Antimicrobial Agents and Chemotherapy

Supplementary Material

Function of the cytochrome P450 enzymes MycCI and MycG in *Micromonospora griseorubida* , a producer of the macrolide antibiotic mycinamicin

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Table S1. PCR primers used in this study

Primer	Sequence (5'-3')	Restricton site
DmycCIF	CTGTCTGACGATGGGCAGAAGATGGTGGTCTGGCCCATGgggtgccagggcgtgccctt	-
DmycCIR	CCAGGACTATCCGCATACCGCACCCCCATTCGTCCGTCAtgtaggctggagctgcttc	-
DmycGF	TAGATATGAGGAGGGCTTTCTGGCAGCCTCGGTCGGGTGgggtgccagggcgtgccctt	-
DmycGR1	ACGTCACATCCTCCACCGCGTAGCGCGGGAACGCCGTTCtgtaggctggagctgcttc	-
DmycGR2	GCGGTCGGGTCGATCCGGCCCGACCACCGGCGCCTGTCAtgtaggctggagctgcttc	-
RecognitionDmycGF	CGGAGGAGAGTCGGGAGGTC	-
RecognitionDmycGR1	CGAACCCGAGATGCTGATTG	-
RecognitionDmycGR2	ACGAGATCGTCGAGATCGAC	-
mycCIproF	GCTGCATGCCAGAAATACCTCCTTGCCTGGTG	*Sph* I
mycCIIR	TGACAAGCTTACTCCTGTTGGCCCACCTGTCCCGTG	*Hin* dIII
myrBpF	GTAAGCTTAACGCTCGGTGATCGGGGTG	*Hin* dIII
myrBpR	GCAAGCTTGATGACATATGTTTCCTTGGGTTC	*Hin* dIII, *Nde* I
myrBpF3	TGAAGCAGCAGGCCCGCCAGTTCCTTC	-
myrBpartialF	ACATGCTCCGGTACGACTTC	-
mycBpR1	CGACCGAGGCTGCCAGAAAG	-
myrBmycGR1	GGCTCGGTCTCCTGCAACTC	-
RecognitionDmycCIF	ACTTCCCGTCAGAATTCCTG	-
RecognitionDmycCIR	CAGCAGTTCCGGTGTCACTG	-
mycGpartialF	GTGCTCGGTGACGGACGCTTC	-
mycGpartialR	CCAGTGCCACCTGGAGTTCCAC	-
mycCIpartialF	TCAGCAGATACGAGCACGTC	-
mycCIpartialR	CGAGGACAGCAGAATTATCAG	-
152attPF	AATCGCTCTTCGTTCGTCTG	-
152intR	AATGCCCGACGAACCTGAAC	-
MGneo860F	TCTCCTGTCATCTCACCTTG	-
MGneo630R	TCATAGCCGAATAGCCTCTC	-

The unique priming sites of disruption cassettes are shown in lower case. The relevant restriction sites for genetic manipulation are underlined.

Table S2. ^{13}C- and ^{1}H-NMR data of F-1 (14-hydroxy-M-III, M-IX), and comparison with ^{13}C-NMR data of mycinamicins.

