

Figure S1. Genetic organization of the FR-008 pathway

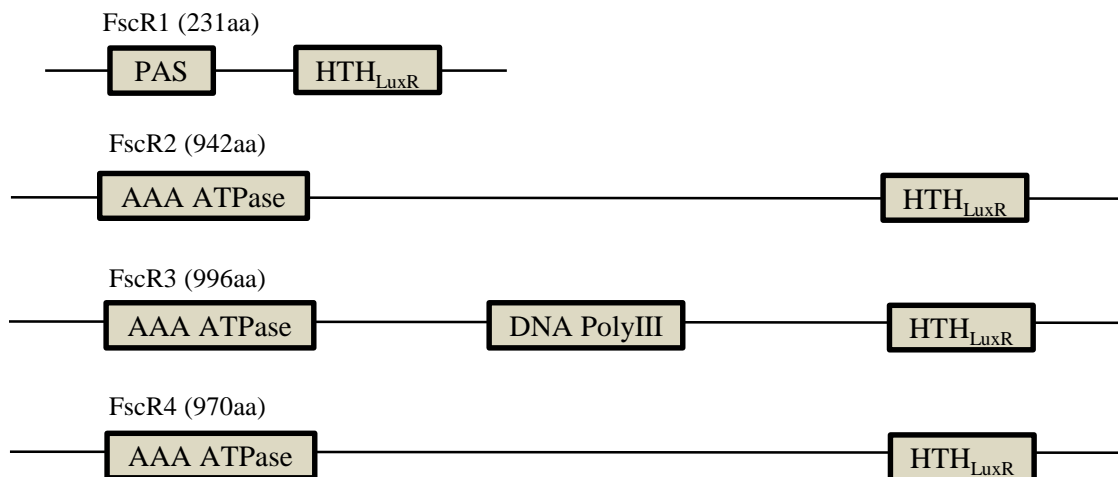


Figure S2. Predicted functional domains for FscR1, FscR2, FscR3, and FscR4. PAS, PAS-like domain; HTH_{LuxR}, helix_turn_helix of LuxR-type; AAA ATPase, Predicted ATPase; DNA PolyIII, DNA polymerase III subunits.

FscRII 1 - **VLLERTTEFLALVDAALGAATEGRSSLVLLTGP LGI** GRSALLQRLSCLADDRDDVRLRA
 NysRIII 1 - **VLLERENELARTRAALDAEAGDSSLTLLI** NGPLCSGRSALLRRIPPELAG- - DGTRVLR
 AmphRIII 1 - **VLLERGAELAAVRQAALSAAAEGRSSHLLVLAGPFCM** GRSTLLHELPRCADEG- TVHVR
 SalRII 1 **VLLERDDLDEAAATLRRAAAGESSLVVOGALGV** GKSTFLEALSELG- REEDTLLRA
 orf1 1 - **VLLERTTEFLALVDAALGAATEGRSSLVLLTGP LGI** GRSALLQRLSCLADDRDDVRLRA
 NppRIII 1 - **VLYDRDP ERLCLRDVAVAGAADGRGAALVVG** GVGITGRITALLDAAAGAAATGTG- LTVLRA

FscRII 60 **HAAPMEQDFAFGVVROLFETLLGDS** PEDARDRWDAHAS- FARHVLADDAAPPAGDCAI A
 NysRIII 58 **SAAWRERDFEFGIAROLF** DHLLSAG- GAGPAERTAGAE- HFSRLMDTGDRPTGTGPAL E
 AmphRIII 59 **NAAPAEQDLAVGVVROLFDSLLTAAS**- GPDREQLADAG- AARRVDDVVP- - - DLEA
 SalRII 60 **QAAAEEESFGLGVVROLFVPLAAAS** TEQARSWRRAAAEGAPPEVRAI AESGLEAPWSAL
 orf1 60 **HAAPMEQDFAFGVVROLFETLLGDS** PEDARDRWDAHAS- FARHVLADDAAPPAGDCAI A
 NppRIII 59 **AAALVERDFEHGVAROLFDPMLAA** AGRGTRSRWLAAGGG- ELPAALAAEPVDP- - - PAPE

FscRII 119 **ATEAVLHGLLSLLANVS** ADSRLLI LVDDLQWS DVPSLRWLTYLAKRLHGLRAVVVCAIRD
 NysRIII 116 **VSQAVLQGAQALLADAS** AERLLI LVDDLQWADGPSLRWL AHTLRRLHGLRALVCTLAD
 AmphRIII 113 **PDCAYLRDLRLLAGLS** ATAPLLI LIDDLQWADAGSLRWLAGAPRLDFGLRMVVCRLRD
 SalRII 120 **TVQGAPRWLAALV** DAMAGGRTLVVI DDLHWCDAESLKVLI HTLACRGGMRI VFAVSVLT
 orf1 119 **ATEAVLHGLLSLLANVS** ADSRLLI LVDDLQWS DVPSLRWLTYLAKRLHGLRAVVVCAIRD
 NppRIII 115 **VRHGRVRELQELLE** TVSAERPVLVLDLQWADTASLRWLNRLAARVPELPVALVGTIVLH

FscRII 179 **GDPRS** HHTLVREIREAATQTLRPASLSLTATHELVREHFGEAGDDEFVQACHEASVGNPL
 NysRIII 176 **GDHRGRYP** LREVAGA AHTVLRDAPLSRDATRVLLAGPQGRPPQDAIVRAVYEASRGNPL
 AmphRIII 173 **GDPGARDP** LVEVVS TAVRVLRFVPLSP AATGELVEAYLGDS PDEAFVRACHDLSRGRPL
 SalRII 180 **GDV** RGNRCYHEDVLALADRTCVLRPLGGDSTRRLVEESCGAPAEDEFFVEAFRVTRVGNPL
 orf1 179 **GDPRS** HHTLVREIREAATQTLRPASLSLTATHELVREHFGEAGDDEFVQACHEASVGNPL
 NppRIII 175 **GDPG** AGRALVRGLAGSATP- LRTRPLGDAAVRGGVADRLGRDGEPEFVQACRDVSACTPS

FscRII 239 **FLLSI** LVGTGFLGRRPLAEHAETARRLRPSQLRERLASI LRTOPAPVRDLAAAI AL LGEQ
 NysRIII 236 **FLT** AFRSALRATGRPPGGDHFCAVRELSPTVLRRLAGHLRI OPQVREVAVAVAALGDH
 AmphRIII 233 **FLRSM** LSELALTCGRPLAENAGLVRTARPTHLRDLNCLLLOPRPVRDAAAALATLAGEQ
 SalRII 240 **L** GALVDEAQRGLRPTAAQAPMVSLRPENVRQRLAGFLRSQPDHLRRAAHALTVLGPD
 orf1 239 **FLMSI** LVGTGFLGRRPLAEHAETARRLRPSQLRERLASI LRTOPAPVRDLAAAI AL LGEQ
 NppRIII 234 **VL** HAVLDDVAATCGCP LAVHAGRVREALPAALRERFARCLRAQDDPARRYLGALAVLGTD

FscRII 299 **SDAPT** LARLAGLDSTGYAGALRALGALGALAAPDEPRFI HRSVRDAAESTLTMVQERMH
 NysRIII 296 **SDP** VLLAQLAGVDEI GFAGARRALVDAGLLARGDRVRFVHGVRDVAVDSLLTLDERERSH
 AmphRIII 293 **EBP** ALLTRLAGLDDVGTTFALVRLHQLGLTAEERARFAHPAVRIVECSMSAAERQCWH
 SalRII 300 **SDPE** LLADLAE LDSAQCAEALRVRLAGLVAEDGWLTSTGSLRDLLELLESMPEERTAMR
 orf1 299 **SDAPT** LARLAGLDSTGYAGALRALGALGALAAPDEPRFI HRSVRDAAESTLTMVQERMH
 NppRIII 294 **ADP** MTVQRLADLDPHGLRTVQGGQLAEQGLLS- EDHTGF AHPLVREVATG- - - PAERERLH

FscRII 359 **DEAAAL** LYAAGSPAEQVAAQLMAVVTTRRQPWAVDVLRAAADTALRRGAP- - - - - - - - DT
 NysRIII 356 **DDAAD** LLYRCGRPAEQVAGHLAVVHPGRP WSEAVLRSAAHNALRAGRP- - - - - - - - AD
 AmphRIII 353 **EAAAE** VLYESGLPAEQVAAQLVAIP TARYPWAVPLRSADAARRRGTG- - - - - - - - EP
 SalRII 360 **SVAAE** LLHRSCHSAELAAANHLNSTLTLPGDKAVDILRTAARSALTRAST- - - - - - - - RD
 orf1 359 **DEAAAL** LYAAGSPAEQVAAQLMAVVTTRRQPWAVDVLRAAADTALRRGAP- - - - - - - - DT
 NppRIII 350 **LRAAW** LTHETGHHVREVAVHLVAVAPLDDWAVEVLRAAAHQAATT AHRPDSADHDGDP A

FscRII 410 **AAGYL** CRALLDSP AAGVGRGRLVVELCTAERGFDP LACERHI AQAVTLLPEPRDRAVAAAL
 NysRIII 407 **AARYL** RRALLHHRTQDGCRAHLLVLELATAERALDPAACVRHVSQAVALLDTSRDAAAVL
 AmphRIII 404 **AARYL** RRALLEVAEQDRKRAHLLVLEL ATVERSFDPAAESHI AQALPLLPAARDRAAVL
 SalRII 411 **AARYL** RRALLEVTSLTGPDRAGLLTELAGAERSFATAASLRHVVEAVPLLDVSRRAAAVV
 orf1 410 **AAGYL** CRALLDSP AAGVGRGRLVVELCTAERGFDP LACERHI AQAVTLLPEPRDRAVAAAL
 NppRIII 410 **AVRYL** RRALLDSDVTGHERGVLLVLELAAAERFTEP GTAVRHVSQAVALLDTSARDRATALT

FscRII 470 **RI** SPTALGPAPLTAVDLLRQAAEDLG- PADG- - LTGTDRLDALRLEARLRHCGHEDPHIEL
 NysRIII 467 **RI** PPSLLAAPS SVAVELVRQAAAGLDEPGQR- - DEEGADELALRLEAWLRHSGHENPVEL
 AmphRIII 464 **RI** SPWTSPTMRSGRALFRRAADDLRDPYTR- - DDP- GAEEVLRLEARLRYTGI DDYAQL
 SalRII 471 **RI** GPTELLMDPSAFRVDAMVREVAEEL AASGTK- - - GPEERELALRLCAREHVS AQDPFAHI
 orf1 470 **RI** SPTALGPAPLTAVDLLRQAAEDLG- PADG- - LTGTDRLDALRLEARLRHCGHEDPHIEL
 NppRIII 470 **LI** DPSTVRDAIDPVRDAVRAADRIDRDGCGTGP ADDDGRAVTVRI RARARRLDEQRPEGL

Figure S3 Amino acid alignment of FscR2 with its homologous proteins. Numbers indicate amino acid position from the N terminus of the protein. Identical amino acid residues are shaded.

