Supplementary information for:

The gamma-butyrolactone system from *Streptomyces filipinensis* reveals novel clues to understand secondary metabolism control.

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Table S1. Deduced function of each gene of the GBL cluster in *S. filipinensis* and orthologous counterparts in other bacterial species.

ORF	Size (pb)	Size (aa)	Predicted function	ID (%)	Protein/Organism
sfb1	1950	649	SARP-like regulator	39	AfsR *S. coelicolor* (BAA14186)
				35	DnrI *S. peucetius* (AAA26736)
sfb2	1050	349	StrR-like regulator	49	KasT *S. kasugaensis* (BAC53615)
				42	StrR *S. griseus* (CAH94333)
sfbR	702	233	TetR-like regulator	57	SpbR *S. pristinaespiralis* (AYO26762)
				56	AvaR1 *S. avermitilis* (AKK07686)
sfb4	1278	425	P450 monooxygenase	57	Orf16* *S. fradiae* (AAD40802)
				56	Cyp *S. avermitilis* (BAC71416)
sfb5	846	281	Dehydrogenase	51	Orf4 *Streptomyces* sp. SBI034 (AKP54263)
				50	ScbC *S. coelicolor* (CAB60186)
sfb6	1482	493	Hypothetical protein	54	Hypothetical protein *S. virginiae* (WP_051762480)
				35	SCO0249 *S. coelicolor* (CAB53289)
sfb7	828	275	SARP-like regulator	66	BulY *S. tsukubaensis* (CBY91988)
				65	FarR4 *S. lavendulae* (BAG71714)
sfbR2	618	205	TetR-like regulator	51	PapR5 *S. pristinaespiralis* (CBW45767)
				47	SgvR1 *S. griseoviridis* (AGN74903)
sfbA	1014	337	AfsA-like protein	57	SrrX *S. rochei* (BAC76543)
				55	Lct9 *S. rishiriensis* (ABX71092)

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                                                                          ┌─helix─┐turn┌─helix─┐
SfbR   --------MA QQERAFRTRR TILTAAAEVF DELGYEAATM SEILGRSGVT KGALYFHFPS 52
ArpA   --------MA KQARAVQTWR SIVDAAASVF DDYGYERAAI SEILRRAKVT KGALYFHFAS 52
BarA   MAVRHERVAV RQERAVRTRQ AIVRAAASVF DEYGFEAATV AEILSRASVT KGAMYFHFAI 60
FarA   --------MA EQVRAIRTRQ AILSAAARVF DERGYQAATI SEILTVAGVT KGALYFHFQS 52
ScbR   --------MA KQDRAIRTRQ TILDAAAQVF EKQGYQAATI TEILKVAGVT KGALYFHFQS 52
SpbR   --------MA RQERAVRTRR AILVAAAEVF DEVGYEAATI SEILKRSGLT KGALYFHFAS 52


SfbR   KEQLAKEVLQ ENVQVVPAVP PQRLKLQEAV DRGLLLAYLM VR---DPVLR GGVRLALEQA 109
ArpA   KEAIAQAIMD EQTST-VEFE QEGSPLQSLV DGGQQFAFAL RH---NSMAR AGTRLSIEGV 108
BarA   KEELARGVLA EQTLH-VAVP ESGSKAQELV DLTMLVAHGM LH---DPILR AGTRLALDQG 116
FarA   KEDLAQGVLT AQNED-LLLP ERPAKLQEVV DAVMLHTHRL RT---NPMVR AGVRLSLDVN 108
ScbR   KEELALGVFD AQEPP-QAVP EQPLRLQELI DMGMLFCHRL RT---NVVAR AGVRLSMDQQ 108
SpbR   KEELAQGVLA EQVHALPDLP EGELMLQTAV DRALLLAHLL RRDTGDPIVR GSVRLTVEQG 112

                                  Ligand binding
                                      *
SfbR   G-HGKVDRSV PFLAWSQESR RMFEQAKLAG ELQPHIDPGE LADVFVGAFA GVQLMSQALT 168
ArpA   F----LGGPH PWGDWIDATA RMLELGQERG EVFPQIDPMV SAKIIVASFT GIQLVSEADS 164
BarA   A--VDFSDAN PFGEWGDICA QLLAEAQERG EVLPHVNPKK TGDFIVGCFT GLQAVSRVTS 174
FarA   A--GGLDRSA PFRNWVDKFT DLLEKAQAQG ELLPHVVPAE TADVITGAYG GVQSMSQALT 166
ScbR   A--HGLDRRG PFRRWHETLL KLLNQAKENG ELLPHVVTTD SADLYVGTFA GIQVVSQTVS 166
SpbR   ALRDGLDRRV PMQAWMEQTQ DLFEQAQAAG EILPHVDLVG AAKTFVGAFT GVQVLSNIMT 172


