The biosynthetic genes encoding for the production of the dynemicin enediyne core in *Micromonospora chersina* ATCC53710

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Supplemental Data

Table S1. Bacterial strains, plasmids and primers used in this study.

Strain/Plasmid	Characteristics	Reference
E. coli		
DH5α	Host for general cloning	Invitrogen
NovaBlue	Host for general cloning	Novagen
XL1-Blue MRF'	Genomic library host	Stratagene
BW25113	Propagation strain for plasmid pIJ790 and *M. chersina* cosmids	This work
S17-1	Donor strain for conjugation between *E.coli* and *Streptomyces*	This work
M. chersina		
ATCC53710	Wild type strain, **8** producer	This work
QGD01	*dynE8* allele mutant, gene disruption, **8** non-producer	This work
QGD08	Δ*orf8* gene disruption mutant, **8** producer	This work
QGDU14	Δ*dynU14* gene disruption mutant, **8** non-producer	This work
QGDU15	Δ*dynU15* gene disruption mutant, **8** non-producer	This work
QGD23	Δ*orf23* gene disruption mutant, **8** analog producer	This work
Plasmids		This work
pEGM®-Teasy	*E. coli* subcloning vector	Promega
pOJ446	*E. coli-Streptomyces* shuttle vector, construction of genomic DNA libarary	This lab
SuperCos1	*E.coli* cloning cosmid vector, Amp^R ,	Stratagene
pJST1009	*M.chersina* ATCC53710 genomic libarary cosmid	This work
pJST1012	*M.chersina* ATCC53710 genomic libarary cosmid	This work
pJST1047	*M.chersina* ATCC53710 genomic libarary cosmid	This work
pJST1059	*M.chersina* ATCC53710 genomic libarary cosmid	This work
pJST1080	*M.chersina* ATCC53710 genomic libarary cosmid	This work
pQG9B01	A 32 kb *Bgl*II fragment from pJST1009 in superCos1, template cosmid for PCR targeting	This work
pQG59B01	A 30 kb *Bgl*II fragment from pJST1059 in superCos1, template cosmid for PCR targeting	This work
pQGD9001	*dynE8* replacement construct in which *dynE8* is replaced by *aac(3)IV* in pQG9B01	This work
pQGD9008	*orf8* replacement construct in which *orf8* is replaced by *aac(3)IV* in pQG9B01	This work
pQGD9U15	*dynU15* replacement construct in which *dynU15* is replaced by *aac(3)IV* in pQG9B01	This work
pQGD9U14	*dynU14* replacement construct in which *dynU14* is replaced by *aac(3)IV* in pQG9B01	This work
pQGD5923	*orf23* replacement construct in which *orf23* is replaced by *aac(3)IV* in pQG59B01	This work
pQGdynA	A 811 bp PCR fragment of *dynA* in pEGM-Teasy	This work