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FscRI I 1 ----- M P P I E L P L L E R D R E L A A L S A V I G E L G S G R P A V V T V T G E P G
NysRI I 1 M P R S K A R N O P T T C T P Q C A P D A H G D P T M L L E C G R E O R L I G D L L H R L G Q G R P S V L S L T G R P G
AmphRI I 1 --- M T G S T P S P Q P L Y O A R P A T A T A P P A G V R A R E R A T Y A A V V D C L G T S G P V L V T L S G R S G
SalRI 1 ----- M T V V T G E R S P H G S G K T G A P P E L V E R E R E L S V I T E A A R R A A A C S P G L I V L E G P D G
Orf2 1 ----- M P P I E L P L L E R D R E L A A L S A V I G E L G S G R P A V V T V T G E P G

FscRI I 41 L G O N D L L R W A A A Y A T D A G L R V L S A H A T P A E H E V R Y G V V A Q L L A E E N - - R A L A P R L F L T D -
NysRI I 61 H A Q N A L V R W G A C R A R H D G L R V L R A C A T P A E R E L R Y G A V L Q L L A V L D G P H G S T L D A A I R H D
AmphRI I 58 F G G S A L V R L A A D R A R A A G L R V L R A R V T P A E S E L P Y T A M T Q L E P L D - A R P A E R P A T S O G
SalRI 55 I G K S A L L R A L V G - A A P A L H V L T A R A E P E Q O O T P L S L A R R L V A P L A E A H R A D F A L P W P V H
Orf2 41 L G O N D L L R W A A A Y A T D A G L R V L G A H A T P A E H E V R Y G V V A Q L L A E E N - - R A L A P R L F L T D -

FscRI I 98 - E Q P G G L P G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
NysRI I 121 G P P P L P V P G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
AmphRI I 116 P N R R E P F P G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
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Orf2 98 - E Q P G G L P G - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

FscRI I 138 A L A R R L - - - - P R A P V A L L T S T T I G T A L T R P E W S V G T P L T G L A T T I E L A L P P L T S G G T A T A V
NysRI I 162 T L L R H L G P D T P L A V L A S S C G D T T A F D T D P K A P A V P G P D T V P V A R F V V P A L T D R G V A A T V
AmphRI I 157 A L L R R S - - - - A G T P L A V V C G G N D T A A A D P C W O S A L G S V P G P L A H H L T L A P L P P E E V A A E V
SalRI 173 H T A R R L P G - O P V L L A L S C R G G H A S A S L D E I A A Q P - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Orf2 138 A L A R R L - - - - P R A P V A L L T S T T I G T A L T R P E W S V G T P L T G L A T T I E L A L P P L T S S G T A T A V

FscRI I 194 L R A F G A P G D P A F T E A L A E A T R G I P A V V H D V L D R F A R A C Y S P R A D R I D P L R A L T A E V V G D H
NysRI I 222 R A V C G T P G D E E F I A A L T S A T A G N P A I L R D A L R A F V D H G L P A D A D H L P E L H A L T A G V V G D H
AmphRI I 213 A R V C G - P A D D A F T A E A R R I S A G H P T V L H D L V Q R F A A L C H R P A A D R V P A L R A I G T E V L G D Q
SalRI 225 Q A L T G S A G A C F O G S C L A V T G G P L L V T R L V S A L R E N G L D L T V E N I T A V D G O G A O S F R S R
Orf2 194 L R A F G A P G D P A F T E A L A E A T R G I P A V V H D V L D R F A R A C H S P R A D R I D P L R A L T A E V V G D H

FscRI I 254 A T R A L S D L R D P A A V D A L R A L A V C G D L L D F P L V C T L A G P H S V S E S R L R A A L A A S G L T T L R D
NysRI I 282 T V R A L D G P - A E V N A V L R A L A V C G D L L D F H R V R A L A G A H S L S E D R I R T L L A S V G L T V S V G
AmphRI I 272 L L Y A V R G L G - P E A G E V V R A L A V C G D L L D L Q V V R T L T R V R S F N E N R L R A A L A R T V G L R T L P
SalRI 285 I A H L S Q O P - D S V L R A V R A M A V L G D G T P T D L C G R L A T L D E P A F A O S L F T L N S L G L V G F T A
Orf2 254 A T R A L S D L R D P A A V D A L R A L A V C G D L L D F P L V C T L A G P H S V S E S R L R A A L A A S G L T T L R D

FscRI I 314 G H - P R V Q D A V V R A R V L E E M P A A D R A E L Y A R A A G L A Q R V A A D D O G I A D L L L L A R P V G - D P
NysRI I 341 D K - - V H I R F P A S K A R V I E D M P A A R A D L V Y R A A E L T H S C G V N D E V A H L L R S S P L G - A P
AmphRI I 331 N G - - R L S V E P E L R V R V L E E M T A D D R T E L H I R A A E L A H R A G A D R A V A E L L L A A R P T G - S A
SalRI 344 G A G A W S F T E P V V R E A V L D G I G D R E G G A A H G R A A R L L H D S G A P T A E V I A Q L L R S Q G T P T E P
Orf2 314 G H - P R V Q D A V V R A R V L E E M P A D R A E L Y A R A A G L A Q R V A A D D O G I A D L L L L A R P V G - D P

FscRI I 371 W A V D T L R R G F T S A L R C G R R D L A V A Y L A R A L D E P L A A E D R A R I E F Q L A S V E N V T A P I A A E R
NysRI I 398 W V P L L R R G F A A A L R R E D H H R A C A C L S R A L C E P L D P R E R S L T L E L A A A E A V A R P E A G D R
AmphRI I 388 W A T R T L R R D A E H A V R E C E H A R A A V L L A R A S E B E Q D P A E H A R L G L E L A A V O L I I E P E A G D L
SalRI 404 W A T T L L R E A A R A M L A S R P E R A V E L L R P C V P E G R E N E C S P A L T E L G V A E G R V D P E A A V G
Orf2 371 W A V D T L R R G F T S A L R C G R R D L A V A Y L A R A L D E P L A A E D R A R I E F Q L A S V E N V T A P A A A E R

FscRI I 431 R L G G L I R - - - - A T R P C P G A G L R A R A T D L C L L C G D T R A A R H A L A G A I D S A P A A P E P R R G P
NysRI I 458 R L G E L V R S T V A D T D P T S S G E G V G V R A I D L C F A R G N S E W V R R T A G E A L P Y A G P A D - - - - -
AmphRI I 448 R I E R L I R - - - - T - - P C T P A A V R L Y A A D L G L T A G G S E T V S R S L A D A L T V T S G T E - - - - -
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Orf2 431 R L G G L I R - - - - A T R P C P G A G L R A R A T D L C L L C G D T R A A R H A L A G A I D S A P A A P E P R R G P

FscRI I 486 T G A P A V P P E A E P A S T P R T H L V P H R P Y A G T P P G A T G P D C R R S A A P D L P G T P R A H D A P R S P G
NysRI I 512 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
AmphRI I 495 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
SalRI 517 - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Orf2 486 T G A P A V P P E A E P P S T S R T H L V P H R P Y A G T P P G A A G P D C R R S A A P D L P G T P R A H D G P R S S G

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Figure S4. Amino acid alignment of FscR3 with its homologous proteins. Numbers indicate amino acid position from the N terminus of the protein. Identical amino acid residues are shaded.