SfbR   GRADLDERVA ALYRVLMAGV AVPSVLVQLD VAVDRGARVN EAVEQQRH-- ---------- 216
ArpA   GRADLRGQVA EMWRHILPSI AHPGVIAHIK PEG-RVDLAA QAREKAEREE QEARIAAEAK 223
BarA   DRQDLGHRIS VMWNHVLPSI VPASMLTWIE TGEERIGKVA AAAEAAEA-- ---------- 222
FarA   EHQDLEHRYA ALLRHLMPSI AQPSVLASLH LGESRAEEVY LEARQLAR-- ---------- 214
ScbR   DYQDLEHRYA LLQKHILPAI AVPSVLAALD LSEERGARLA AELAPTGK-- ---------- 214
SpbR   GRQDMTERVA DLYRFLMTAI AVPGVLVRLD FSPGRGVLAY EEAVRRRD-- ---------- 220


SfbR   GKGQSPPERT AR-LVEHG-- ---------- ---------- ---------- --- 233
ArpA   GAGSDAATDS GSRSGGSGLR GGGSGRGPRA GGAGDEGDEE PAGAGVAAGG VVA 276
BarA   AEASEAASDE ---------- ---------- ---------- ---------- --- 232
FarA   EQADEED--- ---------- ---------- ---------- ---------- --- 221
ScbR   D--------- ---------- ---------- ---------- ---------- --- 215
SpbR   AAPQPAAH-- ---------- ---------- ---------- ---------- --- 228
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Fig S1. Alignment of *S. filipinensis* SfbR with other GBL receptors. Orthologues are ArpA from *S. griseus* (BAA36282), BarA from *S. virginiae* (BAA36282), FarA from *S. lavendulae* (BAG71716), ScbR from *S. coelicolor* (CAB60184) and SpbR from *S. pristinaespiralis* (AAK07686). Identical amino acids in at least four of the six sequences are shaded. The amino acid residues that form the helix-turn-helix motif for DNA binding are indicated and the conserved tryptophan involved in GBL binding (W123 in *S. filipinensis*) is boxed.

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                                                    │helix│ │turn│ │helix│
                                                    └─────┘ └────┘ └─────┘
SfbR2   -MVKQERAVR TRNALIAAAA EDFSRLGYSP SSLLSISRHA GVTSGALHFH FPTKVALASA  59
AvaR2   -MTKQERAAR TRHALIRSAA HAFERQGYTQ ARLADISACA GVSPGALHFH FESKAEVARA  59
BarB    MTPKQERAFR TRTQLVLSAA EAFDRQGFAT ASLTAISNSA GVSNGALHFH FESKEALAAA  60
CprB    -MARQLRAEQ TRATIIGAAA DLFDRRGYES TTLSEIVAHA GVTKGALYFH FAAKEDLAHA  59
FarR2   --MKQERAVR TRQALLESAA TVFGRRGYAE ATLSMISVGA GVSPGALHFH FENKAAVAEA  58
ScbR2   -MTKQERAAR TRRALILSAA EVFDQEGFAP ASLTMISSRA GVSNGALHFH FANKNAVAEA  59


SfbR2   VMAAAIQRLH QIVERCEKRM PPGGALQLLV DAGHELVQQL REDAVLRAGF DLEGD--PGC  117
AvaR2   VEAAAGVSLR RAAWLAQP-- PGTNALQRLT NTSHALAERL RGDVVARAGF RLNCE--SAG  115
BarB    VEAEAAERMR TIVDGAAR-- RGASALQALV DTSHAVMLRL RQDVVVRAGF RLSGD--AAR  116
CprB    VETAGARTLR ATTREVYT-- RRTSALQALA DSSQALAGLL LSDVVARAAF QLNRE--PAY  114
FarR2   ILEIQSRTSR RLAKDLDG-- RGYSSLEALM RLTFGMARLC VQGPVLRAGL RLATAGVPVR  117
ScbR2   VQGEALSVLR QIAHAWPE-- GATPSLQSLV DTSHTLAQRL QDDVVLRAGF GLSGD--TTW  115

