

Construction of plasmids for gene inactivation and ectopic expression

Gene disruption of *sshg_05355* was accomplished using plasmid pOJ5355. For this purpose an *sshg_05355* internal fragment of 1 kb bp was amplified by PCR using primers 5355FW and 5355RV and *S. albus* J1074 chromosomal DNA as a template. The resultant fragment was digested EcoRI-HindIII and cloned into pOJ260 digested with the same enzymes.

Deletion of *sshg_05312* was accomplished by amplification of two DNA fragments of 2.7 and 2.6 kb, respectively. Fragment A, amplified using oligoprimers 5309RplFW and 5312RplRV, was cloned into SpeI-NsiI digested pEFBAoriT leading to pEFBA5312-1. Fragment B, amplified using oligoprimers 5312RplFW and 5315RplRV, was cloned as a BamHI-HpaI fragment into BamHI-EcoRV digested pEFBA5312-1, leading to pEFBA12AG. In this construction fragments A and B are flanking the apramycin resistance gene *aac(3)IV*. Finally, pEFBA12AG was digested with XbaI, and *hyg* from pLHyg was subcloned as a SpeI-NheI fragment to obtain plasmid pEFBA12AGH. The same approach was used for deletion of the following genes, using in each case the corresponding restriction enzymes incorporated into the oligoprimers specified at Table S1: fragments of 2.7 and 2.6 kb, respectively, were amplified for deletion of *plm1* using oligoprimers pairs 5310RplFW/5313RplRV and 5313RplFW/5315RplRV, finally leading to construct pEFBA13AGH; fragments of 2.1 and 2.0 kb, respectively, were amplified for deletion of *plm2* using oligoprimers pairs MRA-F/MRA-R and MRB-F/MRB-R, finally leading to construct pEFBATHMRAB; deletion of *plm3* was accomplished by amplifying two fragments of 2.3 and 2.2 kb using oligoprimers pairs 5312RplFW/5315RplRV and 5315RplFW/5318RplRV, to obtain pEFBA15AGH; fragments of 2.0 kb each for deletion of *plm10* were amplified using oligoprimers pairs MRS1-FW/MRS1-RV and MRS2-FW/MRS2-RV, finally

leading to construct pEFBA22AGH; fragments of 2.0 kb each for deletion of *plm12* were amplified using oligoprimers MR1F/MR1R and MR2F/MR2R, finally leading to construct pEFBATHMR12; *plm19* was deleted by amplifying two fragments of 2.1 and 2.2 kb, respectively, using oligoprimers 5333RplRV/5331RplFW and 5331RplRV/5329RplFW, leading to pEFBA31AGH; *plm22* was deleted by amplifying two fragments of 2.4 and 2.5 kb, respectively, using oligoprimers 5330RplFW/5334RplRV and 5334RplFW/5337RplRV, leading to pEFBA34AGH; fragments of 2.0 kb flanking *plm23* were amplified using oligoprimers MRG1F/MRG1R and MRG2F/MRG2R, leading to construct pEFBATHMRG12; deletion of *plm28* was obtained using plasmid pEFBA40AGH constructed using two fragments of 2.4 and 2.6 kb amplified using primer pairs Rpl5338FW/Rpl5340RV and Rpl5340FW/Rpl5342RV; *plm29* was deleted using plasmid pEFBA41AGH constructed using two fragments of 2.2 kb amplified using oligoprimers 5341RplFW/5343RplRV and 5339RplFW/5341RplRV; *plm30* was deleted using plasmid pEFBATHMRL12 constructed using two fragments of 2.0 kb amplified using oligoprimers MRL1-FW/MRL1-RV and MRL2-FW/MRL2-RV; finally, pEFBASUG for deletion of a DNA region containing *plm40*, *plm41* and *plm42* was generated amplifying two fragments of 2.8 and 2.6 kb, respectively, using oligoprimers 5349RplFW/5352RplRV and 5354RplFW/5357RplRV.

Ectopic expression of selected genes (under the control of *ermE*^{*p}), in *S. albus* J1074 or the appropriate mutant strain, was accomplished by PCR amplification of the corresponding genes using oligoprimers specified at Table S1. After verification by sequencing of each PCR fragment, these were subcloned into BamHI-EcoRI or EcoRI digested pEM4T using in each case the appropriate restriction enzymes sites incorporated into the corresponding oligoprimers (Table S1). Final pEM4T-derived

constructs were digested EcoRV and *hyg* was subcloned as an EcoRV fragment obtained from pLHyg. Constructs generated following this procedure were: pEM4HT5314 (to express *plm2*); pEM4HT5315 (*plm3*); pEM4HTSARP (*plm10*); pEM4HT5324 (*plm12*); pEM4HT5331 (*plm19*); pEM4HT5334 (*plm22*); pEM4HT5335 (*plm23*); pEM4HT5340 (*plm28*); pEM4HT5341 (*plm29*) and pEM4HTLuxR (*plm30*).

