

# Determination of the Protein-Protein Interactions Within Acyl Carrier Protein (MmcB) Dependent Modifications in the Biosynthesis of Mitomycin

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**Supplementary Tables****Table S1.** Strains and plasmids used in this study

Strains and plasmids	Description	Source or reference
<i>Streptomyces</i> sp.		
Strain NRRL 2564	Wild-type producer for mitomycin C	[1]
DJ01	Gene <i>mitE</i> -inactivation mutant	This work
DJ01:: <i>mitE</i>	DJ01 complemented with cloned <i>mitE</i>	This work
DJ03	Gene <i>mmcB</i> -inactivation mutant	This work
DJ03:: <i>mmcB</i>	DJ03 complemented with cloned <i>mmcB</i>	This work
DJ03:: <i>mmcB</i> (S43A)	DJ03 complemented with cloned <i>mmcB</i> (S43A)	This work
DJ05	Gene <i>mitB</i> -inactivation mutant	This work
DJ05:: <i>mitB</i> (originated)	DJ05 complemented with cloned <i>mitB</i> (originated)	This work
DJ07:: <i>mitB</i> (updated)	DJ05 complemented with cloned <i>mitB</i> (updated)	This work
DJ08	Gene <i>mitF</i> -inactivation mutant	This work
DJ08:: <i>mitF</i>	DJ08 complemented with cloned <i>mitF</i>	This work
<i>Escherichia coli</i>		
<i>E. coli</i> DH10B	Cloning host	Invitrogen
<i>E. coli</i> ET12567/pUZ8002	Conjugation donor strain	[2]
<i>E. coli</i> BL21 (DE3)	Protein expression strain	Invitrogen

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<i>E. coli</i> BL21(DE3)/pSV20	Protein expression strain containing of <i>sfp</i>	[3]
Plasmids		
pBluescript SK (+)	<i>ColE, lacZ, bla, oriF1</i>	Stratagene
pJTU1278	<i>rep pIJ101, tsr, bla, oriT</i>	[4]
pSET152	<i>ΦC31, oriT, kasO*p, acc(3)IV</i>	[5]
pET28a	<i>kan, P<sub>T7</sub>, His<sub>6</sub>-tag</i>	Novagen
pJQK401	Construct for <i>mitE</i> deletion	This work
pJQK402	pSET152 with cloned <i>mitE</i>	This work
pJQK406	pET28a with cloned <i>mitE</i>	This work
pJQK408	pSET152 with cloned <i>mitB</i> (originated)	This work
pJQK409	pSET152 with cloned <i>mitB</i> (updated)	This work
pJQK411	pET28a with cloned <i>mitB</i> (updated)	This work
pJQK412	Construct for <i>mitF</i> deletion	This work
pJQK413	pSET152 with cloned <i>mitF</i>	This work
pJQK414	pET28a with cloned <i>mitF</i>	This work
pJQK415	pET28a-TEV with cloned <i>mmcB</i>	This work
pJQK416	pET28a-TEV with cloned <i>mitB</i>	This work

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**Table S2.** Primers used in this study

Primers	Sequences (5'→3')
mitE-petF	ATAC <u>ATATGACTGAACAGGCGACCGGTC</u> , <i>NdeI</i> site underlined
mitE-petR	ATAGA <u>ATTCATGAACCAGGCCTCCTTGGC</u> , <i>EcoRI</i> site underlined
mitB-petF	ATA <u>CATATCGGGCCCCAACGGCACC</u> , <i>NdeI</i> site underlined
mitB-petR	ATAGA <u>ATTCATCGCGCCGCGTCCGTGC</u> , <i>EcoRI</i> site underlined
mmcB-petF	ATAC <u>ATATGGAGACCCTGACGACCGA</u> , <i>NdeI</i> site underlined
mmcB-petR	ATAG <u>CGGCCGCTCATTGGCGGTGCGGT</u> , <i>NotI</i> site underlined
mitB-left armF	ACGG <u>GATCCCAGGACCGAAAGGCTGCTCAATG</u> , <i>BamHI</i> site underlined
mitB-left armR	ATGA <u>AGCTTCAGGACGTCGTCGAGGAAGA</u> , <i>HindIII</i> site underlined
mitE-left armF	AA <u>AGGTACCCACTCATGTCCGTAGCACCC</u> , <i>KpnI</i> site underlined
mitE-left armR	A <u>AGAAGCTTGGTCAACGTCAGGCGGAGGC</u> , <i>HindIII</i> site underlined
mmcB-left armF	ACGG <u>GATCCGGCGGTACGGGATCTATCA</u> , <i>BamHI</i> site underlined
mmcB-left armR	GG <u>GAAGCTTGAGGGAATCGACGAGCACCTT</u> , <i>HindIII</i> site underlined
mitB-right armF	CCC <u>AAGCTTACCCACCGAACCTCACGCAC</u> , <i>HindIII</i> site underlined

mitB-right armR                    CCGGTACCCGACCTTCTCGTGCAGGCTCCC, *KpnI* site underlined

mitE-right armF                    CCCAAGCTTCTGGTCGACGACGTCTCCTGC, *HindIII* site underlined

mitE-right armR                    GCGGATCCAGCCGACACTGCGCACGGT, *BamHI* site underlined

mmcB-right armF                    CCCAAGCTTATCGACAGTCTCGACCTCCTCGC, *HindIII* site underlined

mmcB-pdF                            CAGCATATGGAAACCCTGACCACCGA, *NdeI* site underlined

mmcB-pdR                            CTCGAATTCAATTCCGCCGGTGCCTAACG, *EcoRI* site underlined

mitB-pdF                            CAGCATATGCCGGCCCCAACGGCA, *NdeI* site underlined

mitB-pdR                            CTCGAATTCATCGCGCCGCGTCCCCTGCCGGT, *EcoRI* site underlined