Position	M-I[a]	M-III[b]	M-V[a]	M-IX[a]	F-1 (14-hydroxy-M-III, M-IX)				
	^{13}C (ppm)	^{13}C (ppm)	^{13}C (ppm)	^{13}C (ppm)		^{13}C (ppm)	^{1}H (ppm)	Multiplicity	Coupling (Hz)
1	166.7	166.1	166.3	166.4	C	166.4	-	-	-
2	120.1	120.9	120.7	121.1	CH	120.8	5.83	d	15.4
3	151.5	151.7	151.8	151.9	CH	152.0	6.60	dd	9.8,15.5
4	41.9	41.3	41.3	41.3	CH	41.3	2.71	m	-
5	87.5	87.8	87.7	87.9	CH	87.9	3.26[c]	-	-
6	34.2	34.1	34.1	34.4	CH	34.1	1.22[c]	-	-
7	32.1	32.6	32.7	33.2	CH_2	32.7	1.52	dd	3.4,14.0
							1.57[c]	-	-
8	44.7	44.9	44.8	44.8	CH	44.9	2.54[c]	-	-
9	200.8	203.7	203.8	203.4	C	203.9	-	-	-
10	125.6	123.2	123.8	124.6	CH	124.0	6.27	d	14.9
11	143.7	141.8	141.4	141.3	CH	141.4	7.14	dd	11.2,15.1
12	59.6	133.0	130.5	130.6	CH	130.8	6.47	dd	11.1,15.0
13	59.0	141.3	143.5	143.3	CH	143.4	6.02	d	15.1
14	47.5	49.2	77.4	77.6	CH	77.6	-	-	-
15	72.4	73.7	75.8	76.1	CH	75.7	4.81	dd	2.2,11.0
16	24.7	25.2	21.4	21.6	CH_2	21.5	1.56[c]	-	-
							1.84	m	-
17	8.9	9.7	10.4	10.4	CH_3	10.4	0.91	t	7.5
18	18.9	19.4	19.5	19.5	CH_3	19.6	1.23	d	6.6
19	17.1	17.4	17.4	17.4	CH_3	17.4	0.99	d	6.6
20	17.5	17.7	17.6	17.6	CH_3	17.6	1.14	d	6.9
21	67.1	68.7	75.2	74.7	CH_2	75.6	3.77	d	11.0
							3.88	d	10.0
1'	105.5	104.9	104.8	105.2	CH	104.9	4.24	d	7.3
2'	70.3	70.4	70.4	70.3	CH	69.3	3.49[c]	-	-
3'	65.8	65.8	65.8	66.2	CH	66.0	2.55[c]	-	-
4'	28.5	28.4	28.4	28.9	CH_2	28.7	1.26[c]	-	-
							1.69	dd	1.7,12.7
5'	69.4	69.5	69.4	69.5	CH	69.1	4.21	t	3.1
6'	21.2	21.2	21.2	21.2	CH_3	21.1	1.24	d	6.0
NMe_2	40.1	40.2	40.2	40.4	CH_3	40.3	2.34	s	-
1"	100.9	100.8	101.6	101.4	CH	101.5	4.57	d	7.8
2"	81.9	80.1	81.7	80.3	CH	79.9	3.02	dd	3.0,7.8
3"	79.3	69.8	79.2	70.9	CH	70.4	3.28[c]		-
4"	72.7	72.8	72.5	72.9	CH	72.5	3.21	dd	3.1,9.5
5"	70.6	69.8	70.7	70.9	CH	70.2	3.69	m	-
6"	17.8	17.7	17.6	17.6	CH_3	17.5	1.30	d	6.2
2"-OCH_3	59.0	59.4	59.1	58.9	CH_3	58.9	3.50	s	-
3"-OCH_3	61.6	-	61.7	-	-	-	-	-	-

[a] Kinoshita, K., S. Takenaka, H. Suzuki, T. Morohoshi, and M. Hayashi. 1992. Mycinamicins, new macrolide antibiotics. XIII. Isolation and structures of novel fermentation products from *Micromonospora griseorubida* (FERM BP-705). J. Antibiot. 45:1-9.

[b] Hayashi, M., M. Ohno, K. Kinoshita, S. Satoi, M. Suzuki, and K. Harada. 1981. Mycinamicins, new macrolide antibiotics. III Isolation and structures of mycinamicin aglycones, mycinolide IV and V. J. Antibiot. 34:346-349.

[c] Overlapping

Table S3. ^{13}C- and ^{1}H-NMR data of F-2 (12,13-epoxy-M-III), and comparison with ^{13}C-NMR data of mycinamicins.