pQGdynB	A 893 bp PCR fragment of *dynB* in pEGM-Teasy	This work
pQGdynD	A 1868 bp PCR fragment of *dynD* in pEGM-Teasy	This work
Primers		
dynA- Forward (F)	5'- GCGGTGGCGGTGAGCAGCAAGTAC 3'	Enediyne
dynA- Reverse (R)	5'- GGTCGAAGCGCCAGCCGTCCAGTAC 3'	Enediyne
dynB- F	5'- TGGGCGTCCTCGTCGGACGGCAG 3'	Enediyne
dynB- R	5'- GGCCCGCCGACCACGAGGAACAG 3'	Enediyne
dynD- F	5'- GTGGCGTTCGGCTCGGTGATCG 3'	Enediyne
dynD- R	5- GACGTTCCCCACGAGGTTCGTCTC 3'	Enediyne
DYN-PKSE-gd01F	5'TGACCGCGCCCCTTCCGTACGAGCGAGGAGACCGTGATGattccggggatccgtcgacc 3'	Primer a
DYN-PKSE-gd01R	5'ACCACGTGCCGGTGGACGTAGCTGTCGGGCATCTCCTCAtgtaggctggagctgcttc 3'	Primer a
Dyn-U14-gdF	5' GGACCGGGCCGGCGCCACCCCGGCCGACTACGGCGAGTGattccggggatccgtcgacc 3'	Primer a
Dyn-U14-gdR	5' CGGACACGGTCGACCTCCTGTGGCTCGACGGGTCGGCTAtgtaggctggagctgcttc 3'	Primer a
DynU14-ID1F	5' CGCCACCCGGACCCTGAC 3'	Primer b
DynU14-ID1R	5' GCGCGCCTCGACGGTCTC 3'	Primer b
dynU14F	5' ATGCCCAGTCCCCACCGC 3'	Primer c
dynU14R	5' CTACGCGGTCGGGGTGAG 3'	Primer c
Dyn-U15-gdF	5' TGGAACTTGCGCCGGGCGCAGACCAAGGGGGAGCACATGattccggggatccgtcgacc 3'	Primer a
Dyn-U15-gdR	5' TCAGCGGTGGGGACGCGGGAAGGCGGGGACGCCCGCTCAtgtaggctggagctgcttc 3'	Primer a
dynU15-ID1F	5' GTCTCCCCCGCAGCGTAG 3'	Primer b
dynU15-ID1R	5' TCCACGGTCCGCTGGGTG 3'	Primer b
dynU15F	5' ATGACATCGACGCTCACGCG 3'	Primer c
dynU15R	5' TCAGCCGGCGACGTGCCG 3'	Primer c
Dyn08-gdF	5'TCACGGATCCGTCGGCACCAGGGAAAGGGAATGCAGATGattccggggatccgtcgacc 3'	Primer a
Dyn08-gdR	5'CGCGCGCCGCGCGAAACCGGCCCGCCGCCCGGTGGGTCATgtaggctggagctgcttc 3'	Primer a
Dyn08-ID1F	5' CCCGCCGATCGACTCCAC 3'	Primer b

Dyn08-ID1R	5’ GCGATCTGCCCGTGCATC 3’	Primer b
Dyn08F	5’ ATGCAGCGGCTCATTCGAC 3’	Primer c
Dyn08R	5’ TCAGAAGCAGGTGACCGCC 3’	Primer c
Orf23-gdF	5’ CCGAGGACTGACCGGCCGAGCCGAGGGAGGTGGCGCGTGattccggggatccgtcgacc 3’	Primer a
Orf23-gdR	5’ CAGCAGCTCGACGGACTCCATGCGTCGCTCCCCAGGTCAtgtaggctggagctgcttc 3’	Primer a
Orf23-ID1F	5’ GTGGGTGCGGCTCAACACG 3’	Primer b
Orf23-ID1R	5’ CTGCACGGCGAAGAGCAGG 3’	Primer b
Orf23F	5’ ATGAAGGCCGCGCCCTTCG 3’	Primer c
Orf23R	5’ TCAGTGTGTCGGTCGGGCC 3’	Primer c

Figure S1. Overview of the key cosmids and probes highlighted in the current study. Solid black bars indicate regions of sequenced *M. chersina* genomic DNA (deposited under GenBank accession number **EF552206**).

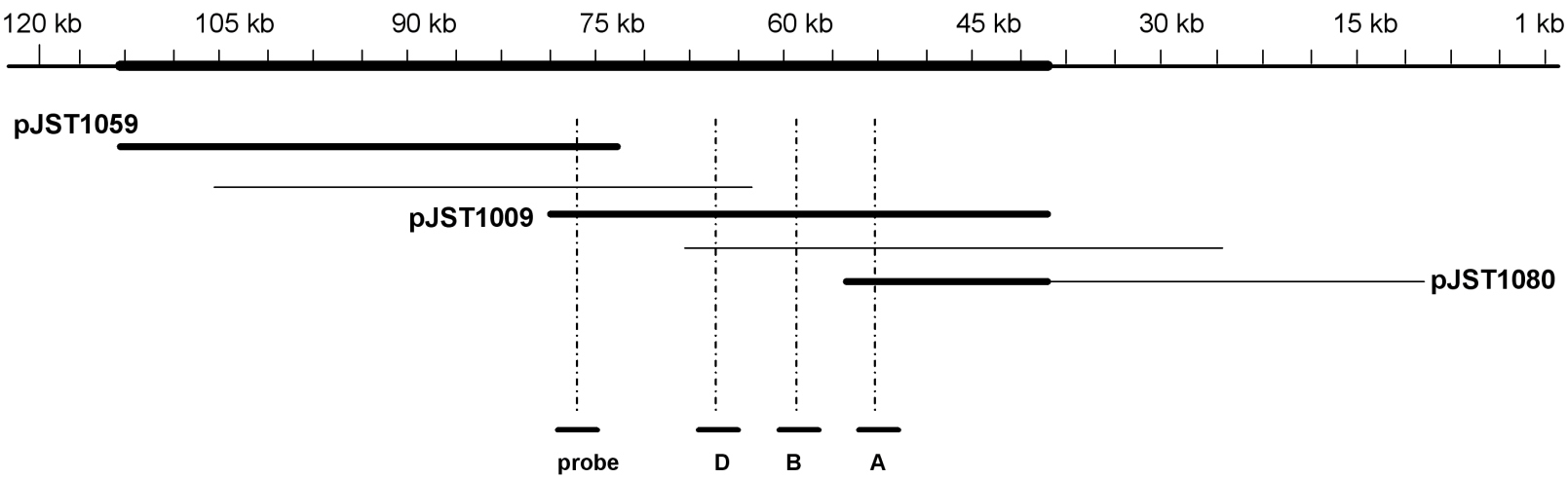
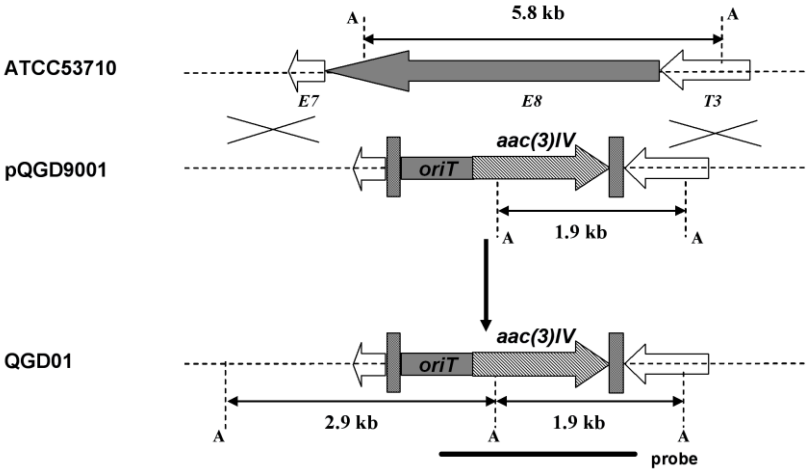


