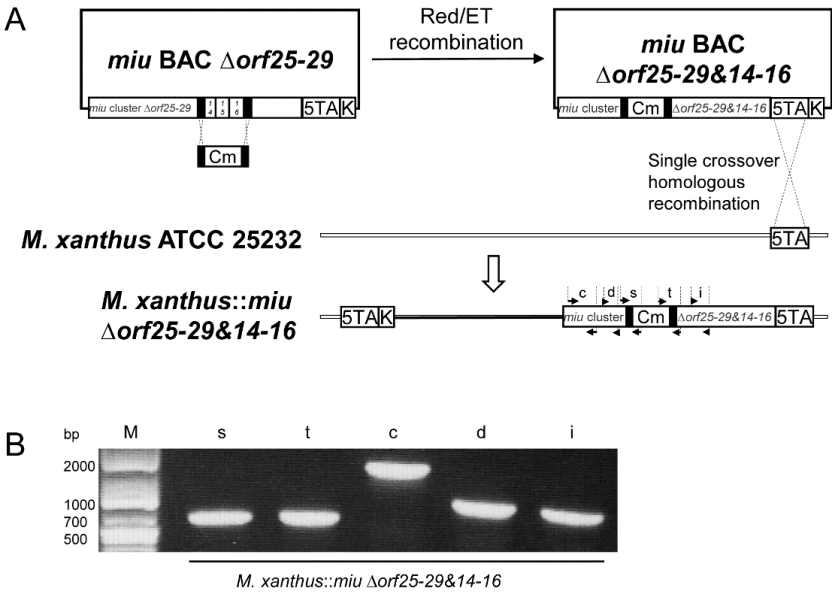

Figure S8. Construction of *M. xanthus*::*miu* Δ*orf25-29&14-16*. (A) The gene region *orf14-16* (*14*|*15*|*16*) in *miu* BAC Δ*orf25-29* was replaced with the chloramphenicol resistance gene (Cm, amplified from pCCI-BAC with primer pair Cm-orf14-F/ Cm-orf16-R) by Red/ET recombination. The generated *miu* BAC Δ*orf25-29&14-16* was integrated into the genome of *M. xanthus* ATCC 25232 by homologous recombination via the 5TA region. (B) PCR verification of *M. xanthus*::*miu* Δ *orf25-29&14-16* by the following primer pairs: s (dorf14 cF/Cm cR, 577 bp), t (Cm cF/dorf16 cR, 591 bp), c (T1PKS1 cF/T1PKS2 cR, 1274 bp), d (T1PKS2 cF/T1PKS2 cR, 783 bp), and i (NPRS cF/NRPS cR, 756 bp). M: DNA marker.

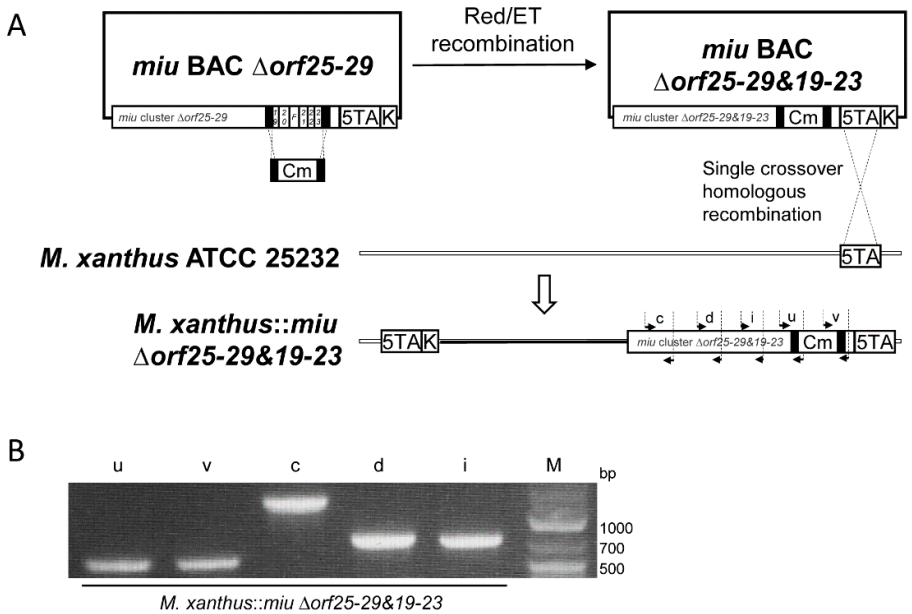

Figure S9. Construction of *M. xanthus*::*miu* Δ*orf25-29&19-23*. (A) The gene region *orf19-23* (*19*|*20*|*F*|*21*|*22*|*23*) in *miu* BAC Δ*orf25-29* was replaced with the chloramphenicol resistance gene (Cm, amplified from pCCIBAC with primer pair Cm-orf19-F/ Cm-orf23-R) by Red/ET recombination. The generated *miu* BAC Δ*orf25-29&19-23* was integrated into the genome of *M. xanthus* ATCC 25232 by homologous recombination via the 5TA region. (B) PCR verification of *M. xanthus*::*miu* Δ *orf25-29&19-23* by the following primer pairs: u (dorf19 cF/Cm cR, 582 bp), v (Cm cF/dorf23 cR, 576 bp), c (T1PKS1 cF/T1PKS2 cR, 1274 bp), d (T1PKS2 cF/T1PKS2 cR, 783 bp), and i (NPRS cF/NRPS cR, 756 bp). M: DNA marker.