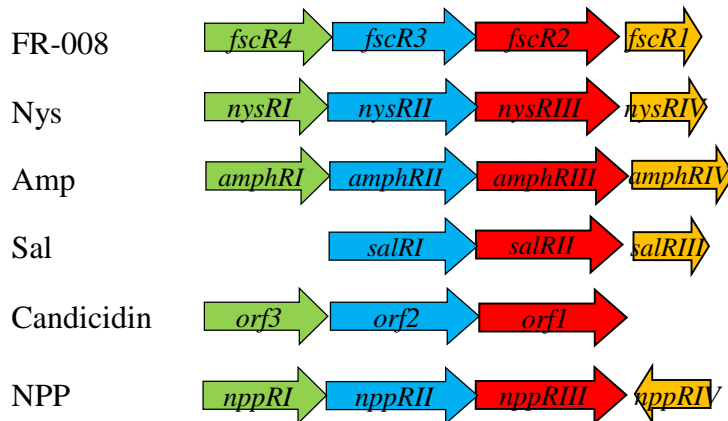
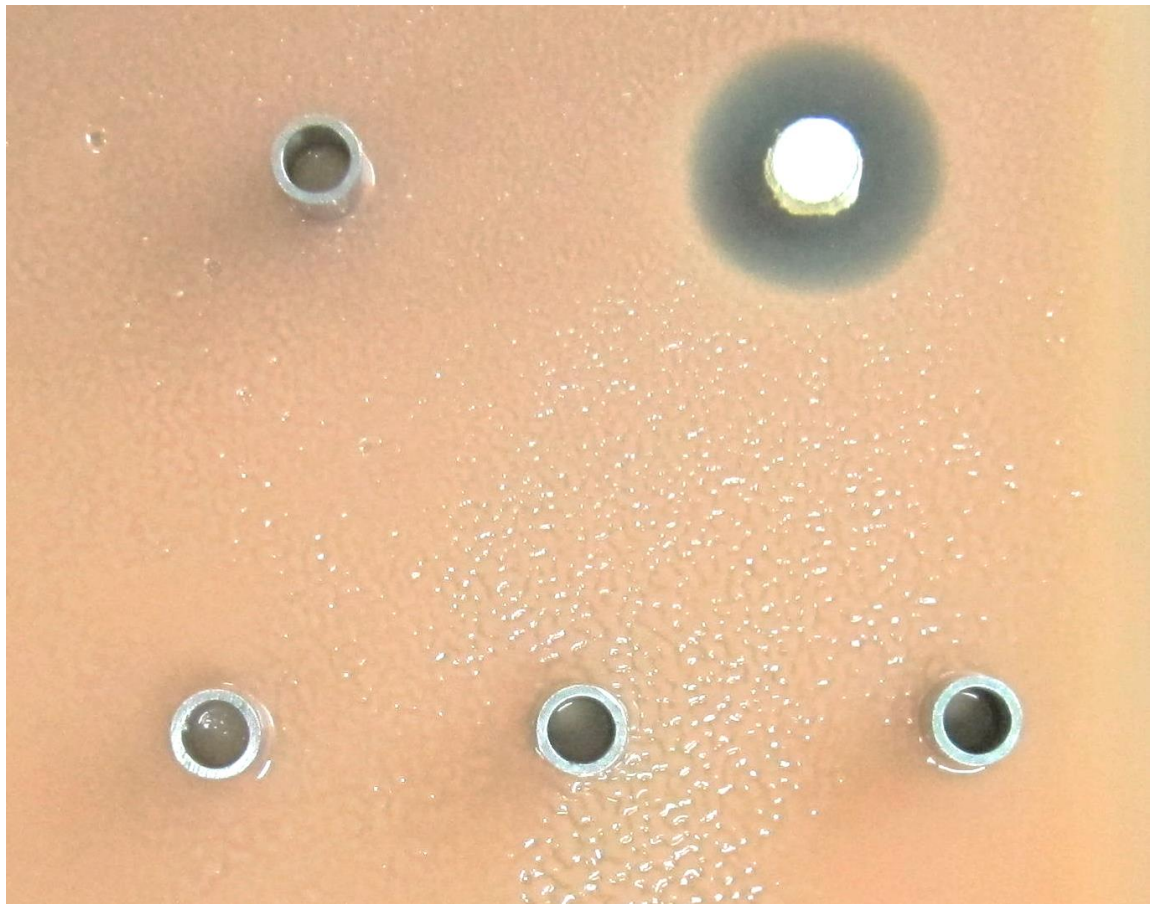


Figure S5. Genetic organization of the four regulatory genes *fscR1-fscR4* in *Streptomyces* sp. FR-008 and syntenic clusters in different polyene pathways. FR-008, FR-008 pathway in *Streptomyces* sp. FR-008; Nys, Nystatin pathway in *Streptomyces noursei* ATCC 11455; Amp, amphotericin pathway in *Streptomyces nodosus*; Sal, salinomycin pathway in *Streptomyces albus* CCM 4719; Can, candicidin pathway in *Streptomyces griseus*; Npp, the nystatin-like *Pseudonocardia* polyene pathway in *Pseudonocardia autotrophica*.

Methanol

FR-008



Antimycin
(0.1 µg/ml)

Antimycin
(1 µg/ml)

Antimycin
(10 µg/ml)

Figure S6. Sensitivity test of the indicator strain *Rhodotorula rubra* to antimycin. One-hundred microliters of different concentrations of antimycin, prepared from a stock solution, or 100 µl methanol (solvent control), was loaded into steel cylinders positioned on the medium seeded with *Rhodotorula rubra*. FR-008, wild-type *Streptomyces* sp. FR-008.

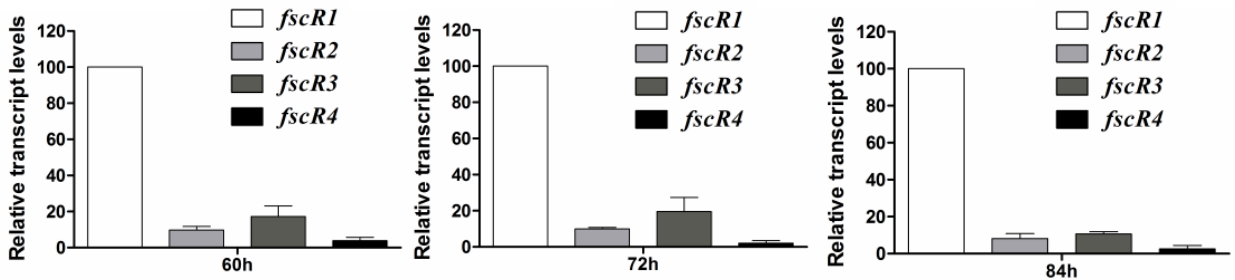


Figure S7. Comparison of the relative expression of *fscR1*-*fscR4* in the wild-type strain during growth in YEME liquid medium. In this assay, RNA samples were isolated at the indicated times, expression of *hrdB*, which encodes the major sigma factor, was used as an internal control, the expression level of *fscR1* at each time point was arbitrarily set to 100, and the y-axis shows the fold change in expression of each gene at the indicated times over the expression of *fscR1*. Results are the mean (\pm SD) from triplicate biological experiments.

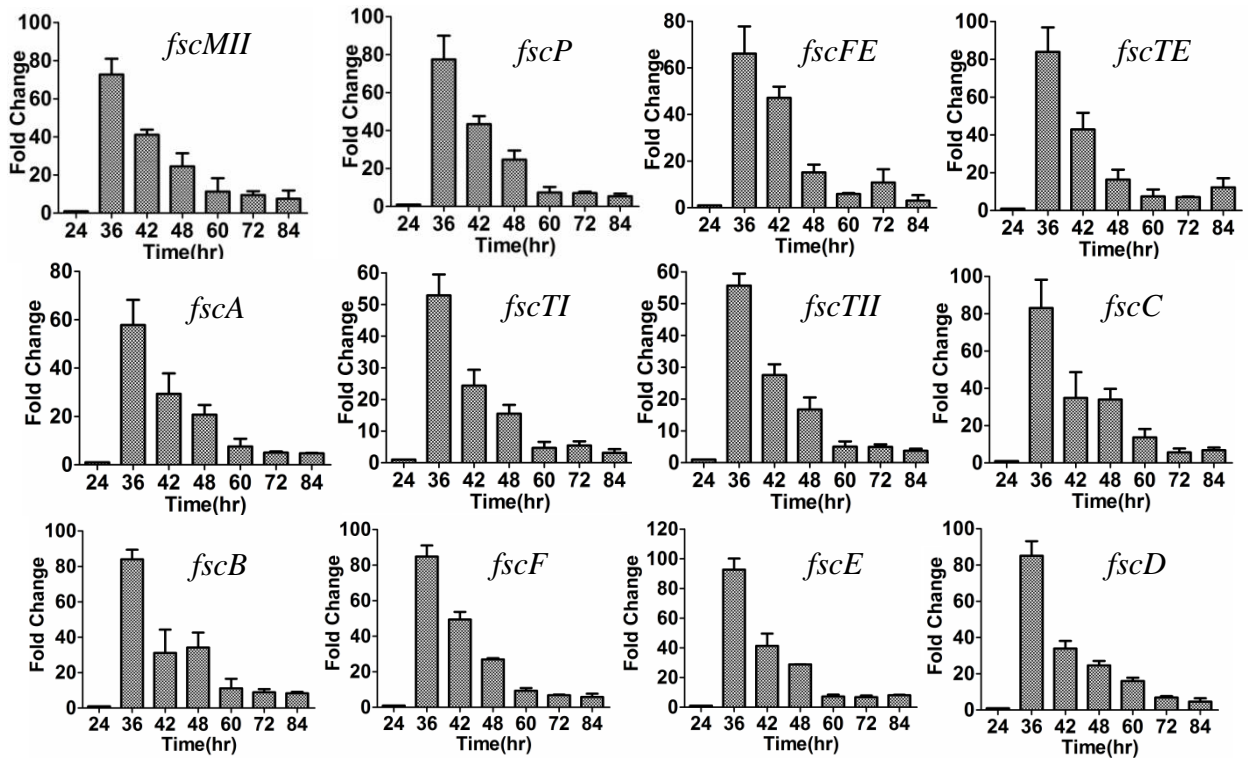


Figure S8. Temporal expression pattern of structural genes of the FR-008 pathway in the wild-type *Streptomyces* sp. FR-008 strain cultured in YEME liquid medium. RNA samples were isolated at the indicated times. Expression of *hrdB*, which encodes the major sigma factor, was used as an internal control. The expression level of each gene at 24 h was arbitrarily set to one. The y-axis shows the fold change in expression level at the indicated times over the level at 24 h. Results are the mean (\pm SD) from triplicate biological experiments.