	M-I[a]	M-III[a]	F-2 (12,13-epoxy-M-III)				
	^{13}C (ppm)	^{13}C (ppm)		^{13}C (ppm)	^{1}H (ppm)	Multiplicity	Coupling (Hz)
1	166.7	166.1	C	165.7	-	-	-
2	120.1	120.9	CH	120.5	5.83	d	
3	151.5	151.7	CH	151.0	6.59	dd	
4	41.9	41.3	CH	41.7	2.72	m	
5	87.5	87.8	CH	87.6	3.32[b]	-	-
6	34.2	34.1	CH	34.2	1.18[b]	-	
7	21.1	32.6	CH_2	32.0	1.47[b]	-	-
					1.62[b]	-	-
8	44.7	44.9	CH	44.6	2.56	m	
9	200.8	203.7	C	200.7	-	-	-
10	125.6	123.2	CH	125.6	6.56	d	
11	143.7	141.8	CH	144.0	6.40	dd	
12	59.6	133.0	CH	59.0	3.25[b]	-	-
13	59.0	141.3	CH	59.0	3.09	dd	
14	47.5	49.2	CH	47.4	1.40	m	
15	72.4	73.7	CH	72.5	5.32	m	
16	24.7	25.2	CH_2	24.8	1.54	m	
					1.86	qd	
17	8.9	9.7	CH_3	8.9	0.88	dd	
18	18.9	19.4	CH_3	19.0	1.21	d	
19	17.1	17.4	CH_3	17.0	0.96	d	
20	17.5	17.7	CH_3	17.5	1.15	d	
21	67.1	68.7	CH_2	67.4	3.60[b]	-	
					4.18	dd	
1'	105.5	104.9	CH	103.6	4.35	d	
2'	70.3	70.4	CH	69.6	3.52	m	
3'	65.8	65.8	CH	66.7	3.30	m	
4'	28.5	28.4	CH_2	31.2	1.47	m	
					1.99	m	
5'	69.4	69.5	CH	67.9	3.57[b]	-	-
6'	21.2	21.2	CH_3	20.8	1.30	d	
7'	40.1	40.2	CH_3	39.0	2.85	br s	
8'	40.1	40.2	CH_3	41.5	2.85	br s	
1"	100.9	100.8	CH	100.7	4.55	d	
2"	81.9	80.1	CH	80.1	3.06	dd	
3"	79.3	69.8	CH	69.5	4.20	dd	
4"	72.7	72.8	CH	72.9	3.24[b]	-	-
5"	70.6	69.8	CH	70.0	3.65	dd	
6"	17.8	17.7	CH_3	17.6	1.30	d	-
2"-OCH_3	59.0	59.4	CH_3	59.3	3.57	s	
3"-OCH_3	61.6	-	-	-	-	-	-

[a] Kinoshita, K., S. Takenaka, H. Suzuki, T. Morohoshi, and M. Hayashi. 1992. Mycinamicins, new macrolide antibiotics. XIII. Isolation and structures of novel fermentation products from *Micromonospora griseorubida* (FERM BP-705). J. Antibiot. 45:1-9.

[b] Overlapping

Table S4. Antibacterial activities of mycinamicins

Microorganism	MIC (μg/ml)			
	14-hydroxy-M-III (M-IX, F-1)	12,13-epoxyl-M-III (F-2)	M-III	M-IV
S. aureus ATCC 25923	0.78	0.20	0.10	0.10
M. luteus ATCC 9341	0.10	0.05	0.02	0.02
E. coli ATCC 25922	100	100	>100	100