Generation of *S. albus* J1074 mutant strains

Construct pOJ5355 was introduced into *S. albus* J1074 by intergeneric conjugation from *E. coli* ET12567 (pUB307) leading to mutant strain SAM5355, which was selected by resistance to apramycin. Constructs pEFBA12AGH, pEFBA13AGH, pEFBATHMRAB, pEFBA22AGH, pEFBATHMR12, pEFBATHMRG12, pEFBA15AG, pEFBA31AGH, pEFBA34AGH, pEFBA40AGH, pEFBA41AGH, pEFBATHMRL12, and pEFBASUG were introduced into *S. albus* J1074 by intergeneric conjugation from *E. coli* ET12567 (pUB307) leading to mutant strains SAM5312, SAM5313, SAM5314, SAM5322, SAM5324, SAM5335, SAM5315, SAM5331, SAM5334; SAM5340, SAM5341, SAM5342, and Δ SUG. In all these cases an apramycin and hygromycin resistant mutant, obtained by a single crossover recombination, was grown in the absence of antibiotics and then screened for the loss of hygromycin resistance, keeping apramycin resistance because of a double crossover recombination.

The *S. albus* J1074 mutant strains generated in this work were analyzed by PCR amplification using oligoprimers: 5312MFW/5312MRV (SAM5312); 5313SobFW/5313SobRV (SAM5313); ApraF/5314R (SAM5314); 5314FW/5316RV (SAM5315); 5322ComFW/5322ComRV (SAM5322); ApraF/MR2R (SAM5324); 5331MFW/5331MRV (SAM5331); 5333MFW/5335MRV (SAM5334); ApraF/5335CP

(SAM5335); 5339FW/5341RV (SAM5340); 5341MFW/5341MRV (SAM5341); 5342ComFW/5342ComRV (SAM5342); 5355RV/M13RP and 5355FW/M13FW (SAM5355); and 5352ComFW/5355ComRV (Δ SUG) (Figure S1 to S4). All PCR products generated were cloned into pCR-BLUNT for sequencing verification.

Plasmids pEM4HT5314, pEM4HT5315, pEM4HTSARP, pEM4HT5324, pEM4HT5331, pEM4HT5334, pEM4HT5335, pEM4HT5340 pEM4HT5341 and pEM4HTLuxR were introduced in *S. albus* J1074 mutant strains SAM5314, SAM5315, SAM5322, SAM5324, SAM5331, SAM5334, SAM5335, SAM5340, SAM5341 and SAM5342, respectively, by intergeneric conjugation and exconjugants were selected for resistance to apramycin and hygromycin. Production of paulomycins in all complemented mutant strains was evaluated by UPLC (Figure S5).

Table S1. Primers used in this work. Amplified PCR products were used in gene inactivation, gene expression and RT-PCR studies.