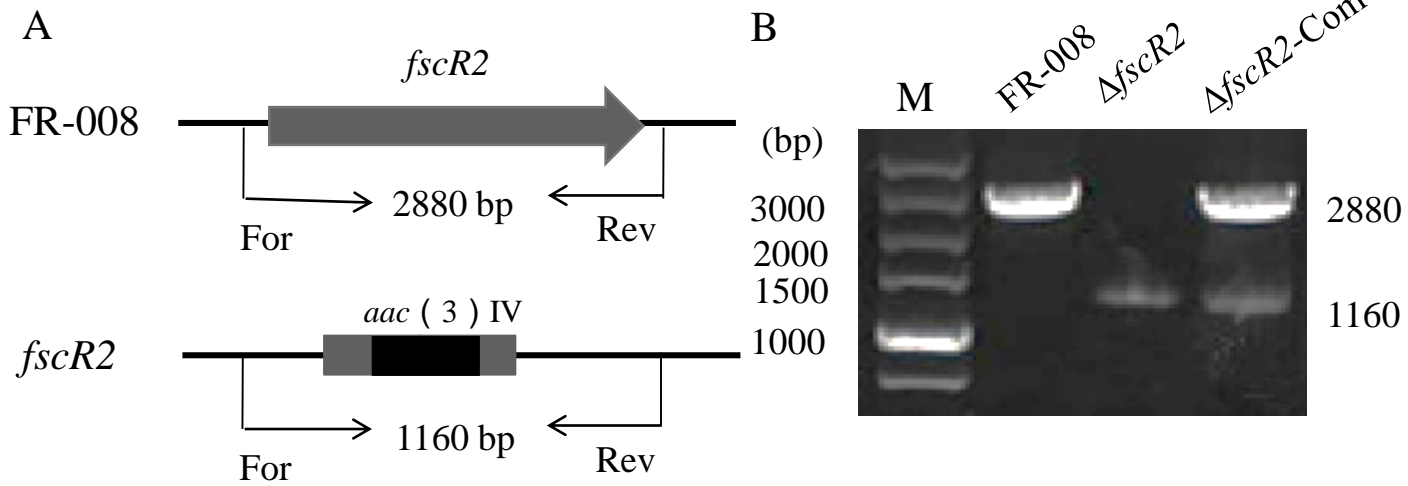


Figure S9. Replacement of an internal region of *fscR2* from *Streptomyces* sp. FR-008. The mutation in $\Delta fscR2$ was confirmed by PCR analysis.

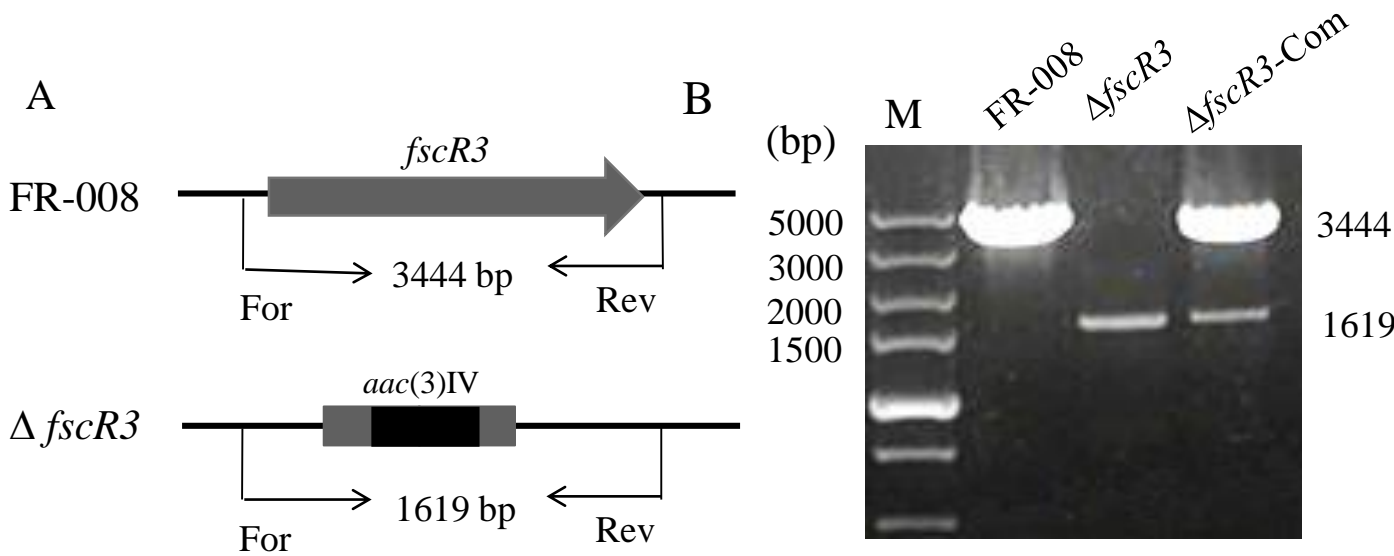


Figure S10. Replacement of an internal region of *fscR3* from *Streptomyces* sp. FR-008. The mutation in $\Delta fscR3$ was confirmed by PCR analysis.

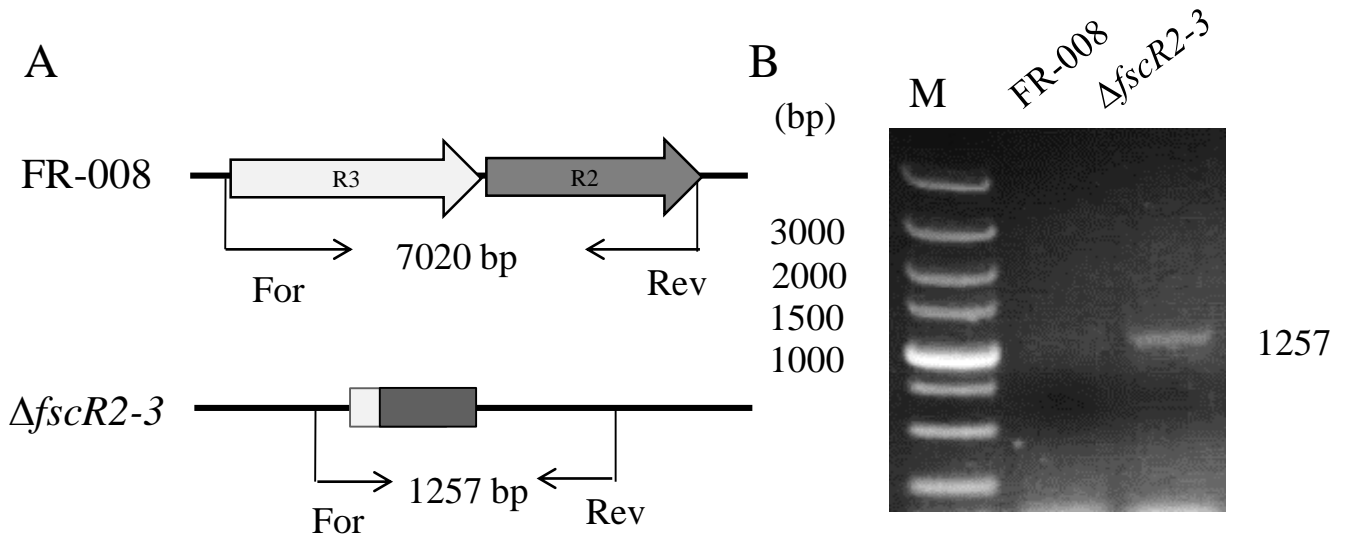


Figure S11. Deletion of the chromosomal region from *fscR2* to *fscR3* from *Streptomyces* sp. FR-008. The deletion in $\Delta fscR2-3$ was confirmed by PCR analysis.

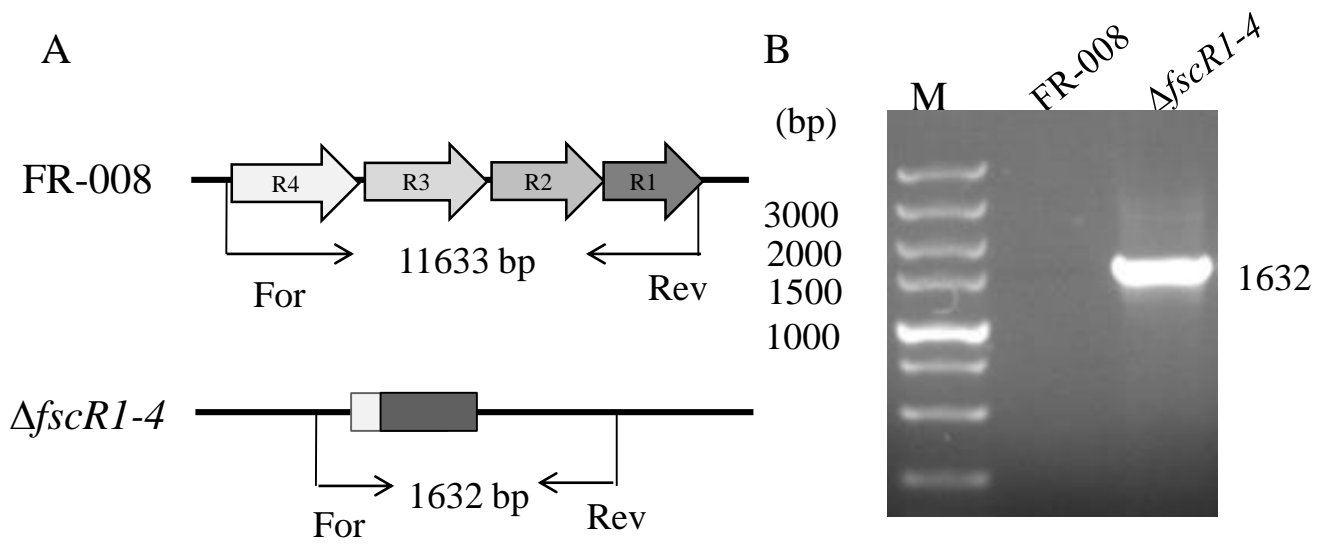


Figure S12. Deletion of the chromosomal region from *fscR1* to *fscR4* from *Streptomyces* sp. FR-008. The deletion in $\Delta fscR1-4$ was confirmed by PCR analysis.