                         Ligand binding
                              *
SfbR2   PRGVGEVRRH WHQWVQATLQ CAEEAGQLRP GVSVEQVASA VFVCTVGIQM LG-RRDTQWV  176
AvaR2   GG-ALNLLRE WQTCVEQLLA EAAEEGLLAR RLVRADTVSA VVAATTGFEL LG-RRDPEWL  173
BarB    QA-THDLPEH WRQSVVRLLE RAGRDGSLTS AVTPSDVAGV VTATVLGFGV LA-RFDSAWL  174
CprB    PP-LPHPFTE WREIATSRLL DAVRQSDVHQ DIDVDSVAHT LVCSVVGTRV VGGTLEPAGR  176
FarR2   PS-SFALPGE WHDYVHRLLL EAAEEGALLP GLNHRNVATT VVAATLGFEA LG-RDDPQWL  172
ScbR2   KE-RADLRRH WVDWVSSGLT VVALDGALAD DVATGDALAV IAATTLGFEA MG-RTDPQWS  173


SfbR2   SRRTLSRFWY LVLPRIAAGE G--DLDAAGT C--------- ---------- -  205
AvaR2   SGQSLAAFWR VLLPRAATAA ALTAVDPDGT CPSRAETRTP ATTAG----- -  218
BarB    ASGSLSGFWK LMLPMIAAGP VERGELDCRP AVPADVRRAP AV-------- -  216
CprB    EPRRLAEMWY ILIRGMVPVT RRARYVTLAA RLEQ------ ---------- -  210
FarR2   APRTLAGFWR VVMPCLAGPA TLRRLDTAGR GS-------- ---------- -  204
ScbR2   TREMFTRLWR LLLPRISADN GTGPVAPEGT SAPGGVVPGP RWWPERQDAP H  224
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Fig S2. Alignment of *S. filipinensis* SfbR2 with other GBL pseudo-receptors. Orthologues are AvaR2 from *S. avermitilis* (BAC71414), BarB from *S. virginiae* (BAA23612), CprB from *S. coelicolor* (BAA28748), FarR2 from *S. lavendulae* (BAG74711) and ScbR2 from *S. coelicolor* (CAC37887). Identical amino acids in at least four of the six sequences are shaded. The amino acid residues that form the helix-turn-helix motif for DNA binding are indicated and the conserved tryptophan likely to be involved in ligand binding (W128 in *S. filipinensis*) is boxed.

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SfbA   ---------M TLLVRQQEPT RAPAWQGVQD LPQLTTTVPR EYVHRASLAE VFLTGCRQLD 51
AfsA   ---------- ---------- -------MDA EAEVVHPVGI EMVHRTRPED AFPRNWVRLG 33
BarX   ---------- ---------- ---------- ---MTSTVPR ELVHRAAVAE VFLTGWSRTA 27
FarX   ---------- ---------- ---------- ---------- -MVHRTSTAQ VLLTDWQRLD 19
ScbA   ---------- ----MPEAVV LINSASDANS IEQTALPVPM ALVHRTRVQD AFPVSWIPKG 46
SrrX   MNVHAFRKQD GDSIRSDRDT RL-SPGDTSW LTPLTTTVPR EYVHRASLAE VFLTRCTRIH 59

                               AfsA domain #1
SfbA   GTRFELTGQW PRAHTFFTSS DGTQHDPMQA AETIRQVGLF LAHSEFGVPL GHHFLLRDME 111
AfsA   RDRFAVEAVL PHDHPFFAPV GDDLHDPLLV AEAMRQAAML AFHAGYGIPL GYHFLLTELD 93
BarX   ENRFALTAQW PRAHSYFTPV N-GCYDPLLA SETIRQVGTL LSHAEFGVSF GDQFLMWDLH 86
FarX   DARFSVTARW PLSHAFFTPV GDGYYDPLMC AETIRQIAYL LGHAEFAVPF GHQFVLWDLS 79
ScbA   GDRFSVTAVL PHDHPFFAPV HGDRHDPLLI AETLRQAAML VFHAGYGVPV GYHFLMATLD 106
SrrX   ETRFLLTGQW PRAHTFFLSP DGRRHDPMQI AETMRQVGLH LAHAEFDVPL GHHFIMWDMS 119