Primer	Sequence (5'-3')	Description
5355FW	AGAATTCACGATCAACGCCCTCAACTCCG	EcoRI ^[a,b]
5355RV	AATAAGCTTGTAGCGGATGCCGACCTGG	HindIII ^[a,b]
5309RplFW	GACTAGTAAGAAATCTCCCGGCGGATG	SpeI ^[a]
5312RplRV	AATATGCATGGTGGCGGTGACTTGGCT	NsiI ^[a]
5312RplFW	ATGGATCCGGCATCTGGGGTGAGGC	BamHI ^[a]
5315RplRV	GCGTTAACACAAGGACCACGAGACCTC	HpaI ^[a]
5310RplFW	GCACTAGTGTCTCTACCTCGTCTTCAA	SpeI ^[a]
5313RplRV	GTTATGCATTGTACCGACCCAGTCCTTCT	NsiI ^[a]
5313RplFW	TATTCTACATATGACCAAGTCGGCGTGATGGA	NdeI ^[a]
5315RplRV	AGGTAAACCTCGATGAAGGTGTGGACCA	HpaI ^[a]
MRA-F	GACGACTAGTATGCTGAGCCAAGTCACCGCCA	SpeI ^[a]
MRA-R	AATTATGCATCAACCCGGCACGAGAGGACTGC	NsiI ^[a]
MRB-F	GGCCCATATGGAACCCCTGAGAGAATCGCTTC	NdeI ^[a]
MRB-R	ATCTGATATCCGGAGGCGGTGTCGATCTTCCG	EcoRV ^[a]
MRS1-FW	AATTATGCATAGAGTTCGGCGCTGAGCGGTCC	NsiI ^[a]
MRS1-RV	ATACACTAGTAACGGATCGTTGCTGATCCCGG	SpeI ^[a]
MRS2-FW	ACGAGATATCCGCTGGTGGCAGAGCTTTACGG	EcoRV ^[a]
MRS2-RV	ATATCATATGGAGGCCATGCTGGCCGCCGACC	NdeI ^[a]
MR1F	ATACTAGTAGGTATCCGCCCGACCGCGTCACG	SpeI ^[a]
MR1R	ATAATGCATATGGCCGGCGACCCGGACCTCG	NsiI ^[a]
MR2F	ATTAGGATCCCCGCGGGGATACGCGCCGACC	BamHI ^[a]
MR2R	ACGTGATATCTTCGTGCACTACCCCATCGCCG	EcoRV ^[a]
MRG1F	ATACTAGTCAGCAACGATGCGCTCGGCCACG	SpeI ^[a]
MRG1R	ATCCTGCAGGTTACGGGCGGCGGTTGCCAGC	SbfI ^[a]
MRG2F	AATTGGATCCCGCGATCGCTGGCCGAGGAGGT	BamHI ^[a]
MRG2R	GCATGATATCTCGCCGAAGCCGACCTAGCCGC	EcoRV ^[a]
5312RplFW	CCACTAGTCCTTCTTGCGAAATCTCTGTC	SpeI ^[a]
5315RplRV	TCCATGCATCCTCAGCACGAAGAAGAAGCT	NsiI ^[a]
5315RplFW	TTAGATCTTCACGGTCTCCCTCGGCCTG	BglII ^[a]
5318RplFW	TGATATCAACGAGTGCAGGGCCACGCC	EcoRV ^[a]
5333RplRV	ATCACTAGTACACCATTCCCGCCGCCA	SpeI ^[a]
5331RplFW	CTATGCATGGCTTCATCGGTTTCGCAC	NsiI ^[a]
5331RplRV	ATGGATCCCCGACATATCCGCACGCG	BamHI ^[a]
5329RplFW	CGGTAACTGATGTGGGCCGCGCGGT	HpaI ^[a]
5330RplFW	CCACTAGTCTGATCCCATGCACGTGCTG	SpeI ^[a]
5334RplRV	GGCACTAGTAAAGAATGACACGCCGACGG	SpeI ^[a]
5334RplFW	TTAGATCTTCGGCAGCGGCGGTGGCAAC	BglII ^[a]
5337RplRV	TGATATCCGGTTCGGCGCCTTCGGCATG	EcoRV ^[a]
Rpl5338FW	TACTAGTACGGCGTTACCCGCGTCTG	SpeI ^[a]
Rpl5340RV	ATTATGCATGGTGGGGTTCGAGGTCGGTA	NsiI ^[a]
Rpl5340FW	ATGGATCCTGAACCCGGACTGGCACCC	BamHI ^[a]
Rpl5342RV	TTTGATATCTCTGCTGGAGGCGGCGGAT	EcoRV ^[a]
5341RplFW	GAAATGCATATCACCGGGAGAGGCGCC	NsiI ^[a]
5343RplRV	TCACTAGTGGTAGGGAGCCACGGTCC	SpeI ^[a]