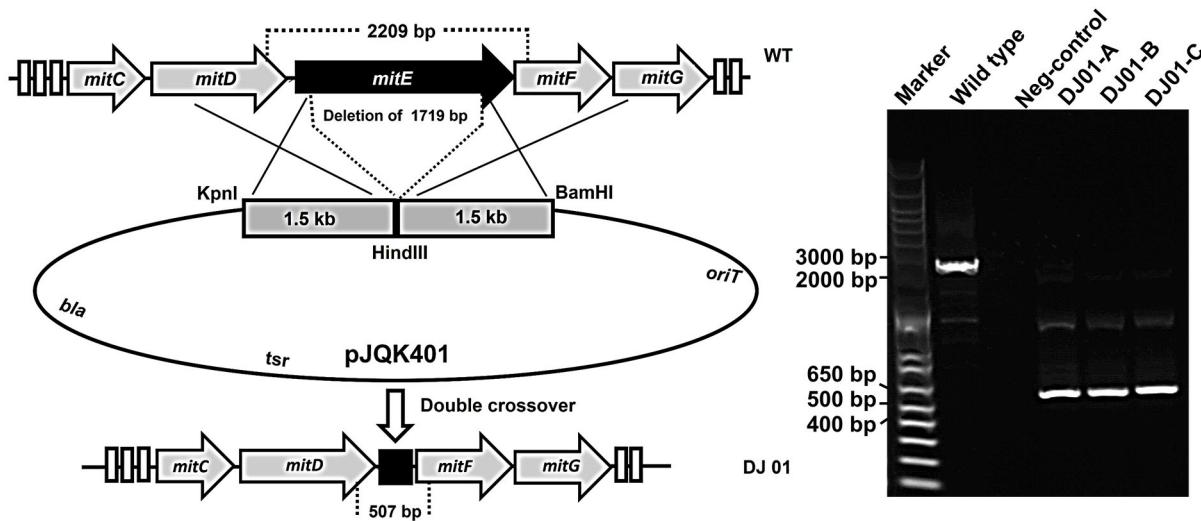
mitF -petF                            CCCATATGAGCACCGTCACCGACCGG, *NdeI* site underlined

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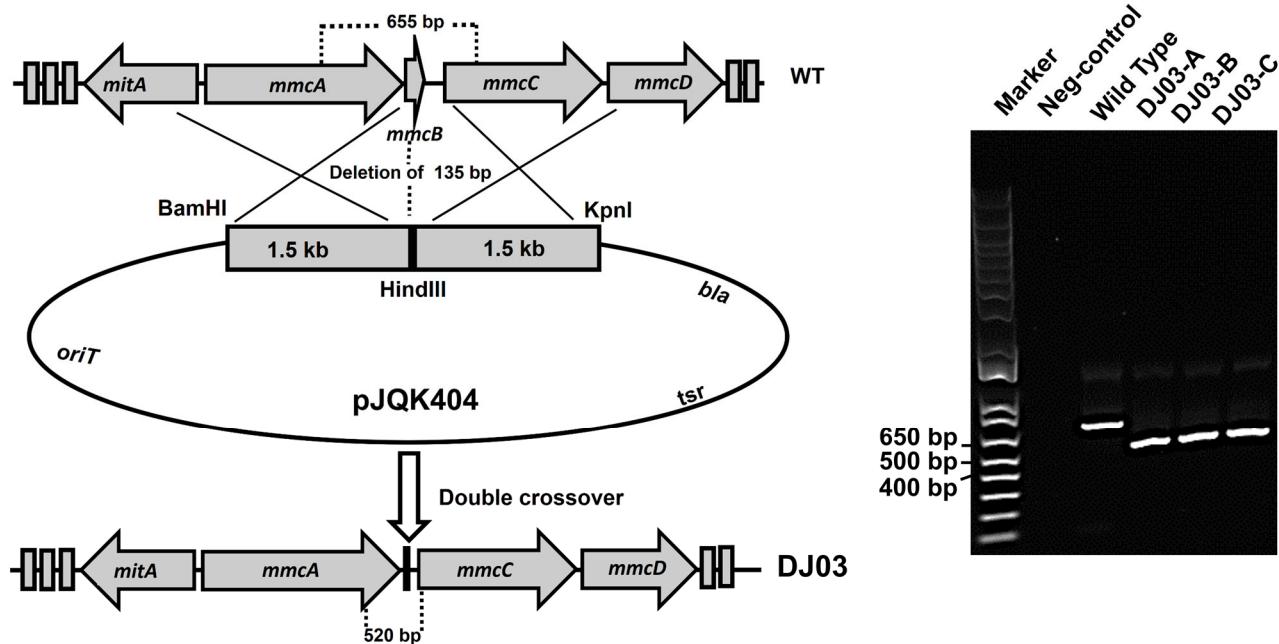
mitF -petR                            CG GAATTC TCAGAAGCGGGCACCAACCG, *EcoRI* site underlined

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## Supplementary Figures



**Figure S1.** The construction of DJ01 mutant. (A). Schematic representation of the in-frame deletion of *mitE*, a 1,719-bp region of *mitE* was deleted through double crossover. (B). Confirmation of the mutant DJ01 through PCR amplification. An approximately 500-bp product was amplified for the DJ01, while a 2,200-bp fragment was obtained from the wild type with the no template-added reaction as the negative control.