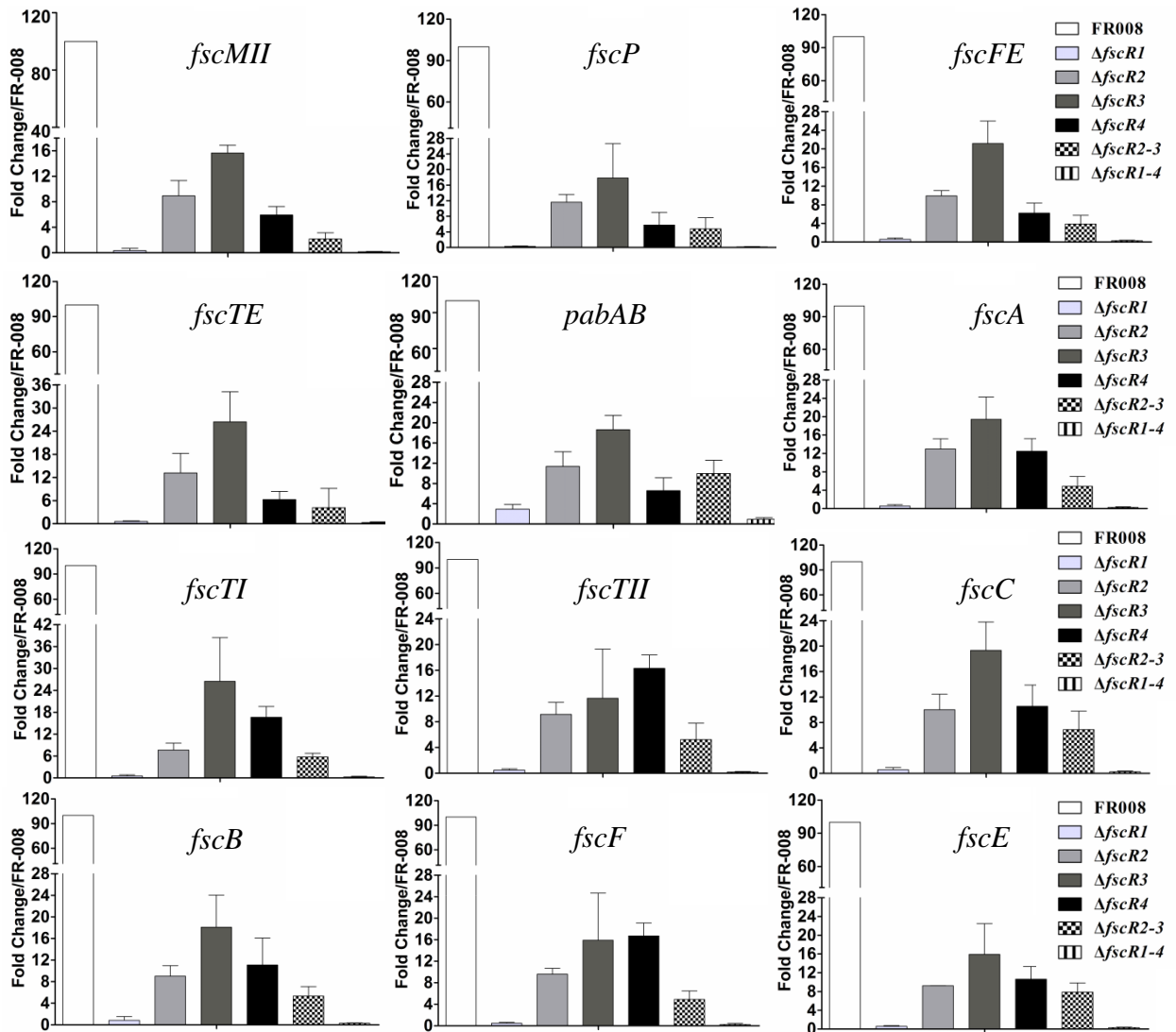


Fig. S13 *fscR1-fscR4* exert different levels of control over structural genes in the FR-008 pathway. RNA samples were isolated from *Streptomyces* sp. FR-008, single mutation strains, and multi-mutation strains grown for three days in YEME liquid medium. Expression of *hrdB*, which encodes the major sigma factor, was used as an internal control. The expression level of each gene in the wild-type strain was arbitrarily set to 100. The y-axis shows the fold change in expression level relative to the control. Results are the mean (\pm SD) from six biological experiments.

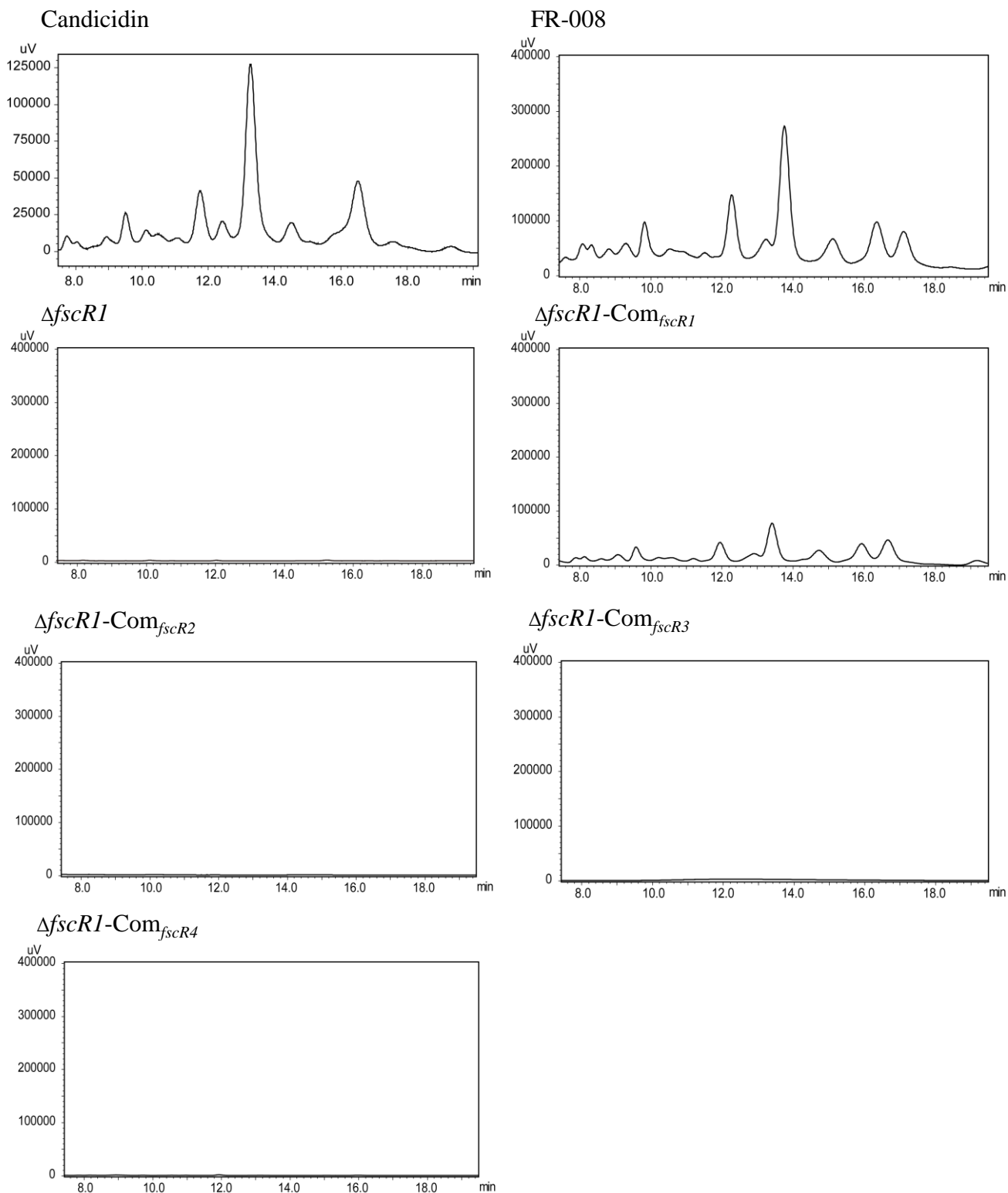


FIG S14. Production of candidin by cross-complemented variants of $\Delta fscR1$. The candidin complex was extracted from strains grown on GS agar medium for 3 days before HPLC analysis. $\Delta fscR1$ was complemented with *fscR1*, *fscR2*, *fscR3*, or *fscR4* as indicated. Top left panel, candidin standard. Top right panel, wild-type *Streptomyces* sp. FR-008 strain.

