

**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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TABLE S1. ¹H NMR and gCOSY data of FDM C in *d*₆-DMSO.

Position	FDM C in <i>d</i> 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 $\Delta fdmV$ 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 $\Delta fdmV$ 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 $\Delta fdmV$ 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 $\Delta fdmV$ mutant strain SB4027 as predicted from a double crossover event between the wild-type and pBS4059.

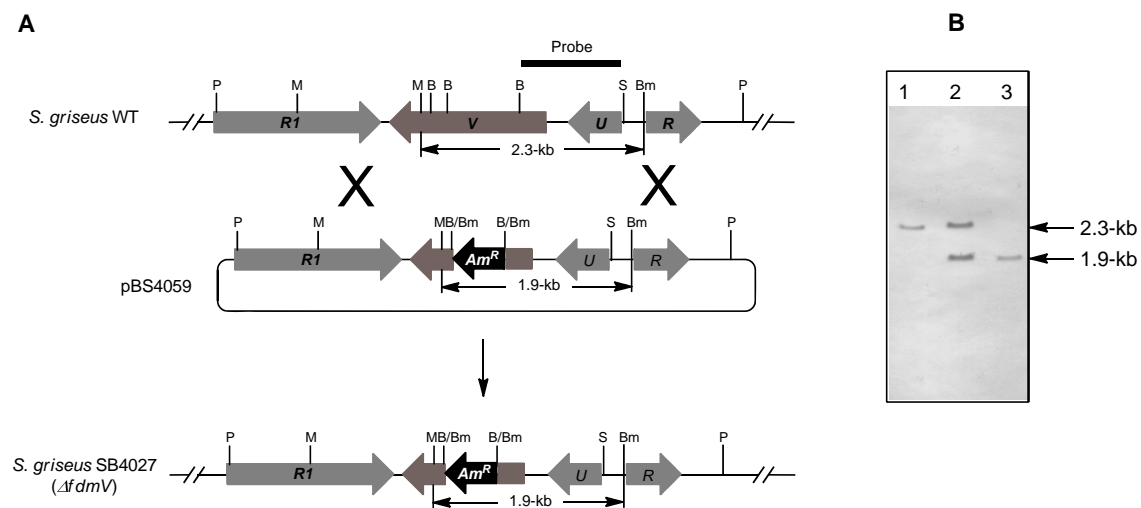


FIGURE S2. Amino acid sequence comparison of FdmV with selected homologs and AS B. The residues conserved in AS B active sites are marked with red asterisks. Accession numbers for each of the enzymes are: FdmV, AAQ08933; LlpA, CAM34350; PdmN, ABK58686; GrhP, AAM33677; RubR, AAM97368; OxyD, AAZ78328;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