5339RplFW	ATGATATCAGGCTCCGCTCCGGCCAT	EcoRV ^[a]
5341RplRV	AAGGATCCGCGCTGACCGGCGTCC	BamHI ^[a]
MRL1-FW	ATTGCATGCGGCGAGGGCGAAGAGCTGGGCC	SphI ^[a]
MRL1-RV	GTCACTAGTAGCAGCGCCCGGACGACGTTTCG	SpeI ^[a]
MRL2-FW	AACTGATATCGATCGTCGCCTGGGCGGAGCGG	EcoRV ^[a]
MRL2-RV	ATATGGATCCCGCCCGCACCCCTGAGCTGCTG	BamHI ^[a]
5349RplFW	AACTAGTCACGCTCGACGGCGCCAA	SpeI ^[a]
5352RplRV	AACATGCATTCCACCAGTAGGCCGCTG	NsiI ^[a]
5354RplFW	ATGGATCCCAAAGGCGCGTGCGGAGG	BamHI ^[a]
5357RplRV	GCGATATCCCGGACGTAGGTGAAGG	EcoRV ^[a]
5312MFW	CGTAGCGAGTGCGCGAGCGG	SAM5312 ^[b]
5312MRV	CCGCCGCCTGGGTAATCCTG	SAM5312 ^[b]
5313SobrFW	CCTATACTGGACGCCTCTGCGG	SAM5313 ^[b]
5313SobrRV	GGCAGGGACGGCGGAGAGG	SAM5313 ^[b]
ApraF	TCATCGGTCAGCTTCTCAACCTT	SAM5314 ^[b]
5314R	AAGGATCCATCGAAGTCCGAGCGGACATCG	SAM5314 ^[b]
5314FW	GCCAGCTCCACCAACGCGTC	SAM5315 ^[b]
5316RV	CGGTACCTGCCAGACGCCCA	SAM5315 ^[b]
5322ComFW	CACCGCGAAGGCCAGCCTC	SAM5322 ^[b]
5322ComRV	GAGAACAAAGACAGCCGGGGCG	SAM5322 ^[b]
MR2R	ACGTGAATTCTTCGTGCACTACCCCATCGCCG	SAM5324 ^[b]
5331MFW	ATGCGTCCAAGGTGCAGTCCCG	SAM5331 ^[b]
5331MRV	CGGGTTGCCGGCGTAGCTGAGT	SAM5331 ^[b]
5333MFW	AAGCTGGCCGAGGGGCTGTC	SAM5334 ^[b]
5335MRV	GAGACGCACACTCCCGGCAG	SAM5334 ^[b]
5335CP	ATGTTTCCTCTTCGTACGGGGCGG	SAM5335 ^[b]
5339FW	ACAGCCACCCCGTGACCGCG	SAM5340 ^[b]
5341RV	GTCGAGGAGGGACGCTGCGT	SAM5340 ^[b]
5341MFW	GGTGGATTCTCTTGCCGCTTCC	SAM5341 ^[b]
5341MRV	CCACCGTCCGACCGACTGGAGT	SAM5341 ^[b]
5342ComFW	CGGGTGGTTGGGACGGCTCC	SAM5342 ^[b]
5342ComRV	GCTCTGCTGGAGGCGGCGGA	SAM5342 ^[b]
M13FW	TGTAAAACGACGGCCAGT	SAM5355 ^[b]
M13RP	CAGGAAACAGCTATGACC	SAM5355 ^[b]
5352ComFW	TACCGCGACGAGAGAGGATGTG	ΔSUG ^[b]
5355ComRV	GACGACTACGCCGACTTCCTCA	ΔSUG ^[b]
5314SobFW	TAGGATCCGCTAGCAGAACACCGCGCCATGAT	BamHI ^[a] <i>sshg_05314</i> ^[c]
5314SobRV	CGCAATTGTCGGTCCGCCTGATGTCCCG	MfeI ^[a] <i>sshg_05314</i> ^[c]
5315CFW	GTGAATTCGTTGATACGTTGCGCGCG	EcoRI ^[a] <i>sshg_05315</i> ^[c]
5315CRV	TGAATTCGGCAGGGACGGCGGAGA	EcoRI ^[a] <i>sshg_05315</i> ^[c]
SarP2FW	CATGACGGATCCGATTCCCTGAGCGGAGTACG	BamHI ^[a] <i>sshg_05322</i> ^[c]
