

Supplementary Material

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References

Table S1. Genes and proteins involved in mycemycin biosynthesis and their putative functions in *S. sp.* FXJ1.235.

Gene	Size (AA)	Protein Homolog and origin (identity/similarity)	Proposed function
<i>orf-1</i>	47	None predicted in NCBI	Unknown
<i>myeQ</i>	394	KynU, NP_250770.1(47/62); <i>Pseudomonas aeruginosa</i> PAO1	Kynureninase
<i>myeC</i>	281	NP_627840.1(86/92); <i>Streptomyces coelicolor</i> A3(2)	Tryptophan 2, 3-dioxygenase
<i>orf-2</i>	139	WP_089100086.1(79/86); <i>Streptomyces hyaluromycini</i>	DUF3151 domain-containing protein
<i>orf-3</i>	489	WP_059203142.1(88/92); <i>Streptomyces griseoruber</i>	MFS transporter
<i>orf-4</i>	27	None predicted in NCBI	Unknown
<i>myeR4</i>	205	SBU95446.1(82/89); <i>Streptomyces</i> sp. OspMP-M45	LuxR family transcriptional regulator
<i>myeJ</i>	280	SCE38941.1(73/80); <i>Streptomyces</i> sp. PpaLS-921	SAM-dependent methyltransferase
<i>myeR1</i>	334	SBU95411.1(88/92); <i>Streptomyces</i> sp. OspMP-M45	Lrp/AsnC family transcriptional regulator
<i>myeK</i>	509	CB02009_orf6, OKJ63402.1(76/84); <i>Streptomyces</i> sp. CB02009	Mltidrug MFS transporter
<i>myeR3</i>	676	SBU95407.1(79/84); <i>Streptomyces</i> sp. OspMP-M45	SARP family transcriptional regulator
<i>myeR5</i>	226	SBU95417.1(76/83); <i>Streptomyces</i> sp. OspMP-M45	TetR family transcriptional regulator
<i>myeP</i>	511	RebH, 4LU6_A (60/72); <i>Lechevalieria aerocolonigenes</i>	Tryptophan halogenase
<i>myeO</i>	181	KtzS, ABV56599.1(58/70); <i>Kutzneria</i> sp. 744	Flavin reductase
<i>myeN</i>	221	SDU28343.1(51/64); <i>Amycolatopsis keratiniphila</i>	Sodium/hydrogen exchanger family
<i>myeA</i>	456	SsfH, ADE34507.1(73/83); <i>Streptomyces</i> sp. SF2575	Salicylate synthase
<i>myeG</i>	350	BomK, ALE27503.1 (57/72); <i>Streptomyces</i> sp. NRRL 12068	Beta-ketoacyl-ACP synthase (amide bond formation)
<i>myeF</i>	521	PchD, NP_252918.1(49/62); <i>Pseudomonas aeruginosa</i> PAO1	2,3-dihydroxybenzoate-AMP ligase
<i>myeE</i>	94	EsmD3, AFB35628.1(55/74); <i>Streptomyces antibioticus</i>	Phosphopantetheine-binding protein
<i>myeD</i>	518	KDQ70109.1(84/89); <i>Streptomyces</i> sp. NTK 937	Amidohydrolase
<i>orf-5</i>	410	WP_078569189.1(81/86); <i>Streptomyces</i>	Phospho-2-dehydro-3-

		sp. NTK 937	deoxyheptonate aldolase
<i>orf-6</i>	256	KFG06189.1(69/81); <i>Streptomyces scabiei</i>	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase

Table S2. Strains used in this study.

Strain	Characteristic	Reference
<i>Escherichia coli</i>		
ETZ12567	<i>E. coli</i> host for conjugation	[1]
Top 10	<i>E. coli</i> host for cloning	Invitrogen
Rosetta(DE3)	<i>E. coli</i> host for heterologous expression	Invitrogen
Rosetta(DE3)/ <i>mymC</i>	<i>E.coli</i> host for MymC heterologous expression	This study
<i>Streptomyces olivaceus</i> FXJ8.012Δ1741		
	Mycemycin C-E producing strain	[2]
<i>Streptomyces</i> sp. FXJ1.235		
FXJ1.235	Mycemycin A-B producing strain	[3]
FXJ1.235Δ <i>myeP</i>	Δ <i>myeP</i> :: <i>neo</i>	This study
FXJ1.235Δ <i>myeO</i>	Δ <i>myeO</i>	This study
FXJ1.235Δ <i>myeG</i>	Δ <i>myeG</i> :: <i>neo</i>	This study
FXJ1.235Δ <i>myeD</i>	Δ <i>myeD</i>	This study

Table S3. Plasmids used in this study.