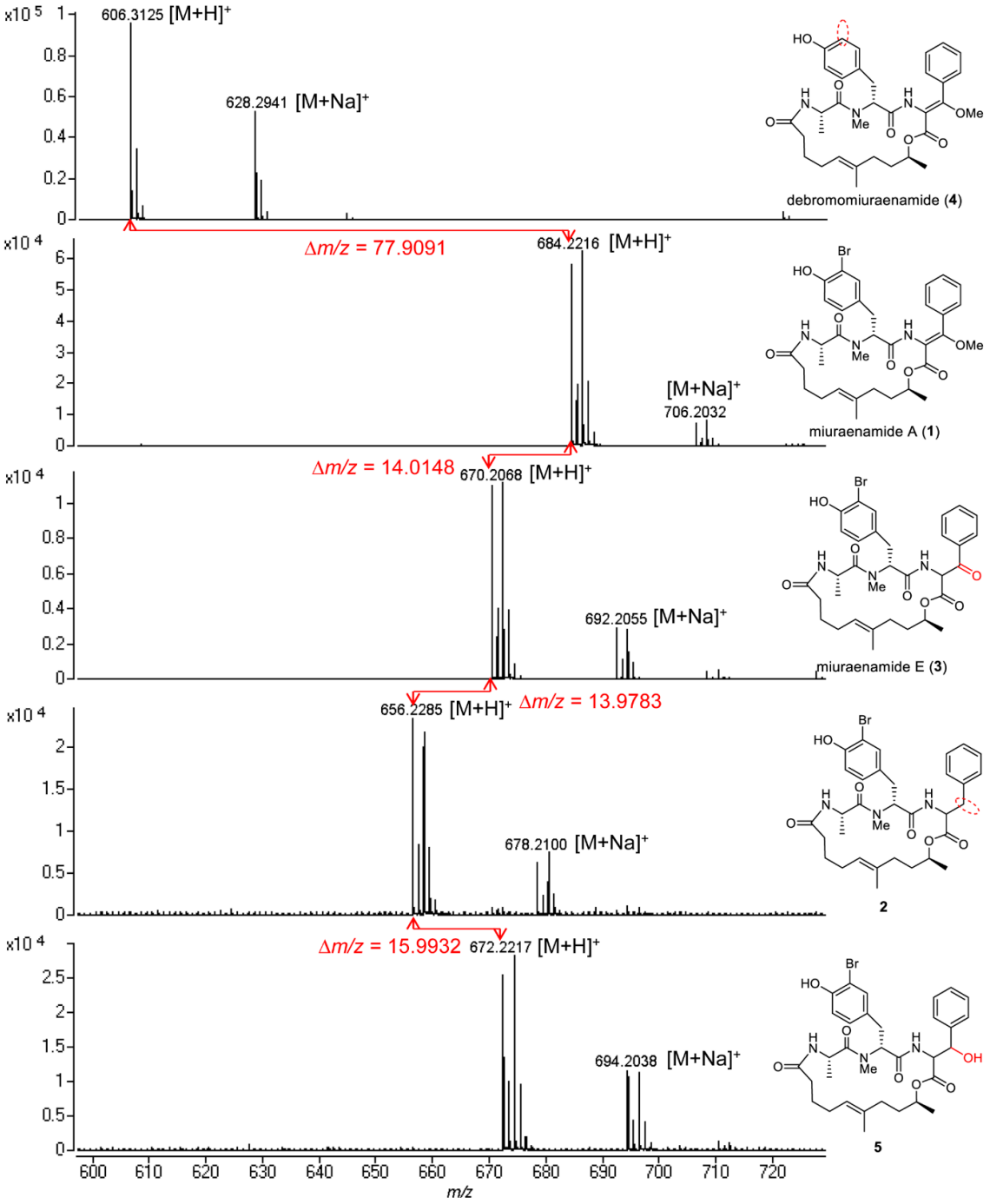

Figure S10. Mass spectra of miuraenamide A and its congeners. The structures of **2**, **4**, **5** are estimated by the molecular formulae compared with those of **1** and **3** and not verified by NMR.