SarP2RV	CTAGCAGAATTCACGACATCGCGAACGCCTGT	EcoRI ^[a] <i>sshg_05322</i> ^[c]
5324CFW	ATAGATCTCGTGTCGTGGGCTCCTGC	BglII ^[a]

		<i>sshg_05324</i> ^[c]
5324CRV	ACGAATTCAGCCGGTCAGCCGCAG	EcoRI ^[a] <i>sshg_05324</i> ^[c]
5331CFW	ATGGATCCTGCCGGCGTAGCTGAGTT	BamHI ^[a] <i>sshg_05331</i> ^[c]
5331CRV	GGCGAATTCGGATATGTCGGAGTTTCTGA	EcoRI ^[a] <i>sshg_05331</i> ^[c]
5334CFW	CGGAATTCGGCAGAGCATACTTTCGC	EcoRI ^[a] <i>sshg_05334</i> ^[c]
5334CRV	GCGAATTCGGATGGACAAGGAACAGG	EcoRI ^[a] <i>sshg_05334</i> ^[c]
5335FW	AAGGATCCACGAGGCGTCACGCGTTC	BamHI ^[a] <i>sshg_05335</i> ^[c]
5335RV	GTGAATTCTTGCGTGACACCGCCCCC	EcoRI ^[a] <i>sshg_05335</i> ^[c]
5340CFW	AAGGATCCCCTAGGAGACCCTCCATGCCCCTG	BamHI ^[a] <i>sshg_05340</i> ^[c]
5340CRV	GGAATTCGCTAGCGGTGCCAGTCCGGGTTC	EcoRI ^[a] <i>sshg_05340</i> ^[c]
5341CFW	ATGGATCCCCTAGGACCGAGTGCTCTGGCCT	BamHI ^[a] <i>sshg_05341</i> ^[c]
5341CRV	TGAATTCGCTAGCTCGCTTTCCCGTATTTGCTGGGC	EcoRI ^[a] <i>sshg_05341</i> ^[c]
LuxRFW	CATGACGGATCCCGTGGCCGCCGACGGACATC	MunI ^[a] <i>sshg_05342</i> ^[c]
LuxRRV	CTAGCACAATTGGACAGGCGCCGGCCGACCCG	BamHI ^[a] <i>sshg_05342</i> ^[c]
5312-F	AAGTCACCGCCACCCGCTAC	<i>sshg_05312</i> ^[d]
5312-R	GGAAGTCGTACCGCTTGGTCAC	<i>sshg_05312</i> ^[e]
5313-F	TTGACGGTCGACAGGATTACCC	<i>sshg_05313</i> ^[d]
5313-R	AGCAAATGCAGGCCCTTGATG	<i>sshg_05313</i> ^[e]
5314-F	TGCTCCTGATGTGGTGCTGATG	<i>sshg_05314</i> ^[d]
5314-R	TGATCCGGCGATTCTGTCTC	<i>sshg_05314</i> ^[e]
5315-F	CTCTGTCCTTCAACGGCGACTG	<i>sshg_05315</i> ^[d]
5315-R	CGGGGAAGAGGTAGACGAACCA	<i>sshg_05315</i> ^[e]
5353-F	CGCGCGAACGGCTGACGAAGTTG	<i>sshg_05353</i> ^[d]
5353-R	GGCTGGTAGAGGTCCAGGTCGAAG	<i>sshg_05353</i> ^[e]
5354-F	GTGTTACGCCCCAGGTCTTC	<i>sshg_05354</i> ^[d]
5354-R	CGCCAGGCAGGTCTCGTACTC	<i>sshg_05354</i> ^[e]
5355-F	TGGTCGACTTCGGCCTGTACTTC	<i>sshg_05355</i> ^[d]
5355-R	CGGTCCTTGTCGTCCTTGACCT	<i>sshg_05355</i> ^[e]
5356-F	GAGGAAGGGGACCTGTGTCTGC	<i>sshg_05356</i> ^[d]
5356-R	GTCGACGATGTTCCGGGTAGGC	<i>sshg_05356</i> ^[e]