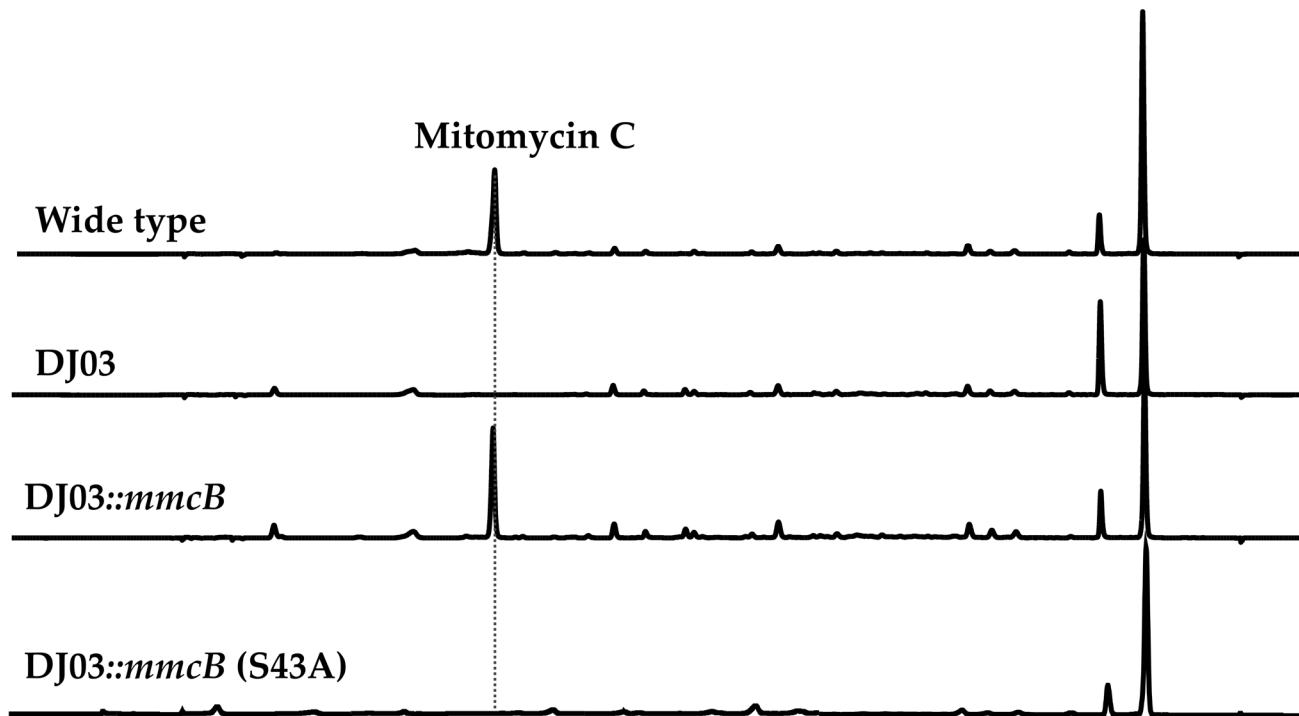


**Figure S2.** The construction of DJ03 mutant. (A). Schematic representation of the in-frame deletion of *mmcB*, a 135-bp region of *mmcB* was deleted through double crossover. (B). Confirmation of the mutant DJ03 through PCR amplification. An approximately 520-bp product was amplified for DJ03, while a 650-bp product was obtained from the wild type with the no template-added reaction as the negative control

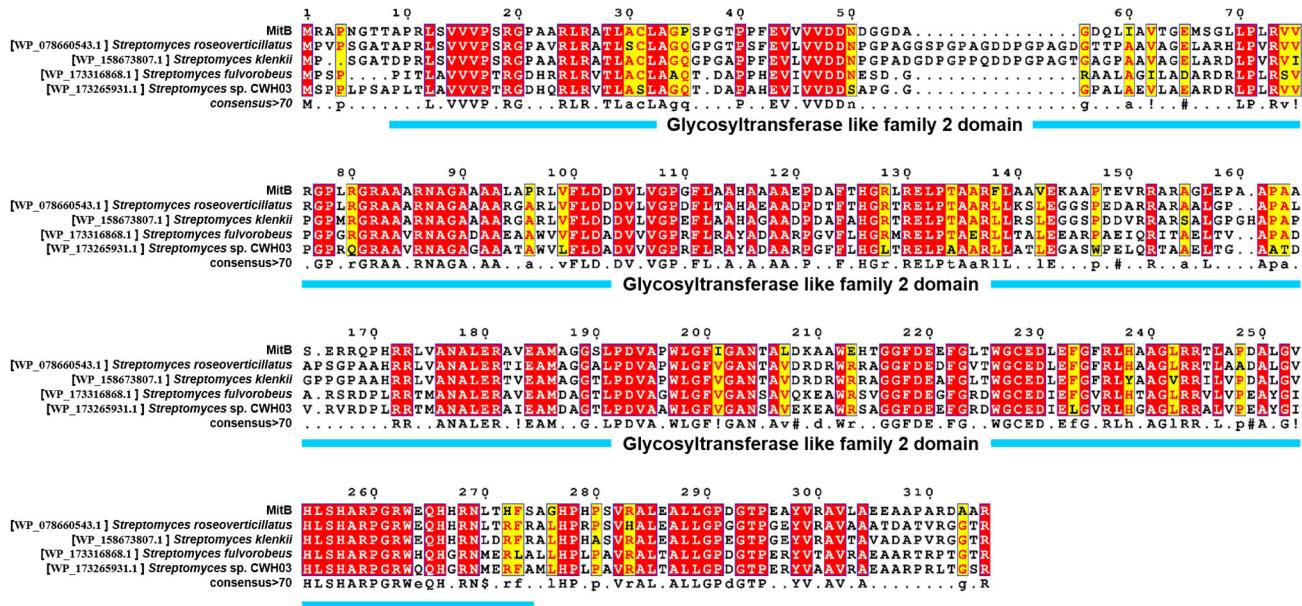
A.