Plasmid	Characteristic	Reference
pET28a	Vector for protein expression, Kan ^R	Invitrogen
pET28a:: <i>mymC</i>	Vector for MymC heterologous expression, Kan ^R	
pUC119:: <i>neo</i>	Source of <i>neo</i> (kan ^R)	[4]
pKC1139	<i>E. coli-Streptomyces</i> shuttle vector, Apr ^R	[5]
pKC1139:: <i>myeP</i> :: <i>neo</i>	<i>MyeP</i> deletion construct, Apr ^R , Kan ^R	This study
pKC1139:: <i>myeO</i>	<i>MyeO</i> deletion construct, Apr ^R	This study
pKC1139:: <i>myeG</i> :: <i>neo</i>	<i>MyeG</i> deletion construct, Apr ^R , Kan ^R	This study
pKC1139:: <i>myeD</i>	<i>MyeD</i> deletion construct, Apr ^R	This study

Apr, apramycin; Kan, kanamycin.

Table S4. Primers used in this study (restriction sites or termini overlaps used are underlined; protective nucleotides are in italics).

Primer	Sequence
Primers for MymC heterologous expression in <i>E. coli</i>	
<i>mymC</i> -EF	<u>GGAATTC</u> GTGGCACGGCCGGACCGGGACGG
<i>mymC</i> -ER	CCCAAGCTTCTACAGGCGGGTGCGGGCGGCC
Primers for mutant construction and confirmation in FXJ1.235	
<i>myeP</i> -LF	<u>GATCCGCGGCCGCGCGCGAT</u> GGACAGGATGGACTGGAAGACG
<i>myeP</i> -LR	<u>GGTATCCAGGGGATAGATCT</u> TACATCTTGCGGACGGTGTCTATA
<i>myeP</i> -RF	<u>CTGGGGTTCGGGTAAGATCT</u> GCCCTCCACGTACGACATCCTG
<i>myeP</i> -RR	<u>GACATGATTACGAATTCGAT</u> GACTCGTTGCTGCGGATGCTG
<i>ΔmyeP</i> -F	CACCACCGACGGTTTCCTCT
<i>ΔmyeP</i> -R	CCATCAACTGCCGCATCCC
<i>myeO</i> -LF	<u>GATCCGCGGCCGCGCGGAT</u> TGTCCCGTACCAACGCACCACACG
<i>myeO</i> -LR	<u>CGTAGAGCAGAGGGGCGGGGCCATCAACTGCCGCATCCCGTCG</u>
<i>myeO</i> -RF	<u>CGACGGGATGCGGCAGTTGATGG</u> CCCCCGCCCCTCTGCTCTACG
<i>myeO</i> -RR	<u>GACATGATTACGAATTCGAT</u> CCTGGAAGTGGACGCTGCTGTG
<i>ΔmyeO</i> -F	TTCGACTTCACGGTGGACTTCATCC
<i>ΔmyeO</i> -R	ACAGCCACGAACGCCAGTCGC
<i>myeG</i> -LF	<u>GATCCGCGGCCGCGCGGAT</u> CGTGGTCGCGCGGAAGCAC
<i>myeG</i> -LR	<u>GGTATCCAGGGGATAGATCT</u> CGAGATGGATTCCGTCGACCTTCA
<i>myeG</i> -RF	<u>CTGGGGTTCGGGTAAGATCT</u> CCGAGAAGCGGGCTGAGCGATA
<i>myeG</i> -RR	<u>GACATGATTACGAATTCGAT</u> CAGGTCGTGCCAGTGGTTGTTGA
<i>ΔmyeG</i> -F	ACAGCATCCGCAGCAACGAGTC
<i>ΔmyeG</i> -R	ACAGGGCGAAGCAGACGATGA
<i>myeD</i> -LF	<u>GATCCGCGGCCGCGCGGAT</u> GAGAAGCGGGGCATCTGGGAG
<i>myeD</i> -LR	<u>GGTATCCAGGGGATAGATCT</u> GTCCGCCGTACAGATGTGTCCG
<i>myeD</i> -RF	<u>CTGGGGTTCGGGTAAGATCT</u> CCGCCCTCTTCGACGACAACCC
<i>myeD</i> -RR	<u>GACATGATTACGAATTCGAT</u> ACGGGACGGGCTCACGGACCA
<i>ΔmyeD</i> -F	GATGATGCGGCTGGTCAACAAG
<i>ΔmyeD</i> -R	GGAGCGGGGTTTGCGGAAC
<i>neo-BglII</i> -F	<u>GAAGATCT</u> ATCCCCTGGATACCGCTCGCCGCAG
<i>neo-BglII</i> -R	<u>GAAGATCT</u> TACCCGAACCCAGAGTCCCG
T7	TAATACGACTCACTATAGGG
T7ter	GCTAGTTATTGCTCAGCGG

Figure S1. HR-ESI-MS spectra of mycemycins A (a) and mycemycin B (b).