FdmV	(1)	MCGVIGVVRQDGRFREDFPAVAEAMAGAMRQGPDECVRGGRTVITVTRNAVIDDLGSR--CPMAADSE---DFPMTIYCGEYVNAARSDIAGRGHGFATRS
LlpA	(1)	MCGVIGVDFPERDLS-EHGSTVTRTAAATLARGPDEEAVWTGRCAALAFRRVAVIDPFGG--CPMVAEEDG---RQLAVLVNNGVYVWRTDDELAARGHGFATRS
PdmN	(1)	MSGFGVVDYERDIR-RSSATTRTNTATMRQGPDEEELWLSERLAFSHRRVAVLDFKGR--CPMRAEHDG---RELAVLAENGVYVWAGVRELAARGHGFATRS
GrhP	(1)	MSAIGVVDPERDLS-FOALVETNAALARGPDRGVVAEGHAAALARRALLDITETGAQFAVHTTGG--AAVAVLIDGALNHTDARRLAARGHGFATRS
RubR	(1)	MSAIGVVDPERDIT-FEAFVVAANAALARGPDRFEEIEGHAAALARRSLGSEHAG-QFATVESAG--SFVAVLIDGSLVNAVARRLAARGHGFATRS
OxyD	(1)	MCGVIGVDFPERDLS-CPRLAWNTDTRARGPDRDLTGGHAAALGHRRLAVIIPAFHR--CPMSTLFD---GTSHTIETSGLVNFRVTELESQGHGFATRS
TcsG	(1)	MCGVIGVDFPAE-HRRDLCAQIDYARGPDAEGLFGRALGLGHR-SVIEPEHR--CPMTAEHE---GRCALITSSGLNFRVRELTSHGHGFATRS
Ant-Orf1	(1)	MCGVIGVDFADPDI-AERRVCAQVDMARGPDEEAVVRFVRFVGRHRSVIDLGG--CPAVTEITLFDGTFPAALSYSGVINGTDRRELAARGHGFATRS
PhzH	(1)	MCGVIGVDFYTRIE-GDPALRMTNMLARGPDAEELKRRRLGHRRLAVIDLGGT--CPMVFYFADG---QEVSVITGTVVNDARDGCRQAQGHGFATRS
AS B	(1)	MSVFCVFIKTIKAV-ELRKALELSRLRARGPDSVSEIYASDNIILRRSIVYVNAAR--RELYNQOK-----THVAVNGVYVWQAARVYEDC-VYQFSGSDC
		* * * * *
FdmV	(106)	EVLRYVYVGERCPFLGGAAADATRSFTRDRFGPVAEIGDGVFGSEPKALVSDVSEFDLGLAVFESMAAFEGCVYFSLDIEFALTRFPG
LlpA	(105)	EVLRYVYVGERACVEHGGAAADDPRELLLRDRGPPVAARVGGVFGSVYALLAHLHEFYVDEGLAELLSYIATPGRALVYRRENRREHITIND
PdmN	(105)	EVLRYVYVGERCAALGGAAADDPSEELLLRDRGCPVAIYVDFGLTASEPKALLAHVNDATVINDRELLSQTPTPAVYKRMVNRRAHVTWRIE
GrhP	(107)	EVLRYVYVGERGDFITLGGAAADDPKQLLRDRGCPSEHVFPGVFGSEPKALLAHFDADVDADGLAEFAQRKPGTGFGRKREIVFSEHAIWR
RubR	(106)	EVLRYVYVGERCAFFELGGAAADDPKQLLRDRGCPGCVLIFPGVFGSEPKALLAHFAPRFDVADGLAEFASRKRPGTGFGRKREIVFSEHAIWR
OxyD	(105)	EVLRYVYVGERLVDRLGGAAADDPKQLLRDRGCPVAIYVDFGLTASEPKALLAHVNDATVINDRELLSQTPTPAVYKRMVNRRAHVTWRIE
TcsG	(104)	EVLRYVYVGERLVEHGGAAADDPSEELLLRDRGPPVAIYVDFGLTASEPKALLAHVNDATVINDRELLSQTPTPAVYKRMVNRRAHVTWRIE
Ant-Orf1	(108)	EVLRYVYVGEREFTVRLGGAAADDPKQLLRDRGCPVAIYVDFGLTASEPKALLAHVNDATVINDRELLSQTPTPAVYKRMVNRRAHVTWRIE
PhzH	(105)	EVLRYVYVGERCCDHLGGAAADDPKQLLRDRGCPVAIYVDFGLTASEPKALLAHVNDATVINDRELLSQTPTPAVYKRMVNRRAHVTWRIE
AS B	(101)	EVLRYVYVGERCCDHLGGAAADDPKQLLRDRGCPVAIYVDFGLTASEPKALLAHVNDATVINDRELLSQTPTPAVYKRMVNRRAHVTWRIE
		* * * * *
FdmV	(216)	DERSLRFVQIEEPEEELAGVATRELESSVAEFLVDPVSVLGGGDSLVAAARAAADGDSSEVETITVSYSEENQPIVYSAIDDFVRAVPE
LlpA	(215)	GEVREKAVTIPREARDWDTVEVRELTESVSHVVDPEITLGGGDSGLAAARQDTFP---ATFAVDREHTTRERERWRHREDFPRAVTRF
PdmN	(215)	RELVTEKAVEGREHTDDEATINRELEESISGRDPEVSLGGGDSGLAAARQDTFP---ATFAVDREHTTRERERWRHREDFPRAVTRF
GrhP	(217)	PE-SRECRVSPREHTISDITVATRELDAAVASHTRDPEVGLMGGGDSGLAAARQDTFP---ATFAVDREHTTRERERWRHREDFPRAVTRF
RubR	(216)	PE-TGHRVSPREHTISDITVATRELDAAVASHTRDPEVGLMGGGDSGLAAARQDTFP---ATFAVDREHTTRERERWRHREDFPRAVTRF
OxyD	(215)	SE-WARRVSPREHTISDITVATRELDAAVASHTRDPEVGLMGGGDSGLAAARQDTFP---ATFAVDREHTTRERERWRHREDFPRAVTRF
TcsG	(214)	GEVREKAVTIPREARDWDTVEVRELTESVSHVVDPEITLGGGDSGLAAARQDTFP---ATFAVDREHTTRERERWRHREDFPRAVTRF