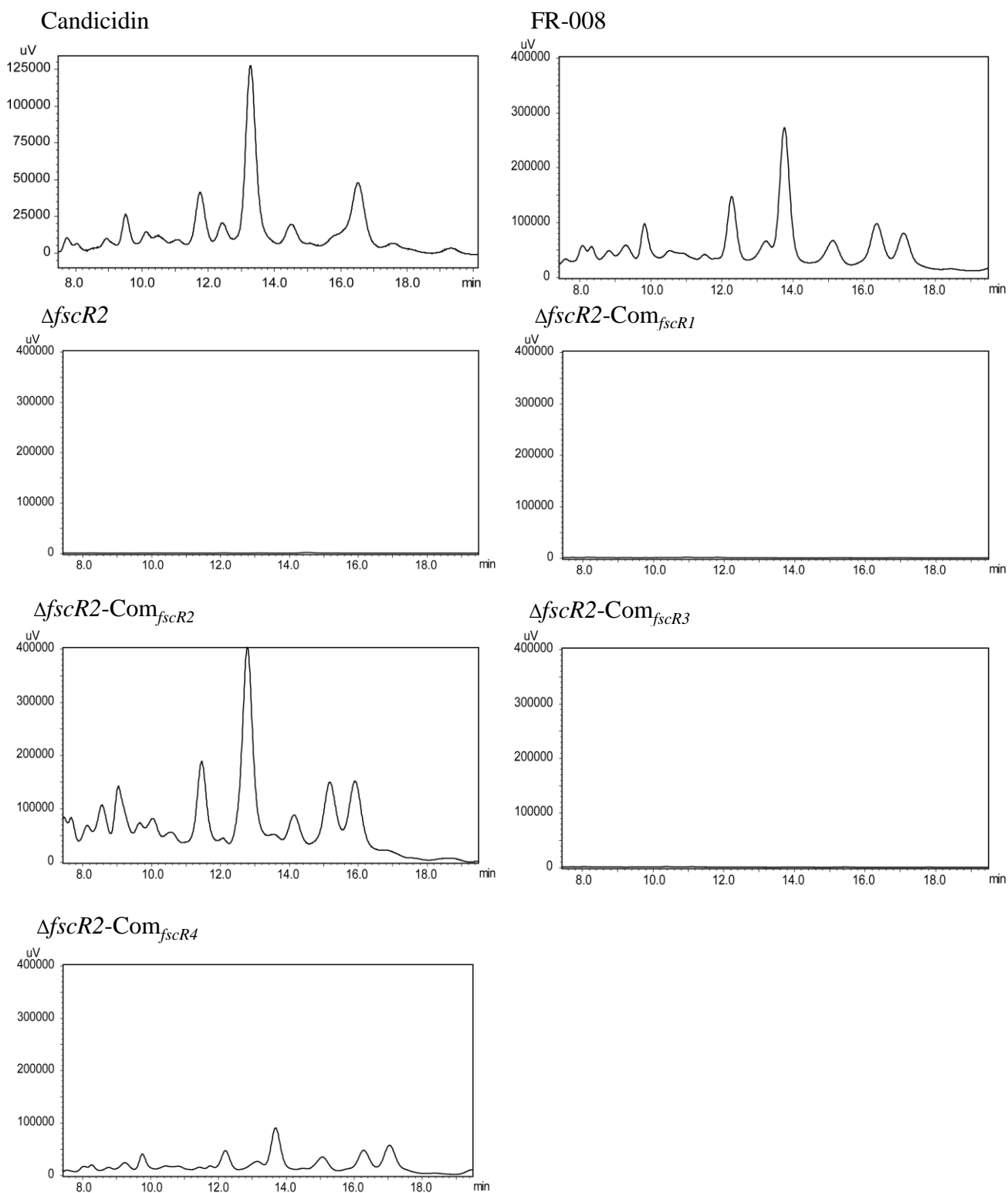


Figure S15. Production of candidin by cross-complemented variants of $\Delta fscR2$. The candidin complex was extracted from strains grown on GS agar medium for 3 days before HPLC analysis. $\Delta fscR2$ was complemented with *fscR1*, *fscR2*, *fscR3*, or *fscR4* as indicated. Top left panel, candidin standard. Top right panel, wild-type *Streptomyces* sp. FR-008 strain.

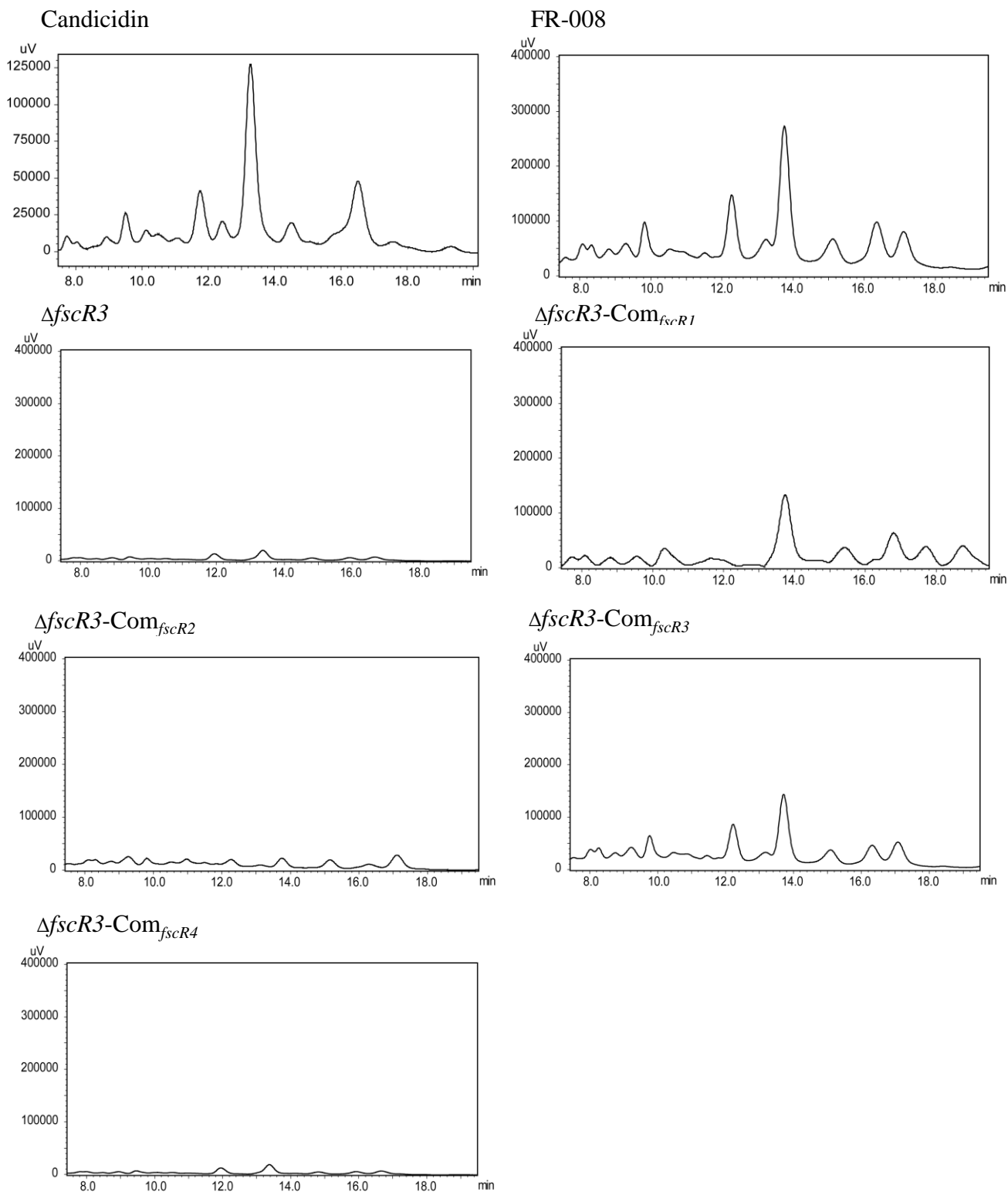


Figure S16. Production of candicidin by cross-complemented variants of $\Delta fscR3$. The candicidin complex was extracted from strains grown on GS agar medium for 3 days before HPLC analysis. $\Delta fscR3$ was complemented with *fscR1*, *fscR2*, *fscR3*, or *fscR4* as indicated. Top left panel, candicidin standard. Top right panel, wild-type *Streptomyces* sp. FR-008 strain.