Species/abbrv	Sequence
1. AAD32725.1_MmcB_Streptomyces_lavendulae	METLI-----TDKIXDRLRKVLVDLSLESLSDPSAVPDEGLVEKLGLDSINTIEFLIVVESEEFGLIEIADEDSLXKLIDSLDLLAGYVSERVNGVTAPAE---
2. WP_106680665.1_hypothetical_protein_Streptosporangium_nondiastaticum	MEKXTENTGKTVTIDEIKDRRLRTVLYDSDLELPMEPSDVDPQGLVDKLGLDSINTIEFLIVVESEEFGLIEIADEDSLXKLIDSLDLLAGYVVERMA-----
3. WP_030369202.1_hypothetical_protein_Streptomyces Roseoverticillatus	MEKTV-----TDEIKDRRLRKVLVDLSLELPMEPSGVPDFGLVDKLGLDSINTIEFLIVVESEEFGLIEIADEDSLXKLIDSLDLLAGYVVERMA-----
4. WP_058042900.1_MULTI SPECIES_hypothetical_protein_Streptomyces	MEKTA-----TDEIKDRRLRKVLVDLSLELPMEPSDVDPQGLVDKLGLDSINTIEFLIVVESEEFGLIEIADEDSLXKLIDSLDLLAGYVVERMA-----
5. WP_063795218.1_hypothetical_protein Streptomyces sp. 150FB	MEETEG-----TKARLRLRKVLVDLSLELSVAPSVDVDRGLVEALGLDSINTIEFLIVVESEEFGLIEIADEDSLXKLIDSLDLLAGYVVERMA-----
6. WP_137968337.1_acyl_carrier_protein Streptomyces_antimycoticus	MNDTDXDRLRRLRVLVESELIDLAPAEAVPDGLREAVGIDSVMLFELIVVNEFFGIQIQQDEDLSVLELYSLDLTIAAYVDEERAQTAARD-----
7. ONT76444.1_hypothetical_protein_ALI144C_36505_Actinosynema_sp._ALI-1_44	-WKGRLRQVLVESIDLRLRFPDIPDNLVAELGLDSINTIEFLIVVESEEFGLIEIADEDSLXKLIDSLDLADYVNSRLKPDSSLADQGS-----
8. WP_047669725.1_hypothetical_protein Nocardiopsis_sp._RV163	MKQPFTEIDPREGNDDYSDVKDRLRKVKWVASLEIKRDPADLPKEIDVDTGLDSINTIEFLIVVNEFFGQIQQDEDLSVRLVBDLDSLRSIAAVVSEERLS-----
9. WP_059145778.1_MULTI SPECIES_acyl_carrier_protein Streptomyces	MNDTDXDRLRRLRVLVESELIDLAPAQVDPGLREAVGIDSVMLFELIVVNEFFGQIQQDEDLSVLELYSLDLTIAAVVDEERAQTAARD-----
10. WP_093466233.1_MULTI SPECIES_acyl_carrier_protein Streptomyces	MNDTDXDRLRRLRVLVESELIDLAPAEAVPDGLREAVGIDSVMLFELIVVNEFFGQIQQDEDLSVLELYSLDLTIAAVVDEERAQTAARD-----
11. WP_044580613.1_acyl_carrier_protein_Amycolatopsis_orientalis	MNTTDXDRLRRLRVLVDSLRIPRPFESIPDGLREELGIDSVMLFELIVVNEFFGQIQQDEDLSVLELYSLDLKIAEVVDSRTAAAG-----
12. WP_076539768.1_acyl_carrier_protein Streptomyces_hygroscopicus	MNETDXDRLRRLRVLVESELIDLAPAEAVPDGLREAVGIDSVMLFELIVVNEFFGQIQQDEDLSVLELYSLDLTIAAVVDEERAQTAARD-----
13. WP_030241113.1_hypothetical_protein Streptomyces_sp._NRRL_S-350	MNDTDXDRLRRLRVLVESELIDLAPAEAVPDGLREAVGIDSVMLFELIVVNEFFGQIQQDEDLSVLELYSLDLTIAAVVDEERAFAAARG-----
14. WP_030112506.1_MULTI SPECIES_acyl_carrier_protein Streptomyces	MATAETDXDRLRKVVEEALRDRDPATVPDEGLRAALDDIDSVAGLELLIVVNEFFGQIQQDEDLSVLLVDSLNVLGDVYVEERLAAADAVPSAGTPG-----
15. WP_030331376.1_acyl_carrier_protein Streptomyces_sp._NRRL_B-1381	MATAETDXDRLRKVVEEALRDRDPATVPDEGLRALDDIDSVAGLELLIVVNEFFGQIQQDEDLSVLLVDSLNVLGDVYVEERLAAADAVPSAGTPG-----
16. AUX26881.1_acyl_carrier_protein Sorangium_cellulosum	MNDVRSRSLRRVIVRGLRDRSPESIPEIQLGIDINSLELLVVNEFDIQQIDDADLSTALVDSLTDLASVYSSARLAAGTAAA-----
17. WP_129354778.1_acyl_carrier_protein Sorangium_cellulosum	MPASSPARNDQQHTGLRGITIMNDVSRSLRRVIVRGLRDRSPESIPEIQLGIDINSLELLVVNEFDIQQIDDADLSTALVDSLTDLASVYSSARLAAGTAAA-----