Ant-Orf1	(218)	GH-RSEELVPEEHTDDVTRVRELELISQADPEITLGGGDSGLAAARQDTFP---ATFAVDREHTTRERERWRHREDFPRAVTRF
PhzH	(215)	NQMKIQSFKVRRQCHRLQENQOTRELVTHALGSEHDPEVSLGGGDSGLAAARQDTFP---ATFAVDREHTTRERERWRHREDFPRAVTRF
AS B	(185)	DERSLRFVQIEEPEEELAGVATRELESSVAEFLVDPVSVLGGGDSLVAAARAAADGDSSEVETITVSYSEENQPIVYSAIDDFVRAVPE
		* * * * *
FdmV	(325)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
LlpA	(319)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
PdmN	(323)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
GrhP	(326)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
RubR	(324)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
OxyD	(319)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
TcsG	(316)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
Ant-Orf1	(326)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
PhzH	(324)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
AS B	(291)	IEAEHLEITADITVARTVRCQVY-APFEMDSTIQALAGR-RERVALGSDLPFGSIVHIDLAHEEQEVAFEQNHETRRSEGGSESPNF
		* * * * *
FdmV	(433)	DFIDMGSYVAEACAMSRILRPRGEEISRAEICDTHLHTRERENRDLVSSEVVRPDCDRLQVAYNIPNANKYFDGSEKSVLRARADLDFRVVDF
LlpA	(425)	EKVYDPPVYAGNADAVRETEVPEPERDALLRAGYEMRNLLTINHEEYVLEVRVYCDHRMEYVFNIPNRFARGEKSEPRANRGLPESVIRRS
PdmN	(430)	ESLIDIPVYRDSEHARLREHVEADPCRRKREITHTVTRVPELRNDREHAYVGLTTPCQPEELCAEENHIEGSEGRKSLRAAVADLLPEVYKRP
GrhP	(435)	KRLDPLDADQHRDAITVPHVVDATAASRAAEVYVATVRAAFELDRADRMSEHVFQLEPPCDRALVEYVYVFAARVRSVYKSLLSAAVADLLPEVYKRP
RubR	(433)	KEIDPMDYADQHRDAITVPHVVDATAASRAAEVYVATVRAAFELDRADRMSEHVFQLEPPCDRALVEYVYVFAARVRSVYKSLLSAAVADLLPEVYKRP
OxyD	(424)	KRLDPLDADQHRDAITVPHVVDATAASRAAEVYVATVRAAFELDRADRMSEHVFQLEPPCDRALVEYVYVFAARVRSVYKSLLSAAVADLLPEVYKRP
TcsG	(421)	KRLDPLDADQHRDAITVPHVVDATAASRAAEVYVATVRAAFELDRADRMSEHVFQLEPPCDRALVEYVYVFAARVRSVYKSLLSAAVADLLPEVYKRP
Ant-Orf1	(431)	AEIINPEYIADQHRDAITVPHVVDATAASRAAEVYVATVRAAFELDRADRMSEHVFQLEPPCDRALVEYVYVFAARVRSVYKSLLSAAVADLLPEVYKRP
PhzH	(424)	RQCDLQYQASDADAQCQEHRAQDPPHRRMRECMHKKMVMVLDKQRLSMANSEVVRPFDHEVYVYVFNVSISKSGEHWLWCKMCFVVEAVVRR
AS B	(377)	KRLDPLDADQHRDAITVPHVVDATAASRAAEVYVATVRAAFELDRADRMSEHVFQLEPPCDRALVEYVYVFAARVRSVYKSLLSAAVADLLPEVYKRP
		* * * * *
FdmV	(543)	XAPFVSDAAYTRAHQTEVLADEASEFLPILLLESAVARE-GGDLQDMIEKMNVMVLOVAWRELESDVEN--
LlpA	(525)	XSEFFVSDAAYTRAHQTEVLADEASEFLPILLLESAVARE-GGDLQDMIEKMNVMVLOVAWRELESDVEN--
PdmN	(530)	XSEFFVSDAAYTRAHQTEVLADEASEFLPILLLESAVARE-GGDLQDMIEKMNVMVLOVAWRELESDVEN--
GrhP	(535)	XSEFFVSDAAYTRAHQTEVLADEASEFLPILLLESAVARE-GGDLQDMIEKMNVMVLOVAWRELESDVEN--
RubR	(533)	XSEFFVSDAAYTRAHQTEVLADEASEFLPILLLESAVARE-GGDLQDMIEKMNVMVLOVAWRELESDVEN--
OxyD	(524)	XSEFFVSDAAYTRAHQTEVLADEASEFLPILLLESAVARE-GGDLQDMIEKMNVMVLOVAWRELESDVEN--
TcsG	(521)	XSEFFVSDAAYTRAHQTEVLADEASEFLPILLLESAVARE-GGDLQDMIEKMNVMVLOVAWRELESDVEN--
Ant-Orf1	(531)	XSEFFVSDAAYTRAHQTEVLADEASEFLPILLLESAVARE-GGDLQDMIEKMNVMVLOVAWRELESDVEN--
PhzH	(524)	XSEFFVSDAAYTRAHQTEVLADEASEFLPILLLESAVARE-GGDLQDMIEKMNVMVLOVAWRELESDVEN--
AS B	(482)	YNTPTREAYVYRETFEELPFPSEACVYGGVSNACSSKAEIWDARFKMDDPFGRAVGMHQSAVK-----