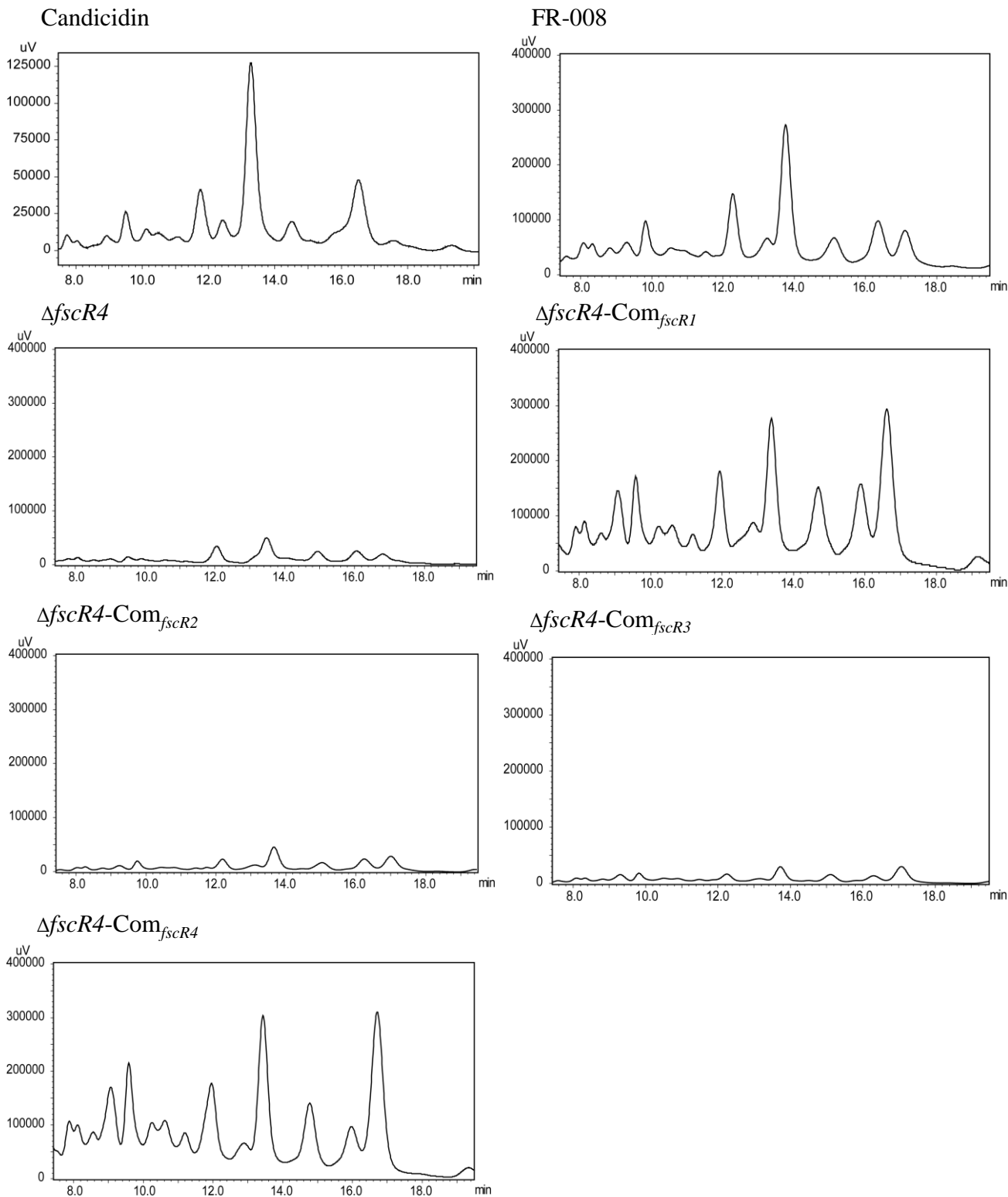


Figure S17. Production of candicidin by cross-complemented variants of $\Delta fscR4$. The candicidin complex was extracted from strains grown on GS agar medium for 3 days before HPLC analysis. $\Delta fscR4$ was complemented with *fscR1*, *fscR2*, *fscR3*, or *fscR4* as indicated. Top left panel, candicidin standard. Top right panel, wild-type *Streptomyces* sp. FR-008 strain.

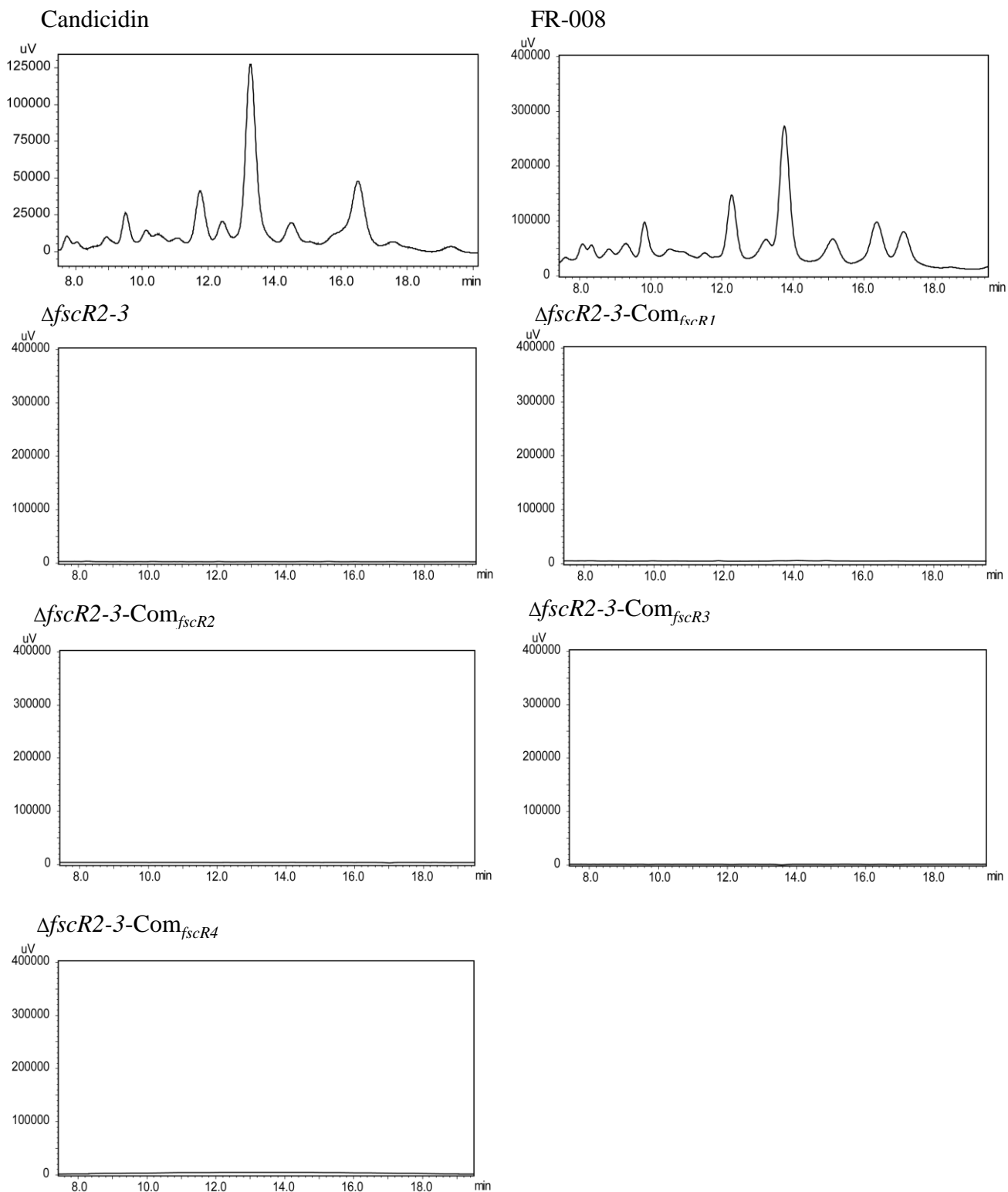


Figure S18. Production of candicidin by cross-complemented variants of $\Delta fscR2-3$. The candicidin complex was extracted from strains grown on GS agar medium for 3 days before HPLC analysis. $\Delta fscR2-3$ was complemented with *fscR1*, *fscR2*, *fscR3*, or *fscR4* as indicated. Top left panel, candicidin standard. Top right panel, wild-type *Streptomyces* sp. FR-008 strain.

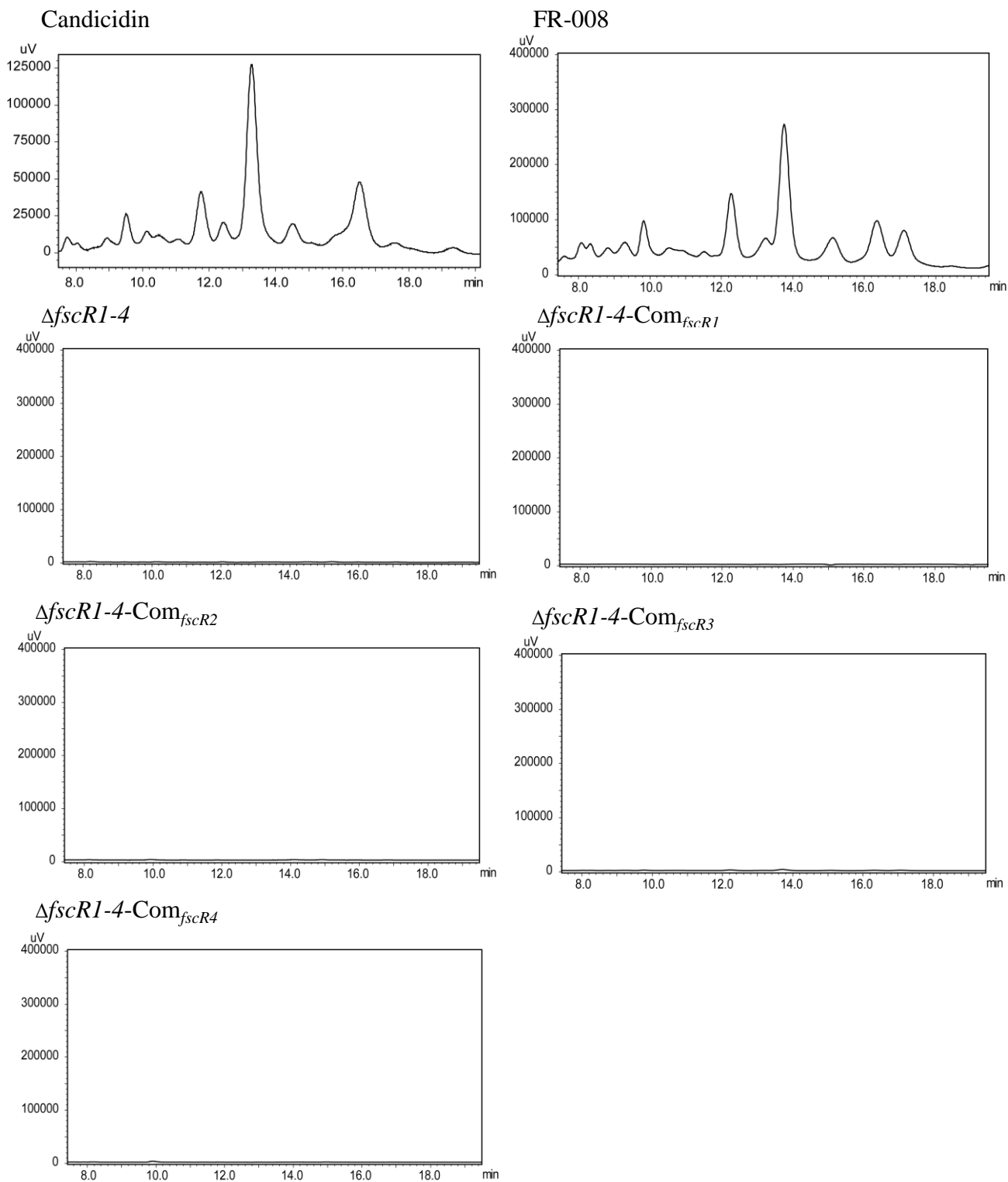


Figure S19. Production of candidin by cross-complemented variants of $\Delta fscR1-4$. The candidin complex was extracted from strains grown on GS agar medium for 3 days before HPLC analysis. $\Delta fscR1-4$ was complemented with $fscR1$, $fscR2$, $fscR3$, or $fscR4$ as indicated. Top left panel, candidin standard. Top right panel, wild-type *Streptomyces* sp. FR-008 strain.