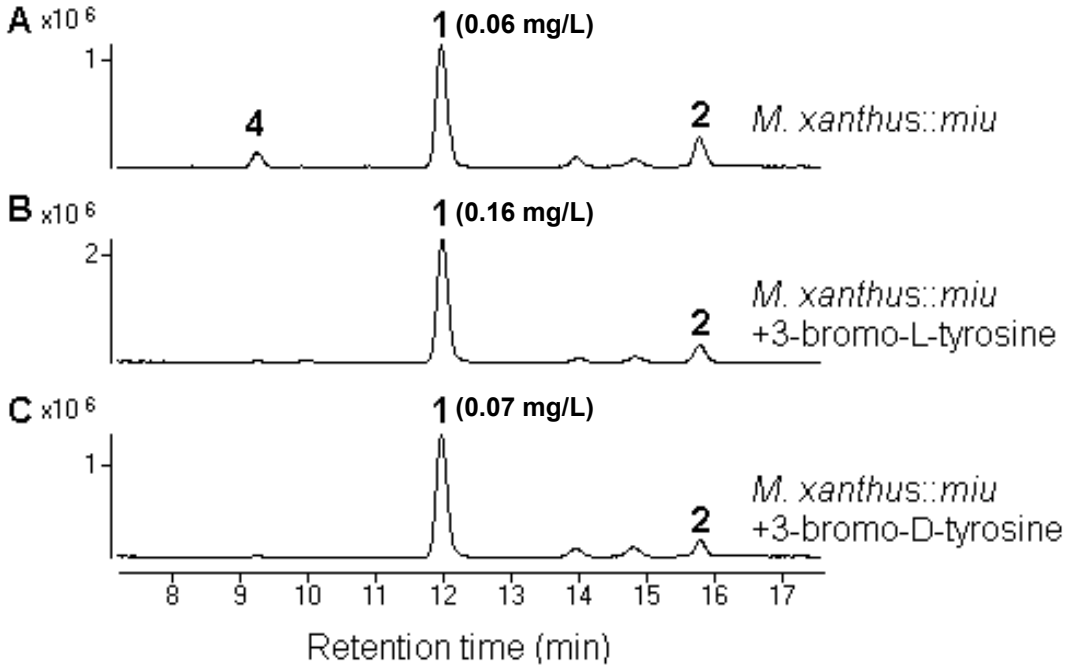

Figure S11. Production of miuraenamide A (**1**) and its congeners by *M. xanthus*::*miu* mutant fed on bromotyrosines. Extracted ion chromatographs (merged from *m/z* 684.2216, 606.3125, 656.2285, 670.2068, and 672.2217) of the extracts of the heterologous producer *M. xanthus*::*miu* (A), fed on 3-bromo-L-tyrosine (B), fed on 3-bromo-D-tyrosine (C).

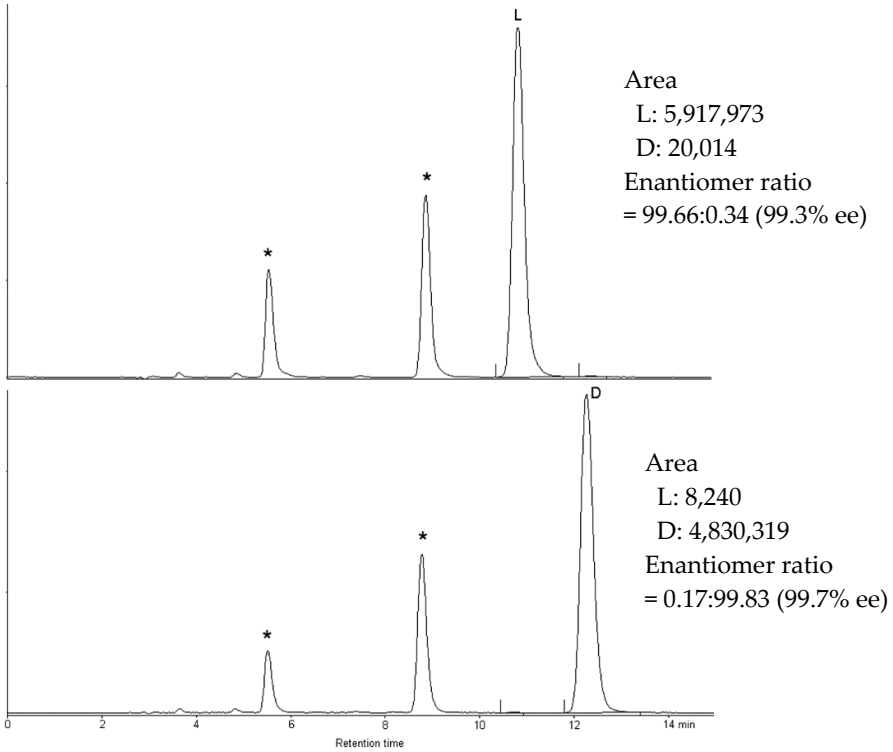

Figure S12. Enantiomeric purity of 3-bromo-D and L-tyrosines determined by Marfey's method. Column: Develosil ODS-UG-5 (4.6 x 250 mm), Solvent: 40% MeCN-0.1% TFA, flow: 1 mL/min, detection: 320 nm. * Impurities originated from Marfey's reagent (FDAA).