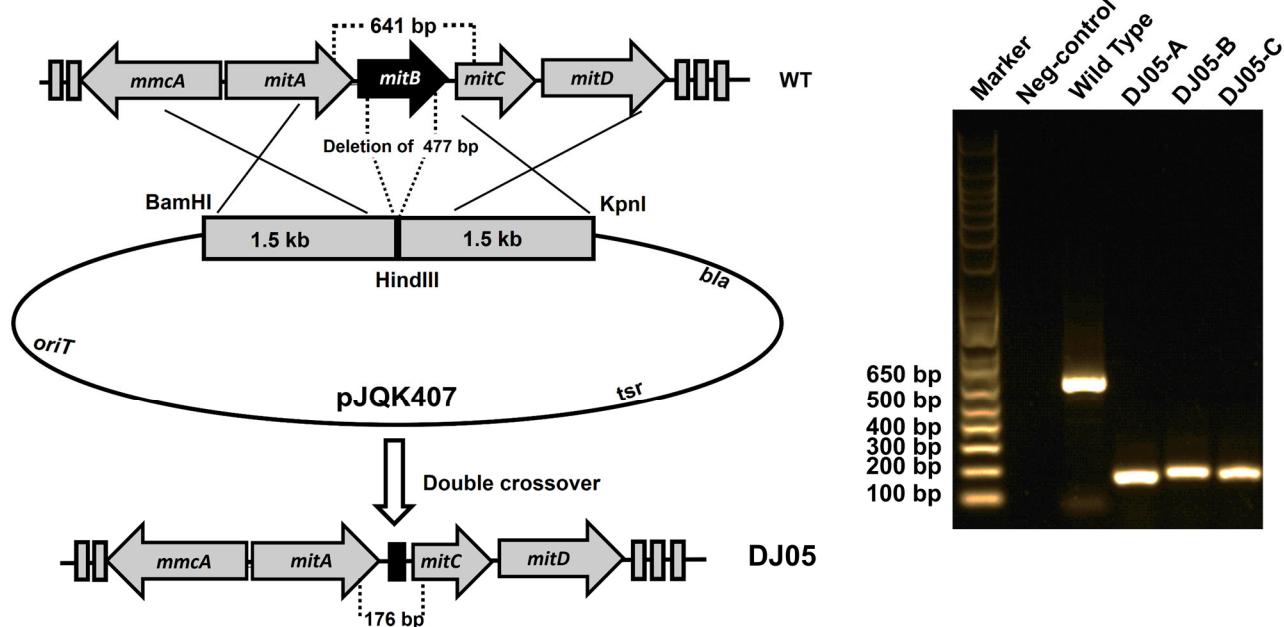
B.



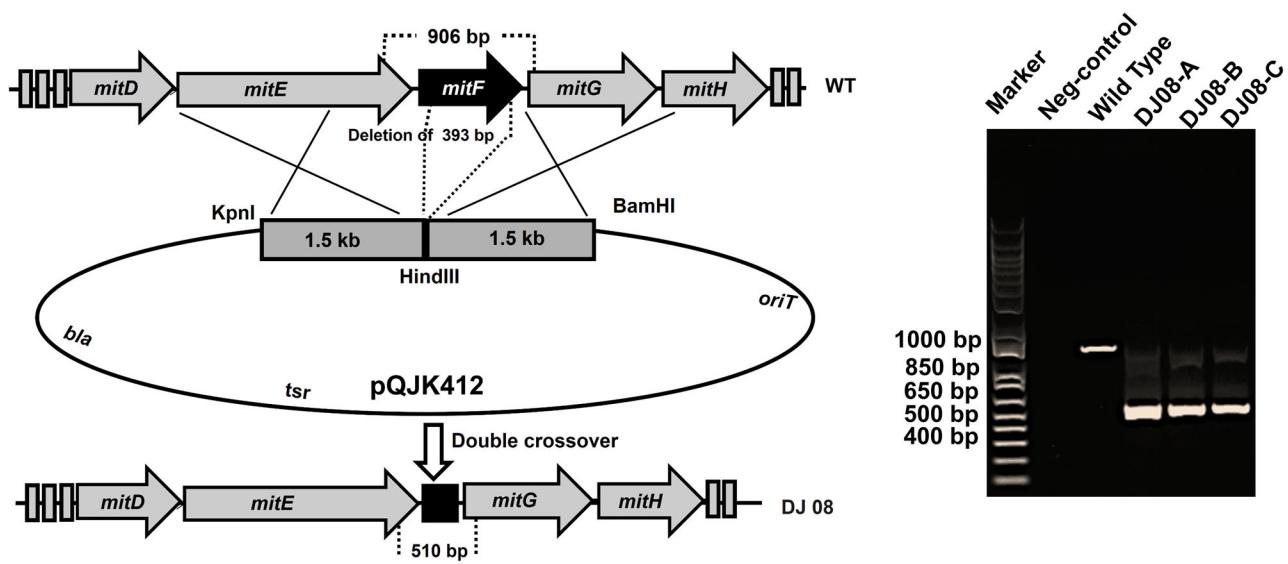
**Figure S3.** Determination of the active site of MmcB. (A) Sequence alignment of MmcB with the homologues, (B) HPLC analysis results of *mmcB* and *mmcB* S43A complementation experiments revealed the active site of Ser 43 residue.