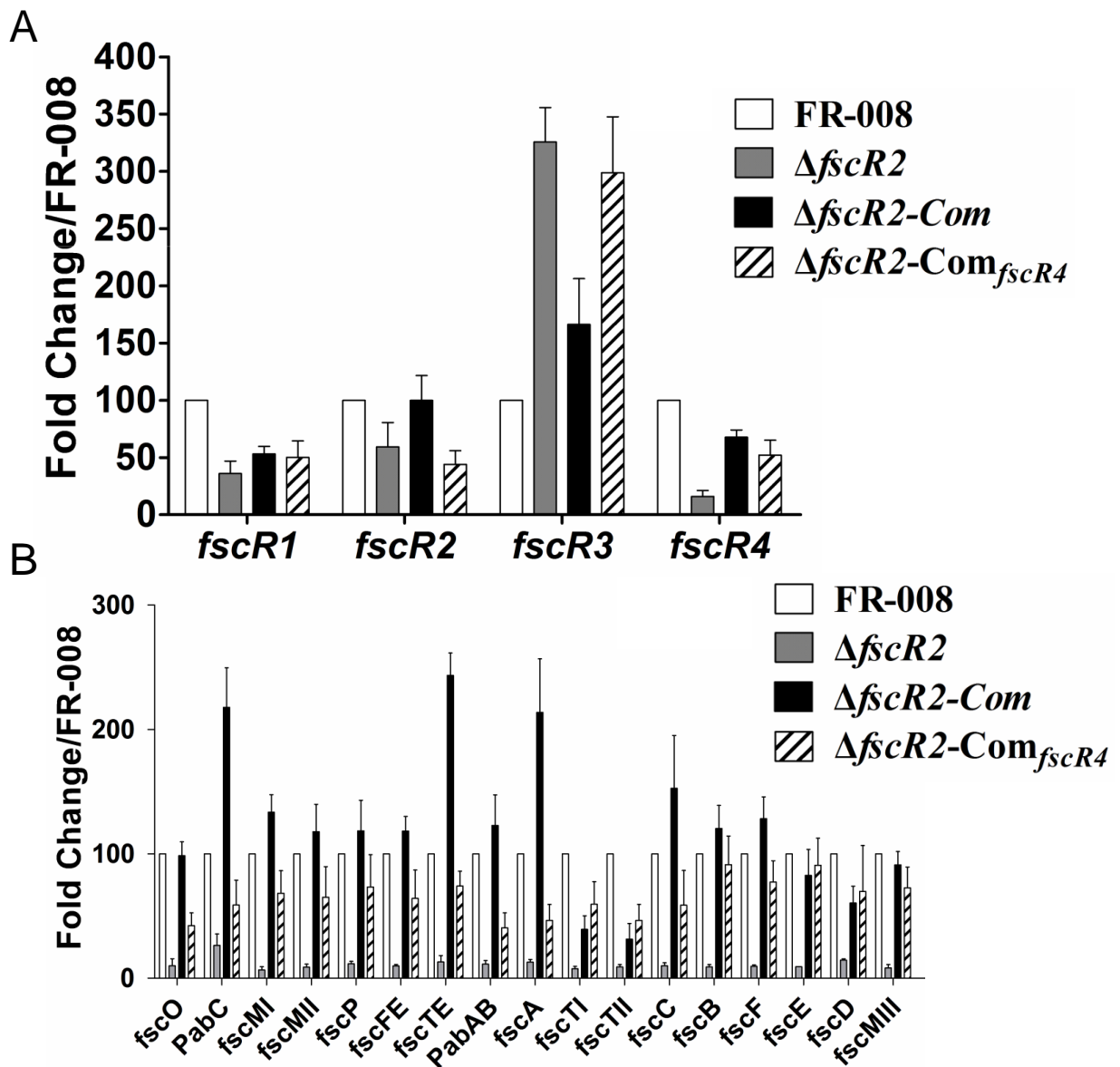


Figure S20. Transcription of FR-008 pathway genes in $\Delta fscR2-Com_{fscR4}$. RNA samples were isolated from *Streptomyces* sp. FR-008 and its derivatives grown for three days in YEME liquid medium. (A) Regulatory genes. (B) Structural genes. Expression of *hrdB*, which encodes the major sigma factor, was used as an internal control. The expression level of each gene in the wild-type strain was arbitrarily set to 100. The y-axis shows the fold change in expression level relative to the control. Results are the mean (\pm SD) from six biological experiments. Primers for regulatory genes were designed to an undeleted portion of the coding sequence.

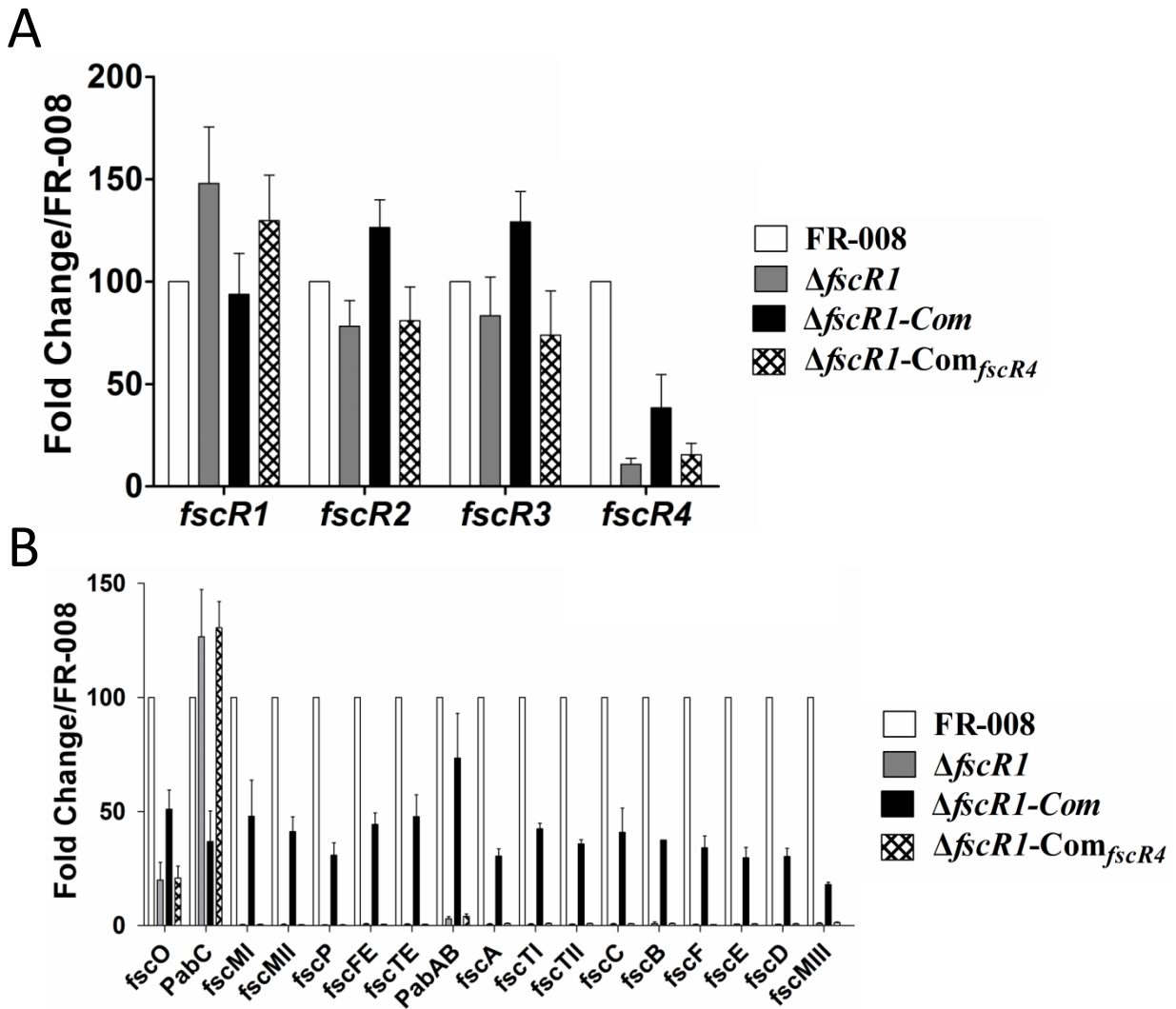


Figure S21. Transcription of FR-008 pathway genes in $\Delta fscR1-Com_{fscR4}$. RNA samples were isolated from *Streptomyces* sp. FR-008 and its derivatives grown for three days in YEME liquid medium. (A) Regulatory genes. (B) Structural genes. Expression of *hrdB*, which encodes the major sigma factor, was used as an internal control. The expression level of each gene in the wild-type strain was arbitrarily set to 100. The y-axis shows the fold change in expression level relative to the control. Results are the mean (\pm SD) from six biological experiments. Primers for regulatory genes were designed to an undeleted portion of the coding sequence.