Figure S2. Inactivation of *dynE8*, *dynU14*, *dynU15*, *orf8*, and *orf23*. **(A)** Schematic overview of *dynE8* gene replacement mutant QGD01 mutant (A = *Afe*I restriction sites) (upper) and Southern analysis of wild-type and Δ*dynE8* (QGD01) *M. chersina* genomic DNA digested with *Afe*I (lower). Lane 1 - molecular weight marker (λ-*Hind*III/*Eco*RI), lanes 2 and 3 - two representative double crossover isolates, lane 4 - a representative single crossover isolate, and lane 5 - wild type. **(B)** Schematic of Δ*dynU14* -inactivation mutant strain QGDU14 (upper) and confirmation of mutant QGDU14 genotype via PCR (lower). Lane 1, 1 kb molecular weight marker; Lane 2 was amplified by primer set "a" (amplification of target disruption cassette) from mutant QGDU14; Lanes 3 and 4 amplified by set "b" (external amplification of target disruption region) from mutant QGDU14 and wild type strain respectively, and Lane 5 by "c" (internal amplification of target gene fragment) from wild type strain. **(C)** Schematic of Δ*dynU15*-inactivation mutant strain QGDU15 (upper) and confirmation of mutant QGDU15 genotype via PCR (lower). Lane 1, 1 kb molecular weight marker; Lane 2 was amplified by primer set "a" (amplification of target disruption cassette) from mutant QGDU15; Lanes 3 and 4 amplified by set "b" from mutant QGDU15 and wild type strain respectively, and Lane 5 by "c" from wild type strain. **(D)** Schematic of *Δorf8* -inactivation mutant strain QGD01 (upper) and confirmation of mutant QGD08 genotype via PCR (lower). Lane 1, 1 kb molecular weight marker; 2-4, 5-7, and 8-10, three individual double crossover isolates of QGD08; Lane 11-13, wild type. Lanes 2, 5, 8 and 11 were amplified by primer set "a"; Lanes 3, 6, 9 and 12 were amplified by primer set "b" and Lanes 4, 7, 10 and 13 were amplified by primer set "c''. **(E)** Schematic of Δ*orf23* -inactivation mutant strain QGD23 (upper) and confirmation of mutant QGD23 genotype via PCR (lower). Lane 1, 1 kb molecular weight marker; Lane 2 was amplified by primer set "a" (amplification of target disruption cassette) from mutant QGD23, Lanes 3 and 4 were amplified by primer set "b" (external amplification of target disruption region) from mutant QGD23 and wild type strain respectively, and Lane 5 was amplified by primer set "c'' (internal amplification of *orf23* fragment) from wild type.