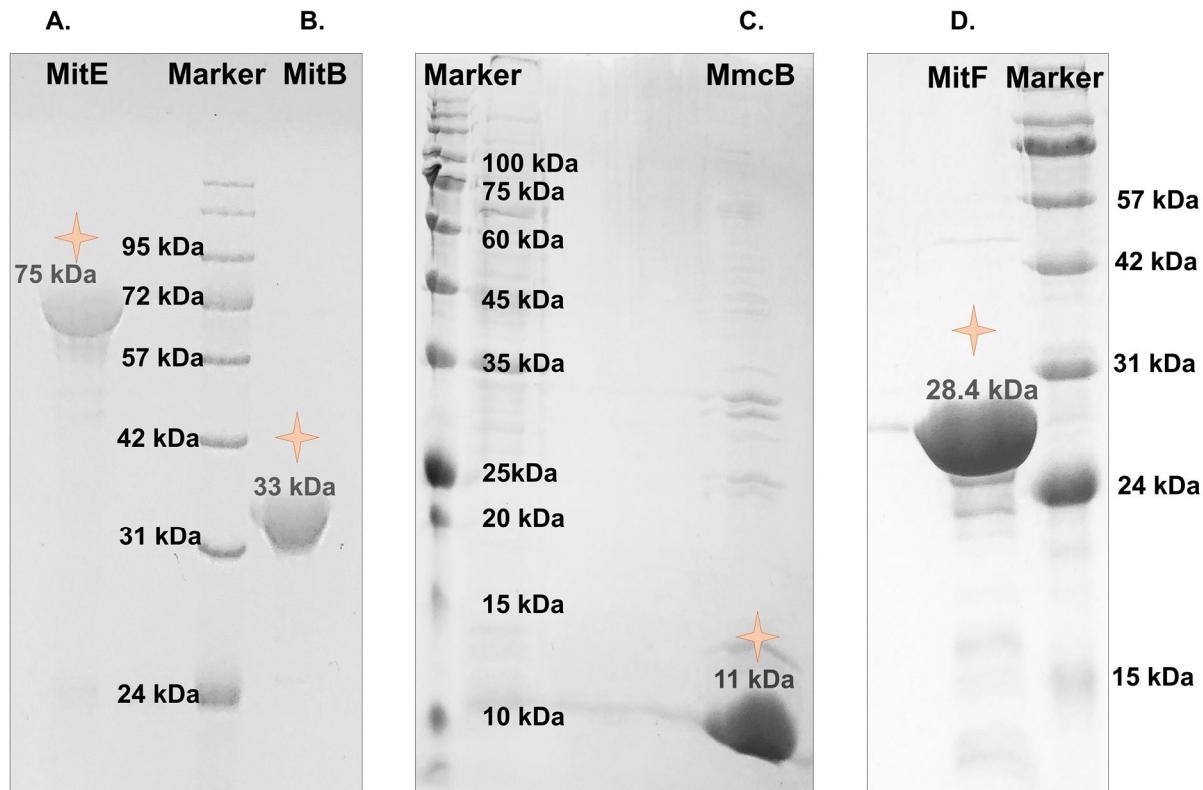
**Figure S4.** The sequence alignment of MitB with its homologies. The targeted family 2 glycosyltransferases including of WP\_078660543.1 from *Streptomyces roseoverticillatus*, WP\_158673807.1 from *Streptomyces klenkii*, WP\_173316868.1 from *Streptomyces fulvorobeus*, and WP\_173265931.1 from *Streptomyces* sp. CWH03.



**Figure S5.** The construction of DJ05 mutant. (A). Schematic representation of the in-frame deletion of *mitB*, a 477-bp region of *mitB* was deleted through double crossover. (B). Confirmation of the mutant DJ05 through PCR amplification. An approximately 170-bp product was amplified for DJ03, while a 640-bp product was obtained from the wild type with the no template-added reaction as the negative control.



**Figure S6.** The construction of DJ08 mutant. (A). Schematic representation of the in-frame deletion of *mitF*, a 393-bp region of *mitF* was deleted through double crossover. (B). Confirmation of the mutant DJ08 through PCR amplification. An approximately 510-bp product was amplified for DJ03, while a 906-bp product was obtained from the wild type with the no template-added reaction as the negative control.



**Figure S7.** SDS-PAGE analysis of the purified MitE (A), MitB (B), MmcB (C) and MitF (D) expressed in *E. coli* BL21(DE3)