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

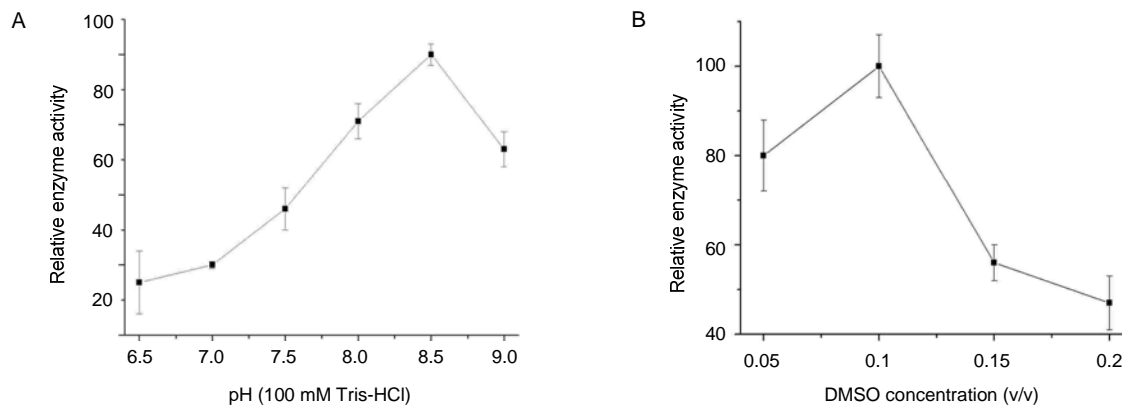


FIGURE S4. Structures of benzoic acids tested as substrate analogs for FdmV-catalyzed amidation.

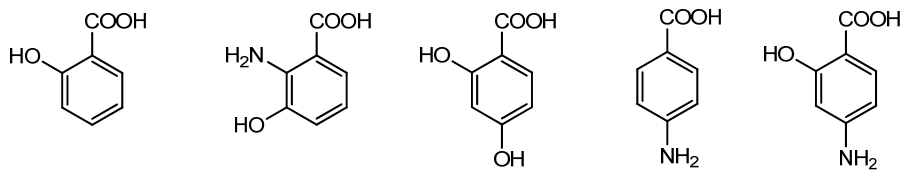


FIGURE S5. FdmV-catalyzed amidation of FDM M-3 to FDM M-6 as analyzed by HPLC: (I) completed assay with boiled FdmV as a control and (II) completed assay with FdmV under the optimized assay condition with L-Gln as an ammonia source for 3 hrs. (●) FDM M-3; (◆) FDM M-6.