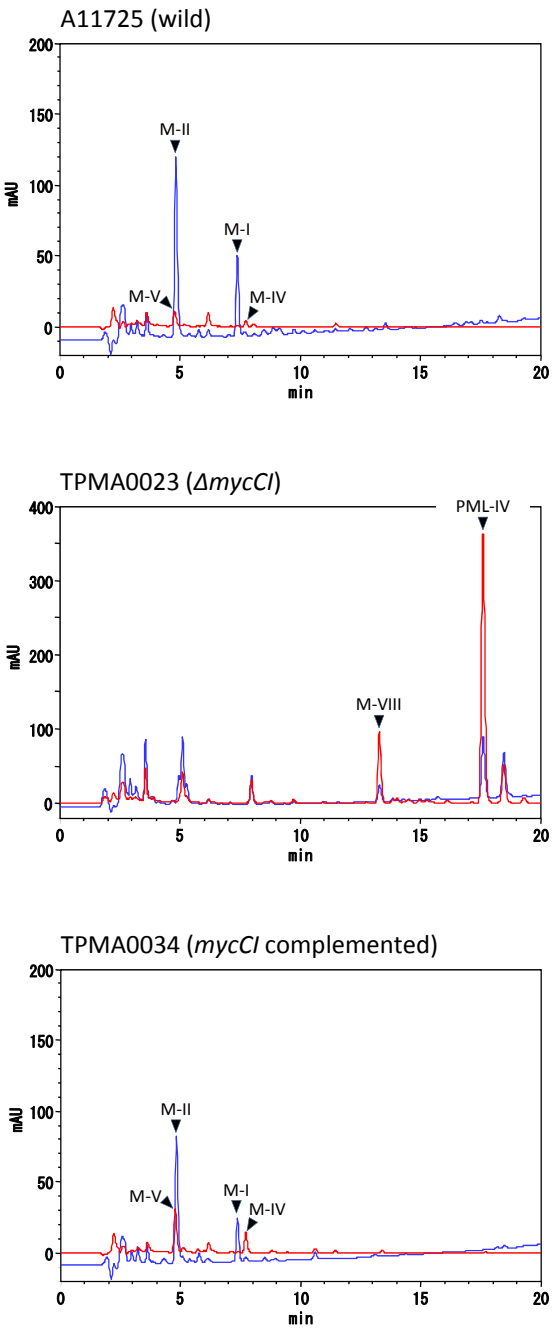

Figure S1. HPLC chromatograms of the EtOAc extract obtained from the culture broth of *M. griseorubida* A11725 (wild), TPMA0023 (*ΔmycCI*), and TPMA0034 (*mycCI* complemented). The EtOAc extract from the culture broth of TPMA0023 was not treated with 0.1% TFA. HPLC conditions: column, ODS-80T_M (Tosoh); mobile phase, a 20-min. linear gradient from 35% MeCN to 70% MeCN containing 0.1% TFA; flow rate, 0.8 mL/min; UV wavelength, 200–300 nm. Blue line, at 240 nm; red line, at 280 nm.

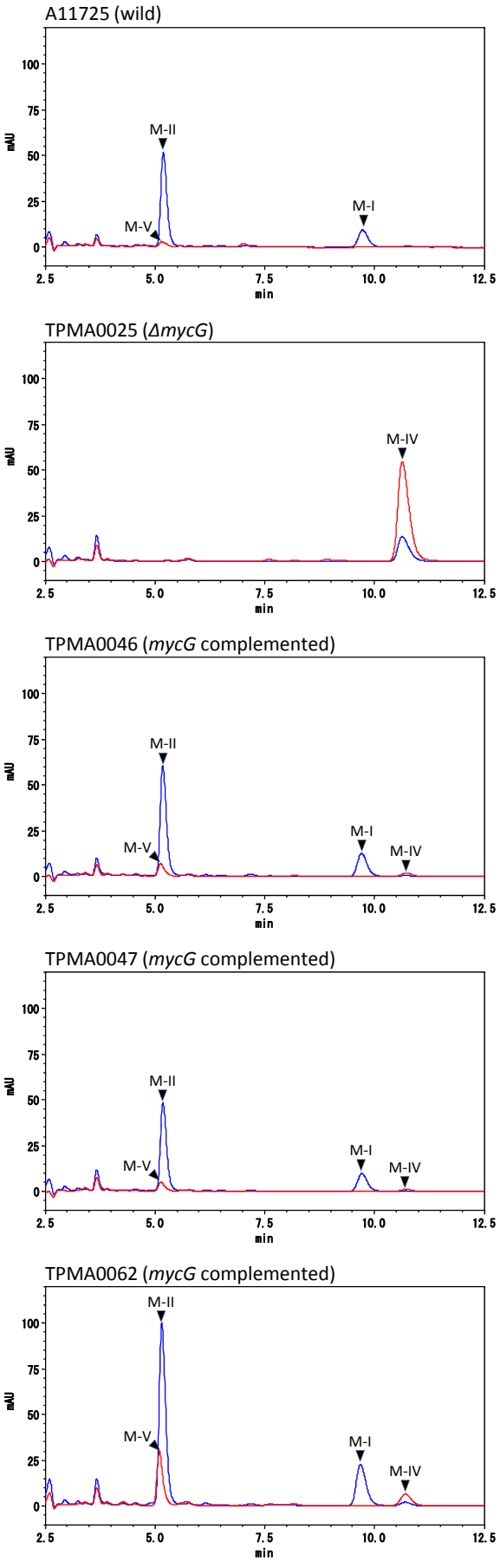

Figure S2. HPLC chromatograms of the EtOAc extract obtained from the culture broth of wild strain *M. griseorubida* A11725, *mycG* disruption mutant TPMA0025, and *mycG* complementation strains TPMA0034, TPMA0047, and TPMA0062. HPLC conditions: column, ODS-80T_M (Tosoh); mobile phase, MeCN, 0.06% TFA (35:65); flow rate, 0.8 mL/min; UV wavelength, 200–300 nm. Blue line, at 240 nm; Red line, at 280 nm.