(A)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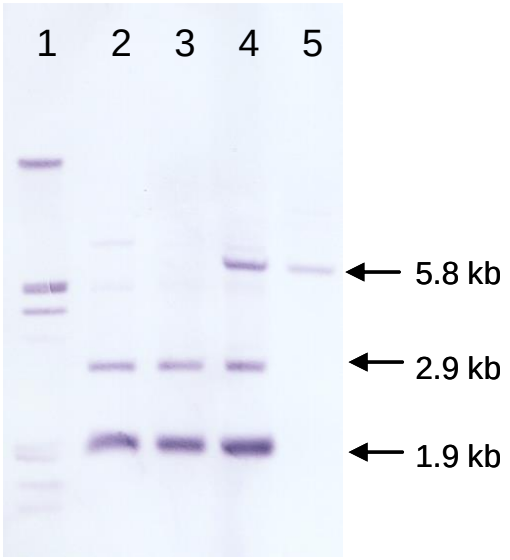
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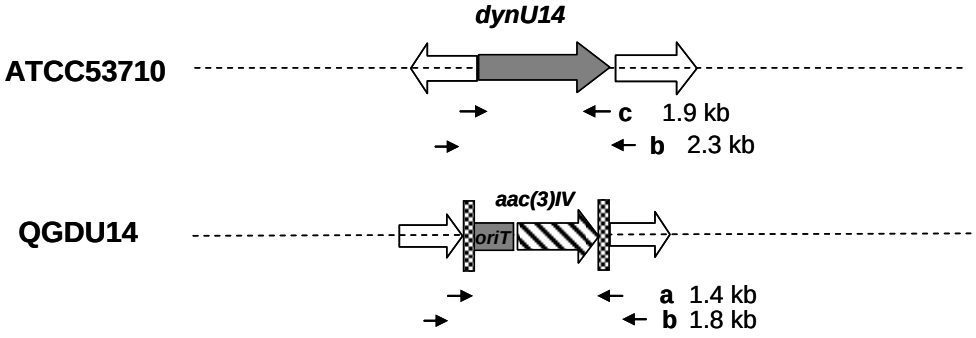
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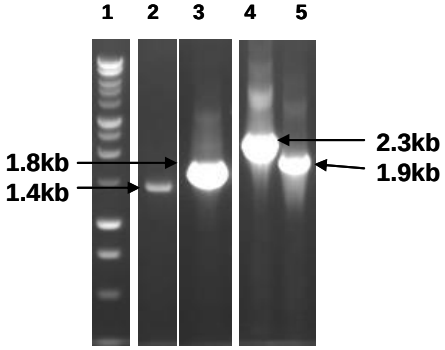