[a] Restriction site in bold in primer sequence

[b] Primers to verify mutant strains by PCR

[c] Primers to amplify genes used for ectopic expression

[d] Forward primer used for RT-PCR studies

[e] Reverse primer used for RT-PCR studies

Figure S1. PCR analysis of SAM5355, SAM5312 and SAM5313 mutant strains. Chromosomal DNA from each mutant was used to verify the correct location of the resistance marker. The oligoprimers used are listed at Table S1. Lambda DNA digested with *Pst*I has been used as molecular-weight size marker. All PCR products generated were sequenced.

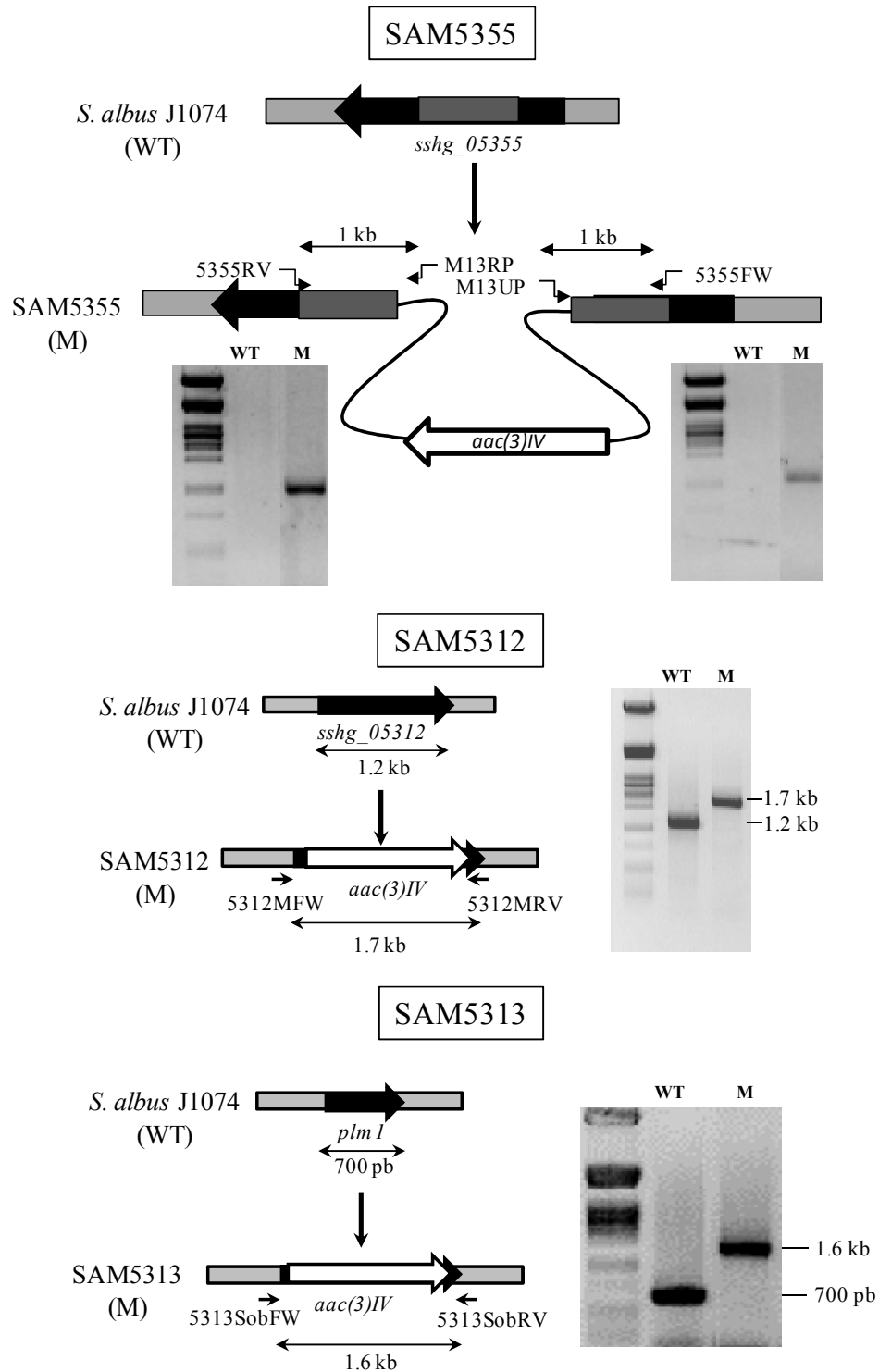


Figure S2. PCR analysis of SAM5314, SAM5315 and SAM5322 mutant strains. Chromosomal DNA from each mutant was used to verify the correct location of the resistance marker. The oligoprimers used are listed at Table S1. Lambda DNA digested with *Pst*I has been used as molecular-weight size marker. All PCR products generated were sequenced.