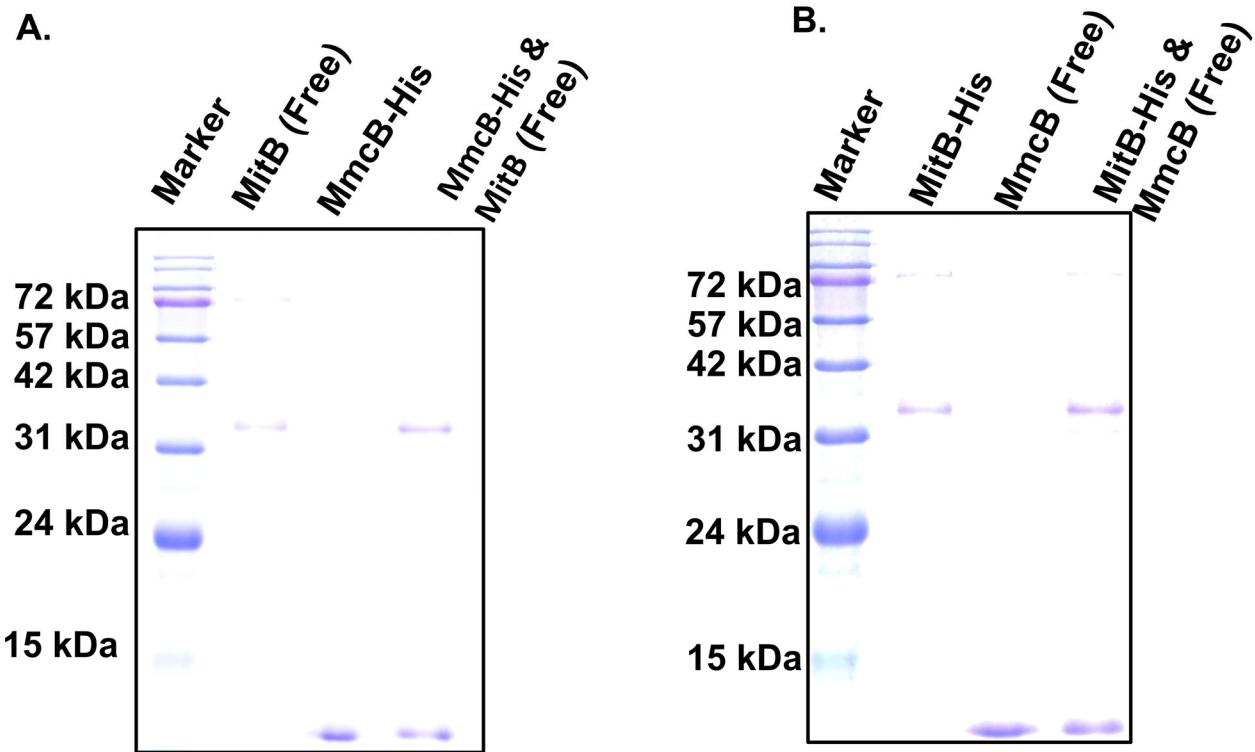


Figure S8. The *in vivo* pull-down assay between MmcB and MitB

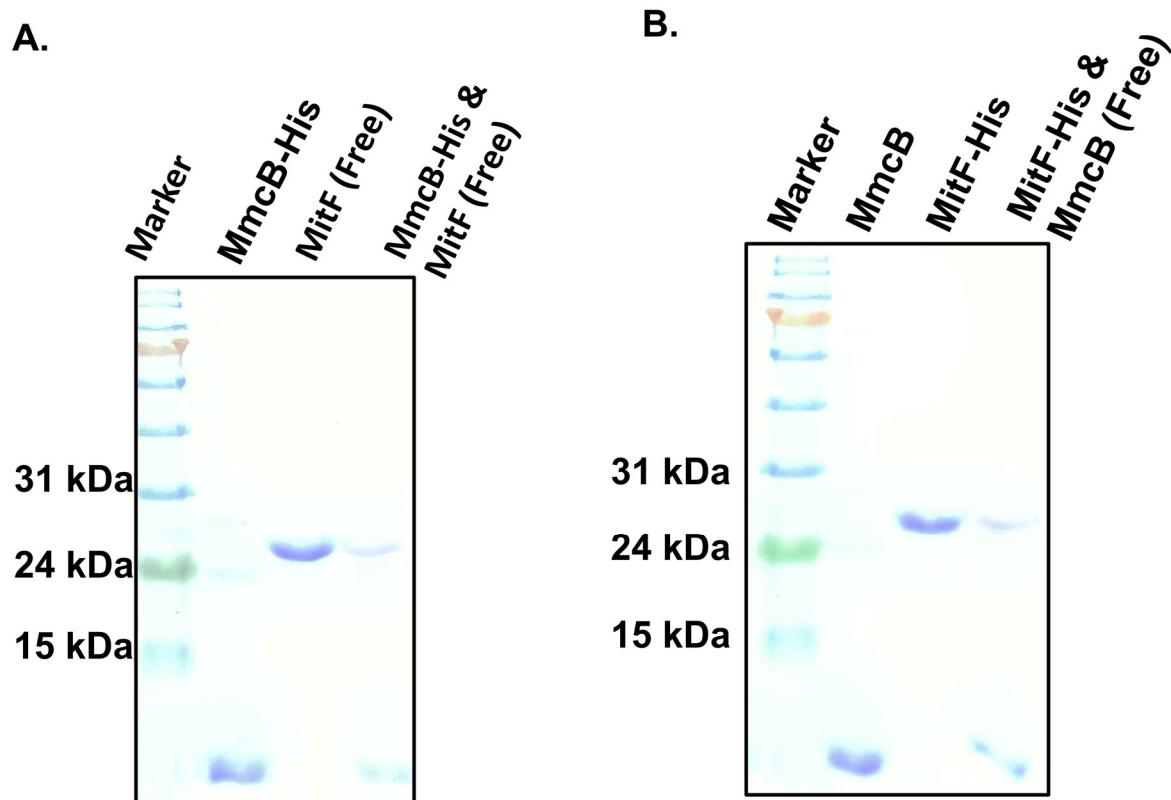
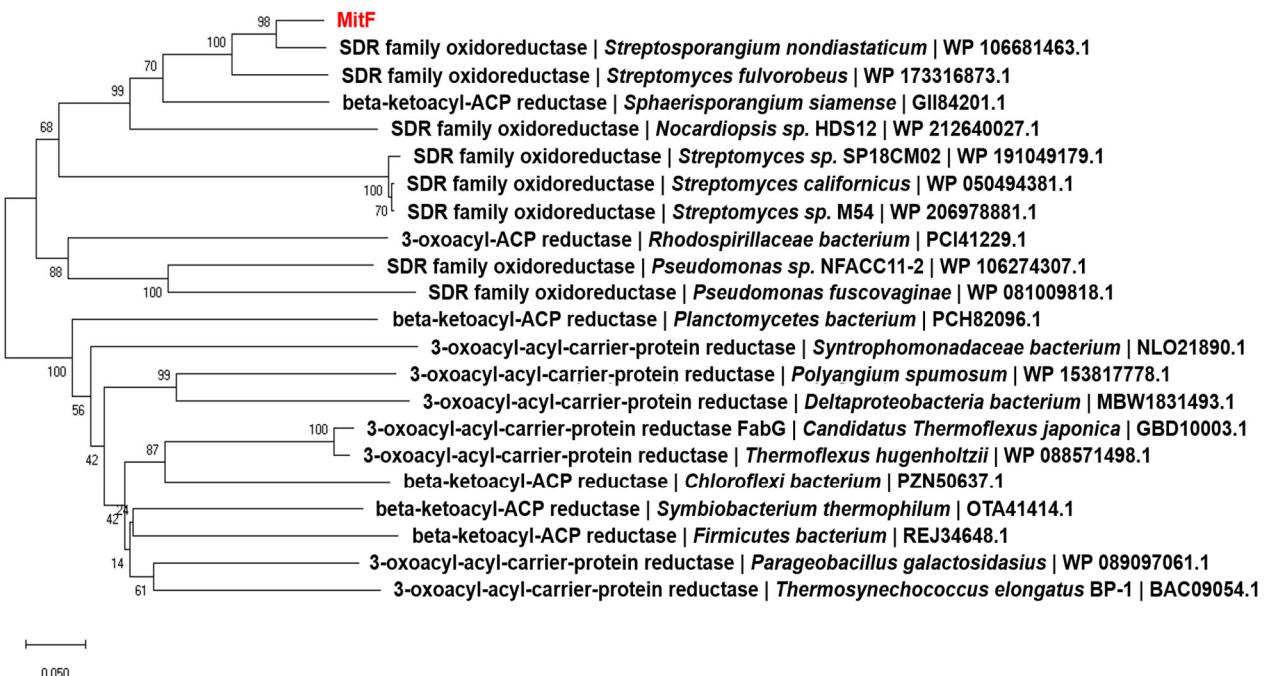
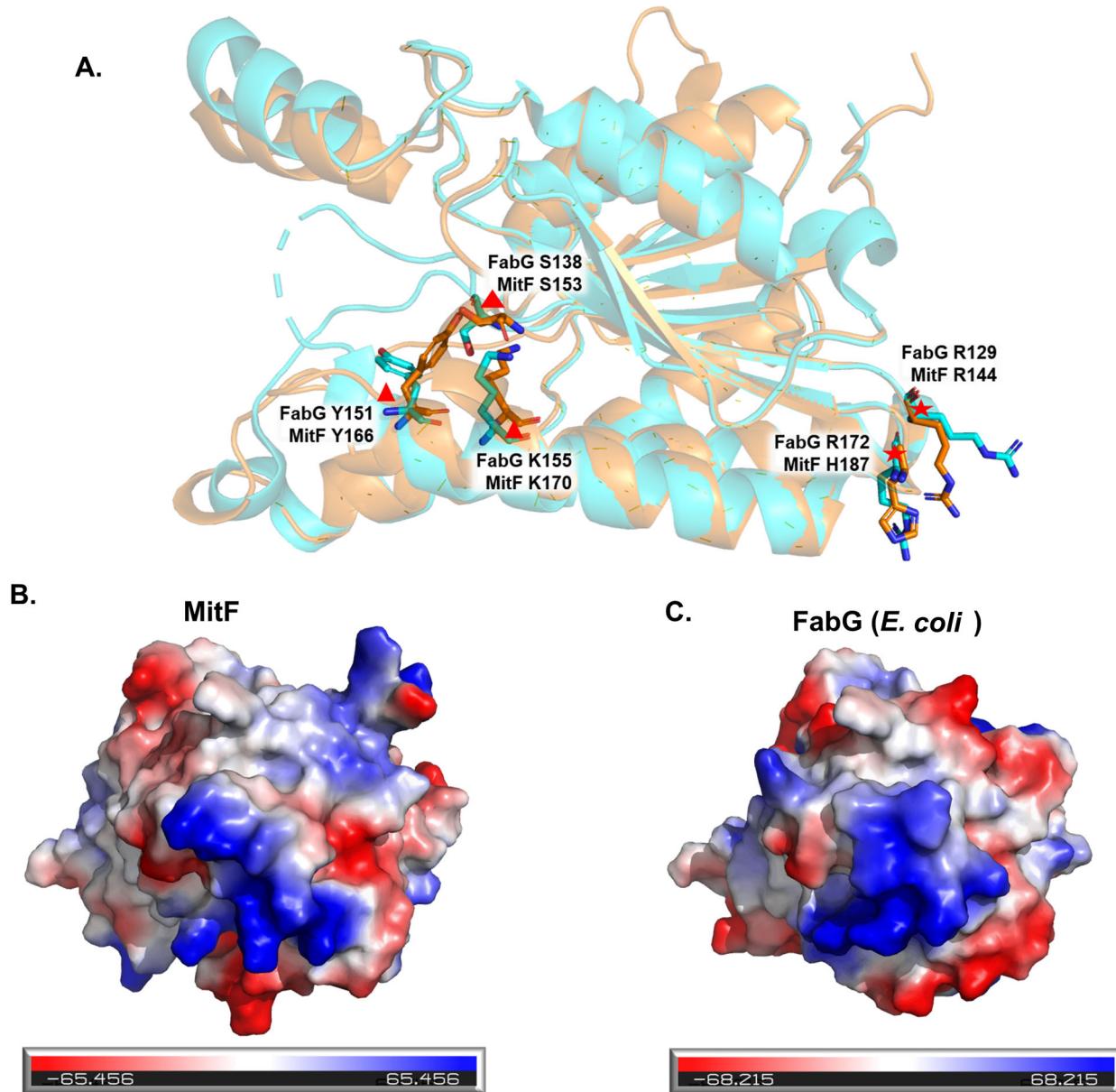


Figure S9. The *in vivo* pull-down assay between MmcB and MitF



**Figure S10.** Phylogenetic analysis of MitF with its homologous. The analysis was performed in MEGA 11 software. The amino acid sequences alignment of MitF and homologs were conducted by using ClustalW, and then phylogenetic analyses were further proceeding by the neighbor-joining method. The parameters of phylogeny were set as follows: bootstrap test (1000 replicates), *p*-distance mode, and complete deletion. The numbers adjacent to branches represent bootstrap values.



**Figure S11.** The models of MitF with the FabG (PDB: 1Q7B) by SWISS-MODEL server. (A) The superimposition of the MitF with FabG revealed the conserved activity center, (B) Characteristic electron cloud map of the MitF, (C) Characteristic electron cloud map of the FabG.

## References

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