Characterization of FdmV as an amide synthetase for fredericamycin A biosynthesis in *Streptomyces griseus* ATCC 43944

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Running title: FdmV as an amide synthetase

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SUPPLEMENTAL DATA

TABLE S1. ^{1}H NMR and gCOSY data of FDM C in d_6-DMSO.

Position	FDM C in d_6-DMSO (500 Hz)	
	δ_H (J (Hz))	gCOSY(H→H)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
OCH_3-11	3.94 (3H, s)	
12	6.93 (1H, s)	
13		
14		
15		
16		
17		
18		
19	2.80 (2H, br)	20
20	2.69 (2H, br)	19
21		
22	6.75 (1H, s)	
23		
24	4.25 (2H, s)	
25		
26	6.17 (1H, d, 15.5)	27
27	7.24 (1H, dd, 15.5, 9.0)	26, 28
28	6.31 (1H, m)	27
29	6.32 (1H, m)	30
30	1.85 (3H, d, 4.5)	29
COOH-1	-[a]	
OH-3	12.69 (1H, s)[b]	
OH-6	12.85 (1H, s)[b]	
OH-10	-[b]	
OH-13	-[b]	
OH-17	-[b]	

[a] not observed

[b] interchangeable.

FIGURE S1. Construction and confirmation of the *ΔfdmV* mutant strain SB4027. (A) Inactivation of *fdmV* by replacing an internal fragment with the *aac(3)IV* Am resistance gene as in pBS4059 and construction of the *ΔfdmV* mutant strain SB4027 via a double crossover homologous recombination event. B, *Bgl*II; Bm, *Bam*HI; M, *Mlu*I; P, *Pst*I; S, *Sph*I. (B) Confirmation of the genotype of the *ΔfdmV* mutant strain SB4027 by Southern analysis of genomic DNAs digested with *Mlu*I and *Bam*HI with the 0.8-kb *Bgl*II-*Sph*I fragment containing *fdmU* as a probe. Lane 1, *S. griseus* wild-type; lane 2, recombinant strain resulting from a single crossover event between the wild-type and pBS4059; lane 3, the desired *ΔfdmV* mutant strain SB4027 as predicted from a double crossover event between the wild-type and pBS4059.

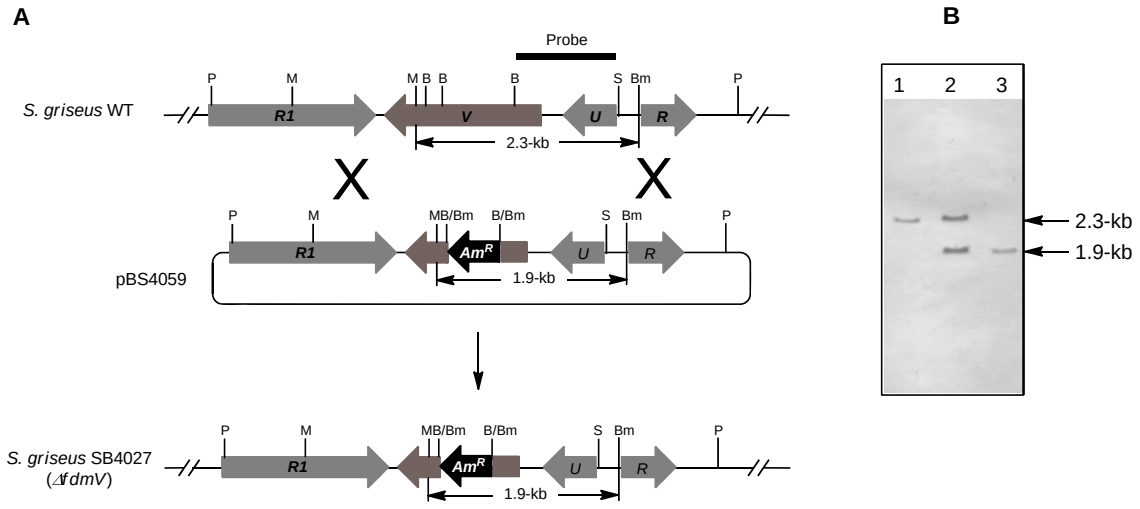

FIGURE S2. Amino acid sequence comparison of FdmV with selected homologs and AS B. The residues conserved in AS B active sites are marked with red asterisks. Accession numbers for each of the enzymes are: FdmV, AAQ08933; LlpA, CAM34350; PdmN, ABK58686; GrhP, AAM33677; RubR, AAM97368; OxyD, AAZ78328; TcsG, BAB12569; Ant-Orf1, ABW71832; PhzH, AAF17502; AS B, NP_415200.