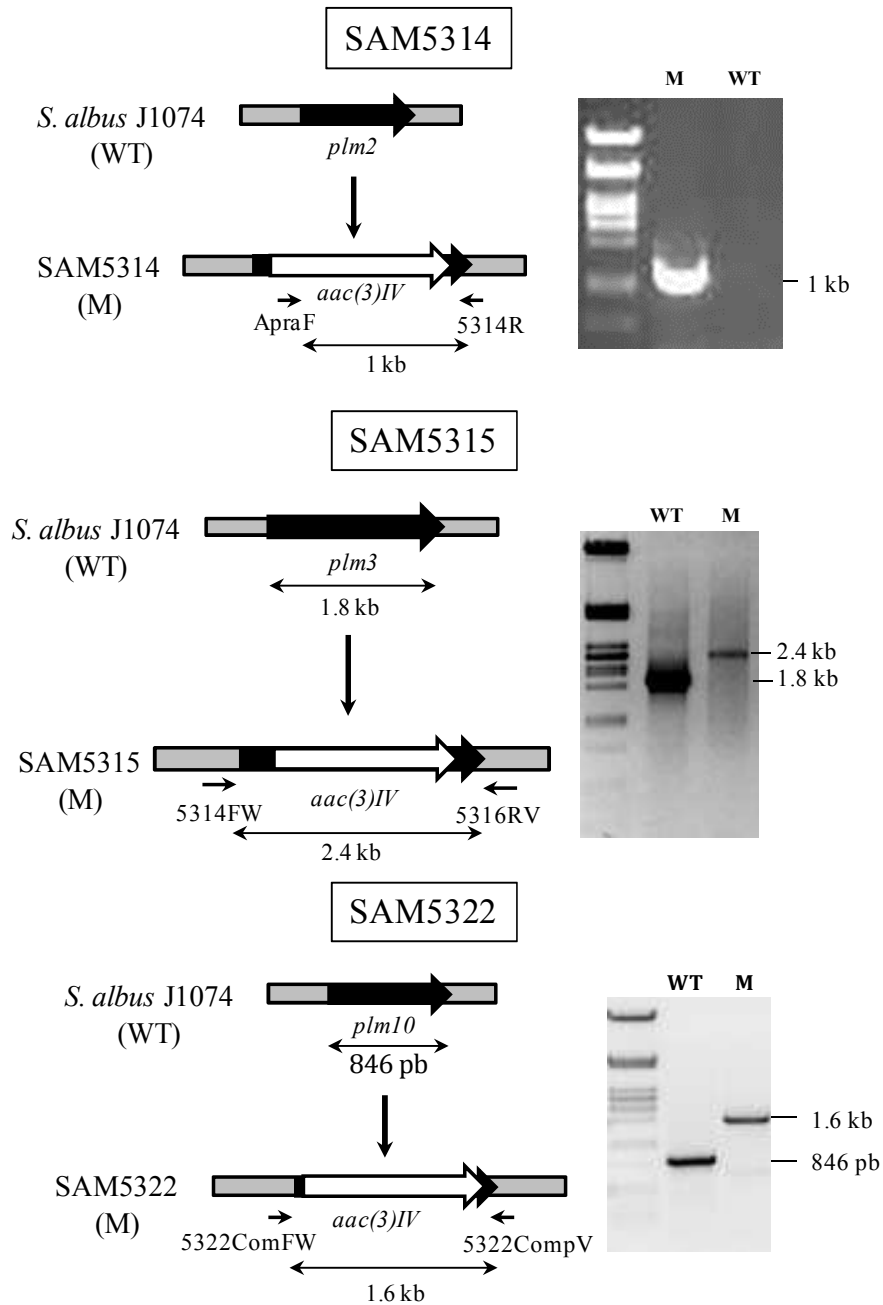


Figure S3. PCR analysis of SAM5324, SAM5331, SAM5334 and SAM5335 mutant strains. Chromosomal DNA from each mutant was used to verify the correct location of the resistance marker. The oligoprimers used are listed at Table S1. Lambda DNA digested with *Pst*I has been used as molecular-weight size marker. All PCR products generated were sequenced.

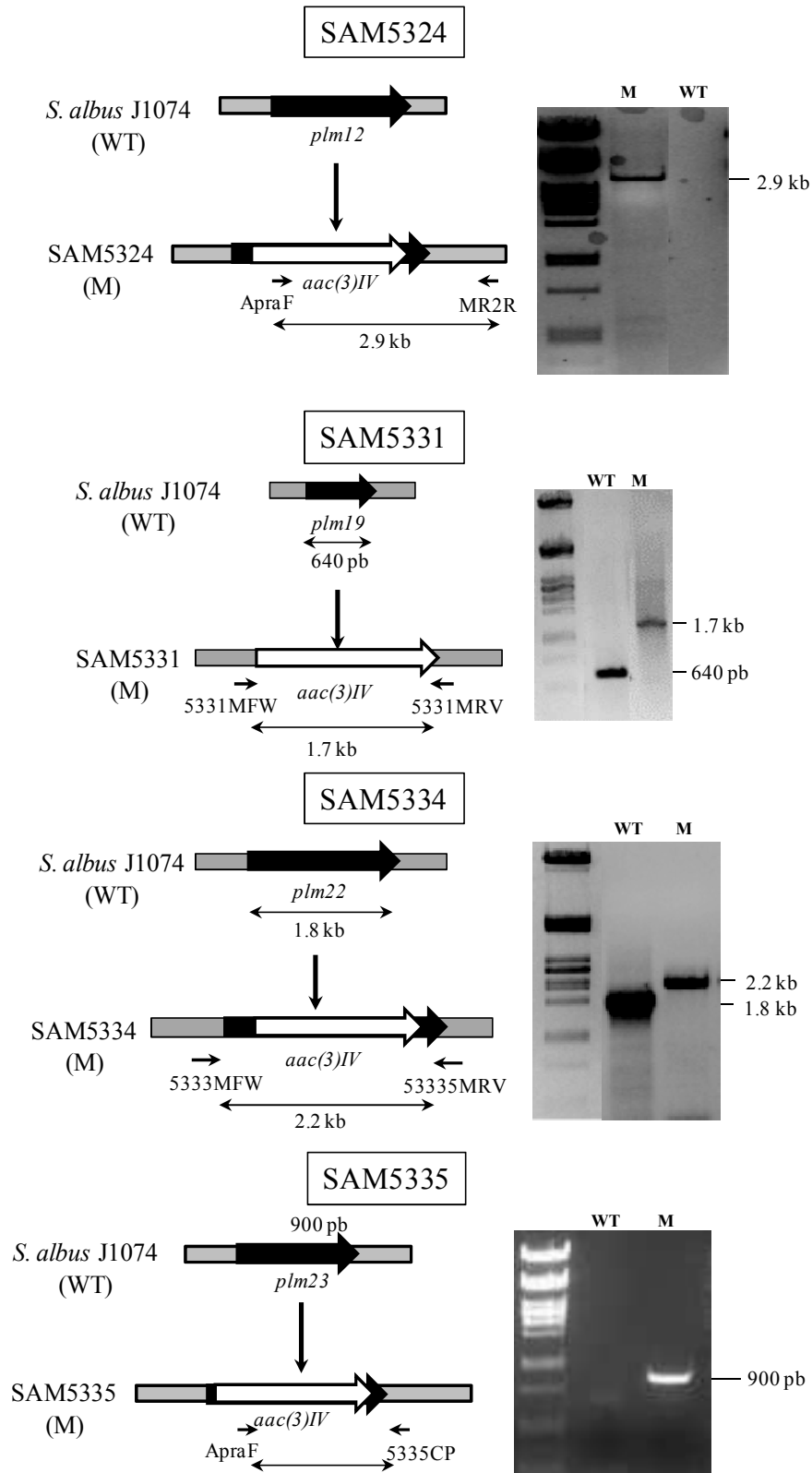


Figure S4. PCR analysis of SAM5340, SAM5341, SAM5342 and Δ SUG mutant strains. Chromosomal DNA from each mutant was used to verify the correct location of the resistance marker. The oligoprimers used are listed at Table S1. Lambda DNA digested with *Pst*I has been used as molecular-weight size marker. All PCR products generated were sequenced.