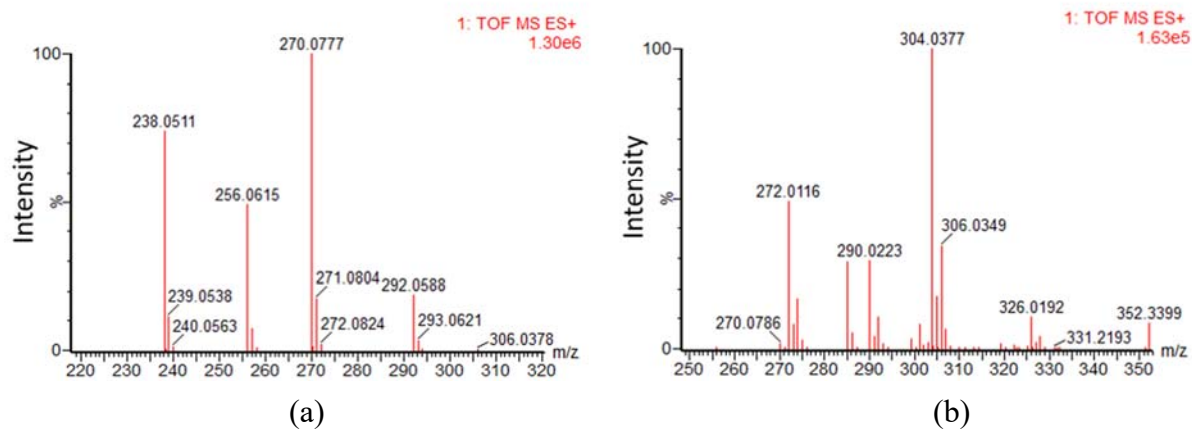


Figure S2. UV spectrum of 5-Cl-anthranilic acid.

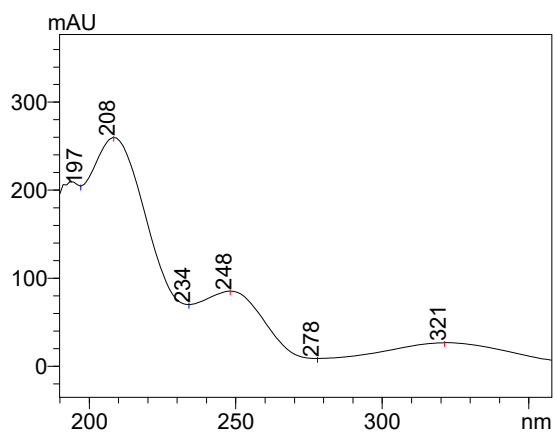
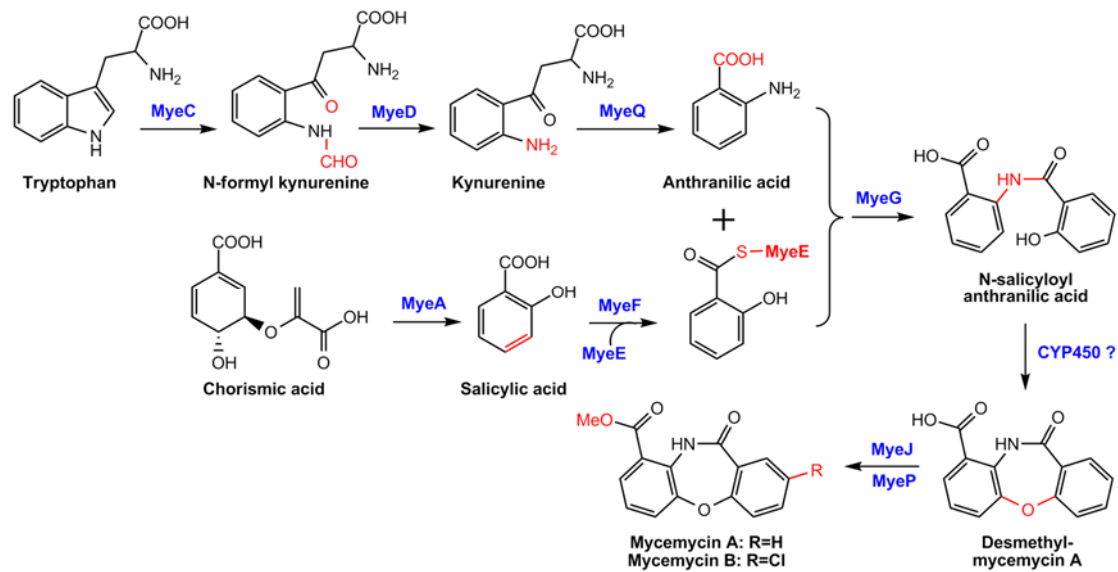


Figure S3. Proposed pathway for the biosynthesis of mycemycins A and B in *S. sp.* FXJ1.235.



References

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