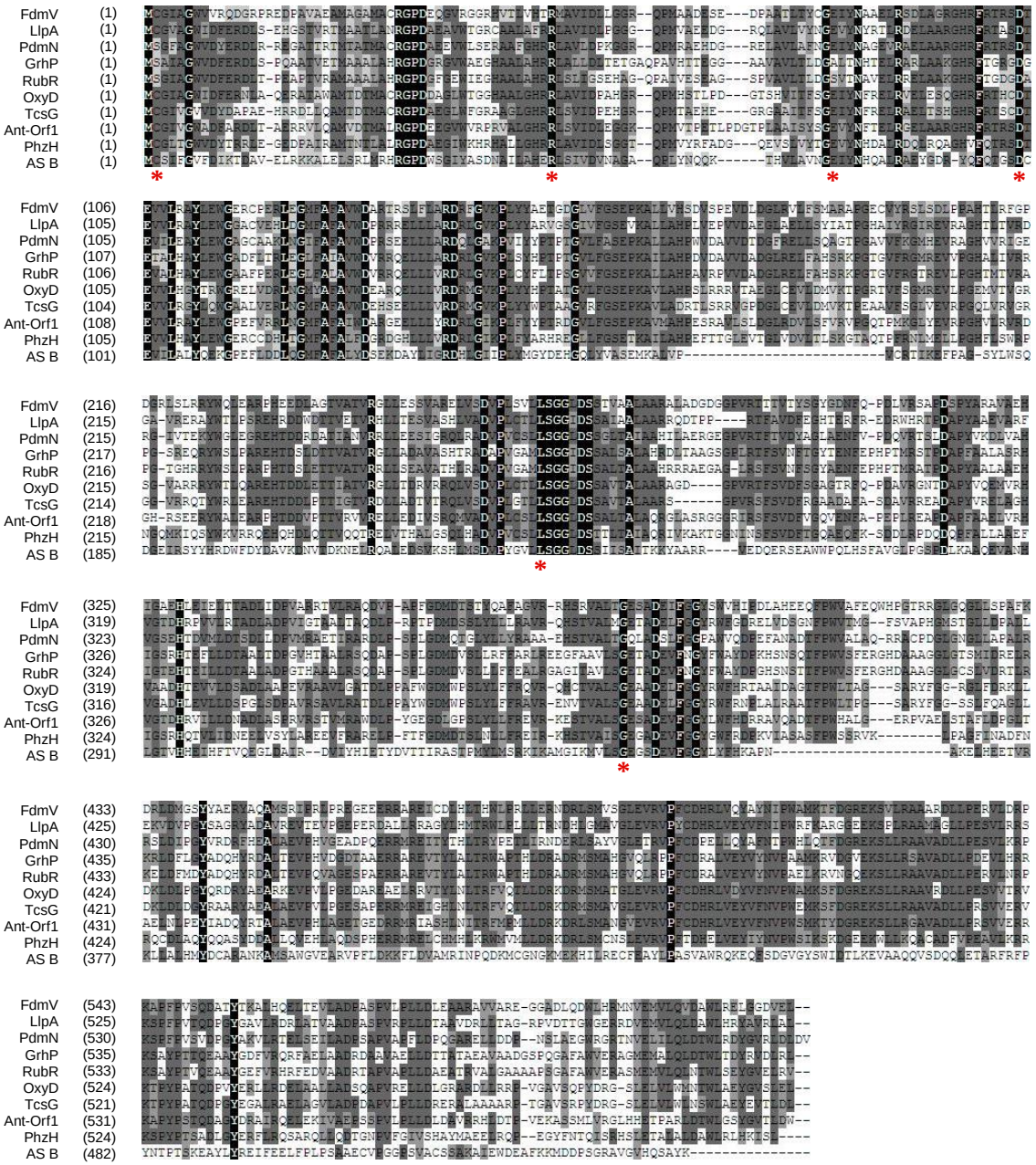

FIGURE S3. Optimization of FdmV assay conditions with varying (A) pH (100 mM Tris-HCl) and (B) DMSO concentration.

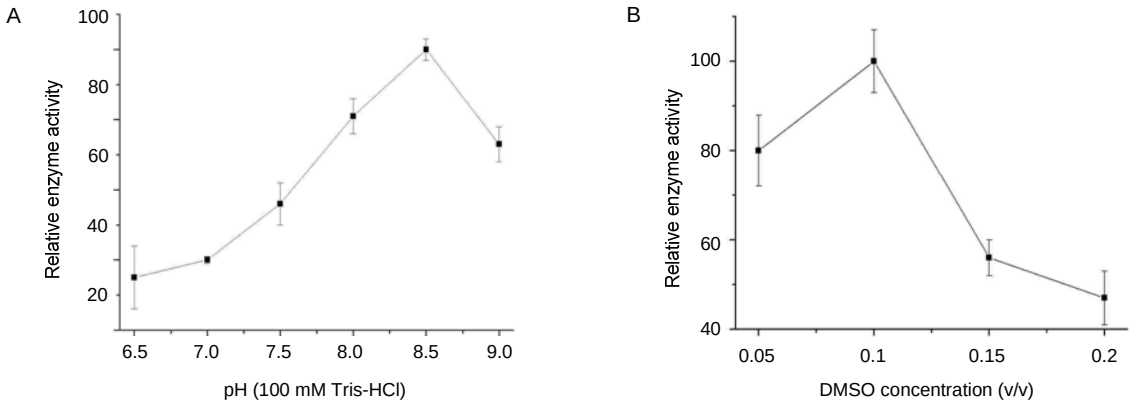

FIGURE S4. Structures of benzoic acids tested as substrate analogs for FdmV-catalyzed amidation.

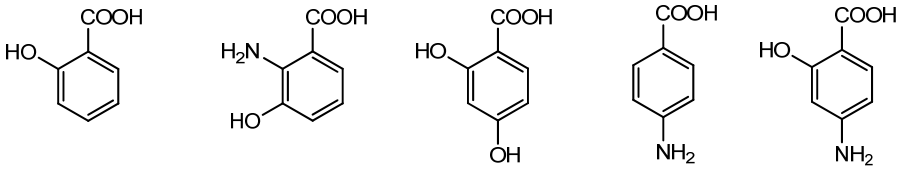

FIGURE S5. FdmV-catalyzed amidation of FDM M-3 to FDM M-6 as analyzed by HPLC: (I) completed assay with boiled FdmV as a control and (II) completed assay with FdmV under the optimized assay condition with L-Gln as an ammonia source for 3 hrs. (●) FDM M-3; (♦) FDM M-6.