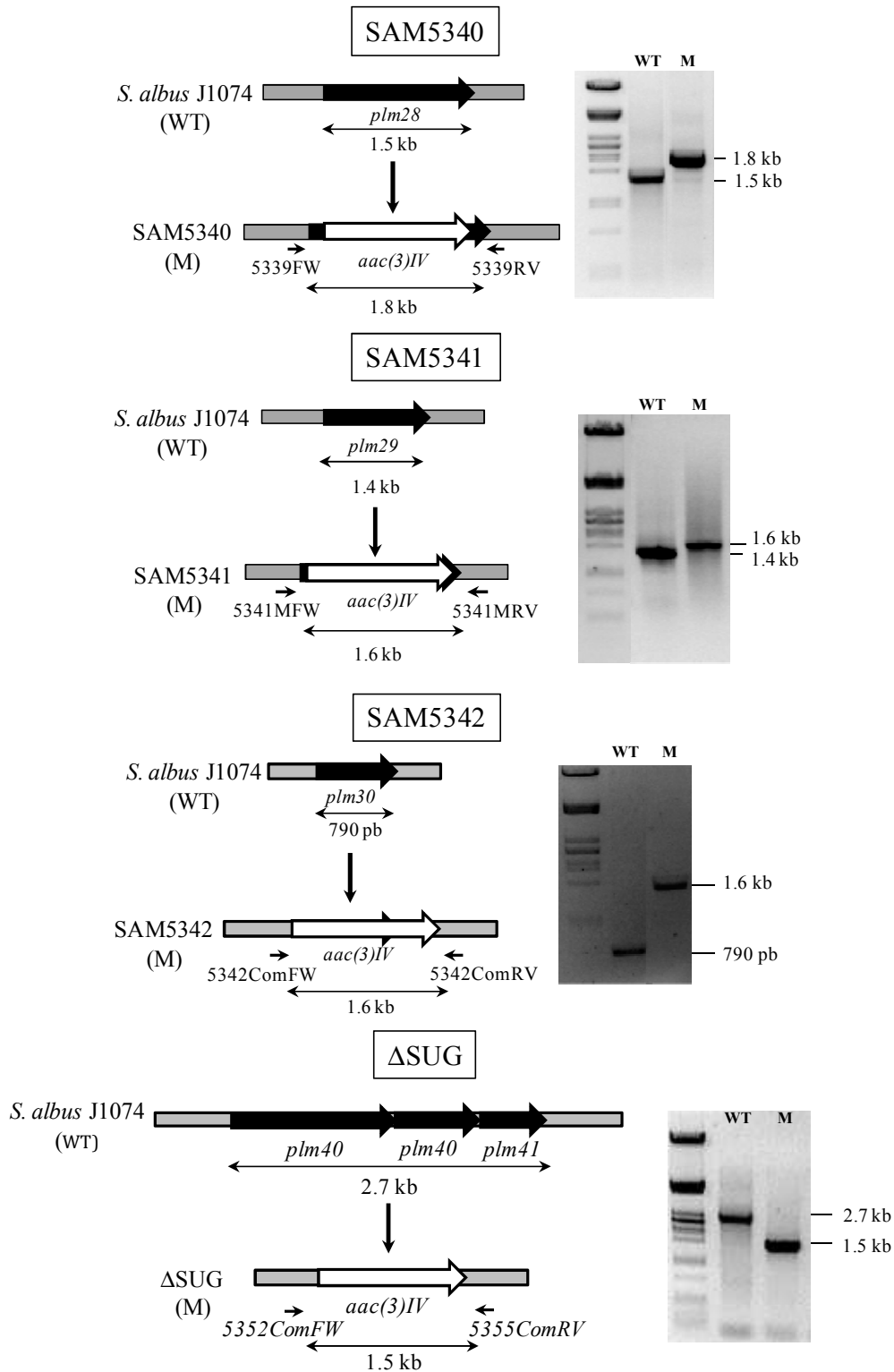


Figure S5. Genetic complementation of *S. albus* J1074 mutant strains. UPLC traces of paulomycins and paulomenols from each mutant strain complemented with the appropriate plasmid. Each plasmid is expressing the appropriate intact gene under the control of *ermE***p*.