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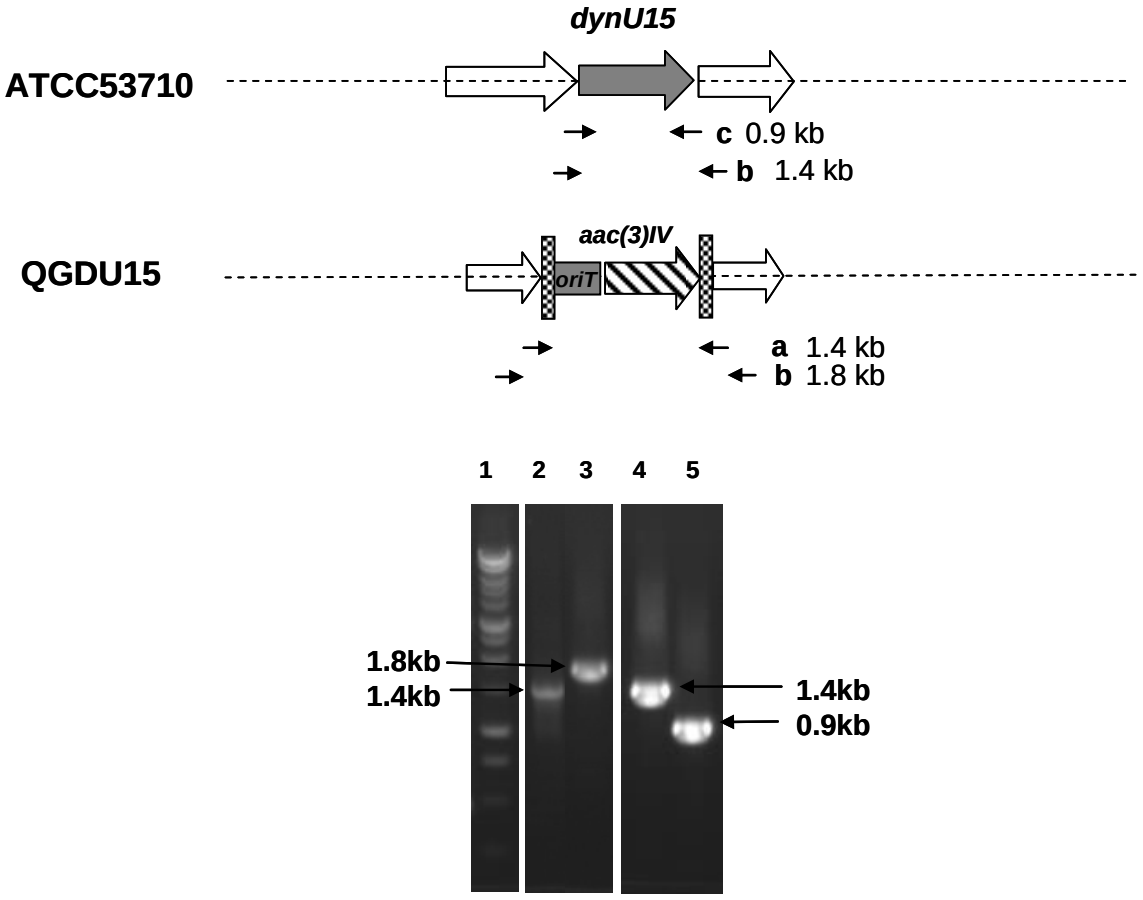
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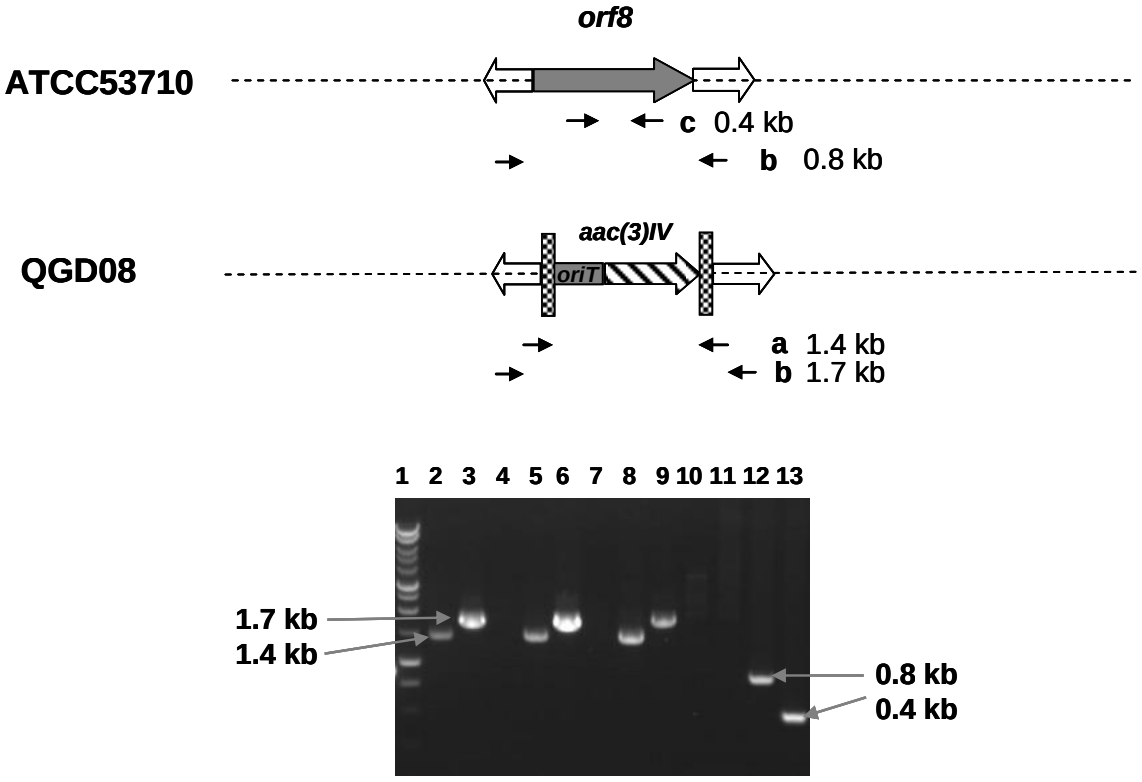
(D)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(E)

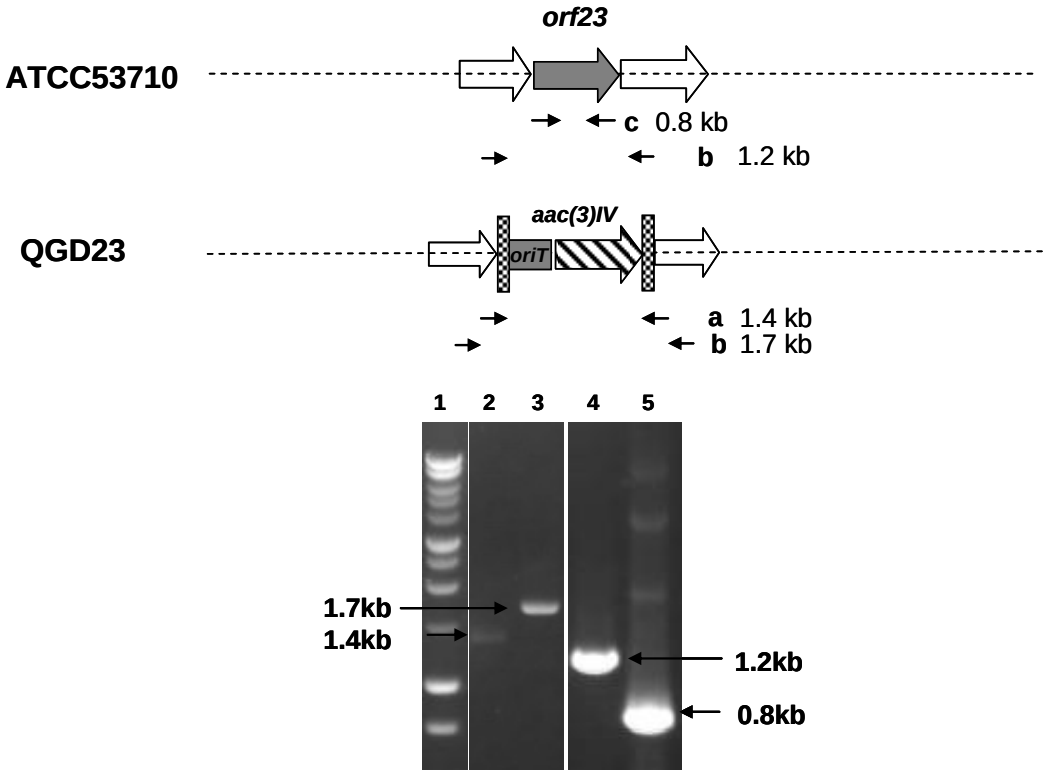
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Figure S3. HPLC analysis of fermentation extracts from wild-type, Δ*dynU14* (QGDU14), and Δ*dynU15* (QGDU15) *M. chersina* prodigy: (i) wild-type, (ii) QGDU14, (iii) QGDU15. Parameters for analytical HPLC and product characterization are described in Experimental Procedures.