SfbA   FS-VIPDNLG IGARPSELTL QAACTDVKWR GNRLVQFAMR ITIERDGRLA ARGSGHFTCV 170
AfsA   YV-CHPEYLG VGGEPTEIGL EVFCSDLKWR AGLPAQGRVG WAVHRGDRLA ATGVAATRFS 152
BarX   HS-VRPEQAG VGAAPADLEL DVICSDIRRR GRRLAGMRYE VTLYCGGQVI ATGGAAFDCT 145
FarX   VSVVRPELLR VGLVPATVDL AITCVEIKRR AGRLSGLGYE AVVRRDGQVV ATGRASVTCT 139
ScbA   YT-CHLDHLG VSGEVAELEV EVACSQLKFR GGQPVQGQVD WAVRRAGRLA ATGTATTRFT 165
SrrX   FV-SRVEHLG VGRTPTDLDV EATCVDVVRR RGKLVEFRLV ITIERDGHLV ANGGGRFTCI 178

SfbA   APAAYHRLRG AGHTETG-TV PIPRPLPVEP WRVGRSSVAD VVLSATDQPG RWLLSPDPRH 229
AfsA   TPKAYRRMRG DVPVEGI--- SLPETAPVPA SPAGRARVED VVLSGTGREG VWELRVDTRH 209
BarX   SPAVYQRLRG DRVGATG-VR PLP--QPLAP ASVGRFLTTD VVLSATERPL EWQLRVDEQH 202
FarX   SPAVYQRIRP EHVLTPE-HR PLPLTAPAAP QSVARLSPTD VVLSPLDREN RWQLRVDTNH 198
ScbA   SPQVYRRMRG DFATPTA--- SVPGTAPVPA ARAGRTRDED VVLSASSQQD TWRLRVDTSH 222
SrrX   TEAMYRRLRR SAPATTAHQA ASHQPAPLPP SDFGRTAPRD VVLAPGGAPN RWRLNADTSH 238

                               AfsA domain #2
SfbA   PILFDHGGDH VPGMVLIEAA RQAACGLLE- GDTFLPVRAI TEFHRYAEFA TPCWIDAVLA 288
AfsA   LTLFQRPNDH VPGMLLLEAA RQAACLVAG- PAGIVPVEAR TRFHRYSEFG SPCWIGAVVQ 268
BarX   PVLFDHPVDH VPGMVLMESA RQAAQAIDP- SRPFLPTTMR SEFSRYAELD RPCWIQAEPL 261
FarX   PVLFDHWVDH VPGMVLMEAA RQAAASALG- RPSFMPLGVA GEFKRYVELD APCVIESERL 257
ScbA   PTLFQRPNDH VPGMLLLEAA RQAACLVTG- PAPFVPSIGG TRFVRYAEFD SPCWIQATVR 281
SrrX   PILFDHEGDH VPGMVLLESA RQAACALLPP GSTLIPATVS TEFRRYVEFT SPCWIEASGL 298

SfbA   HPEQPG-TRS VYVTGHQDGN EVFRTRLDGE CAHHGAPREQ RAGAAQDVGA 337
AfsA   PGTDED-TVT VRVTGHQDGE TVFSTVLSGP RAHG------ ---------- 301
BarX   PAADNG-DRQ VRVTGHQDDT TVFSCLIGTR GAAE------ ---------- 294
FarX   FQDVPGAEEV VRVTGHQNGE LTFVGTVTAS SYGY------ ---------- 291
ScbA   PGPAAG-LTT VRVTGHQDGS LVFLTTLSGP AFSG------ ---------- 314
SrrX   AVTGSG-TFH ALITGRQDDD EVFTARISGP VVQD------ ---------- 331
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Fig S3. Alignment of *S. filipinensis* SfbA with other GBL synthases. Orthologues are AfsA from *S. griseus* (BAH47547), BarX from *S. virginiae* (BAA23611), FarX from *S. lavendulae* (BAA21858), ScbA from *S. coelicolor* (CAA07627) and SrrX from *S. rochei* (BAC76543). Identical amino acids in at least four of the six sequences are shaded. The amino acid residues likely to be involved in catalysis (E83, R86, Q87 and E247, R250, Q251 in *S. filipinensis*) are boxed. The two AfsA domains characteristic of these proteins (Pfam03756) are indicated.
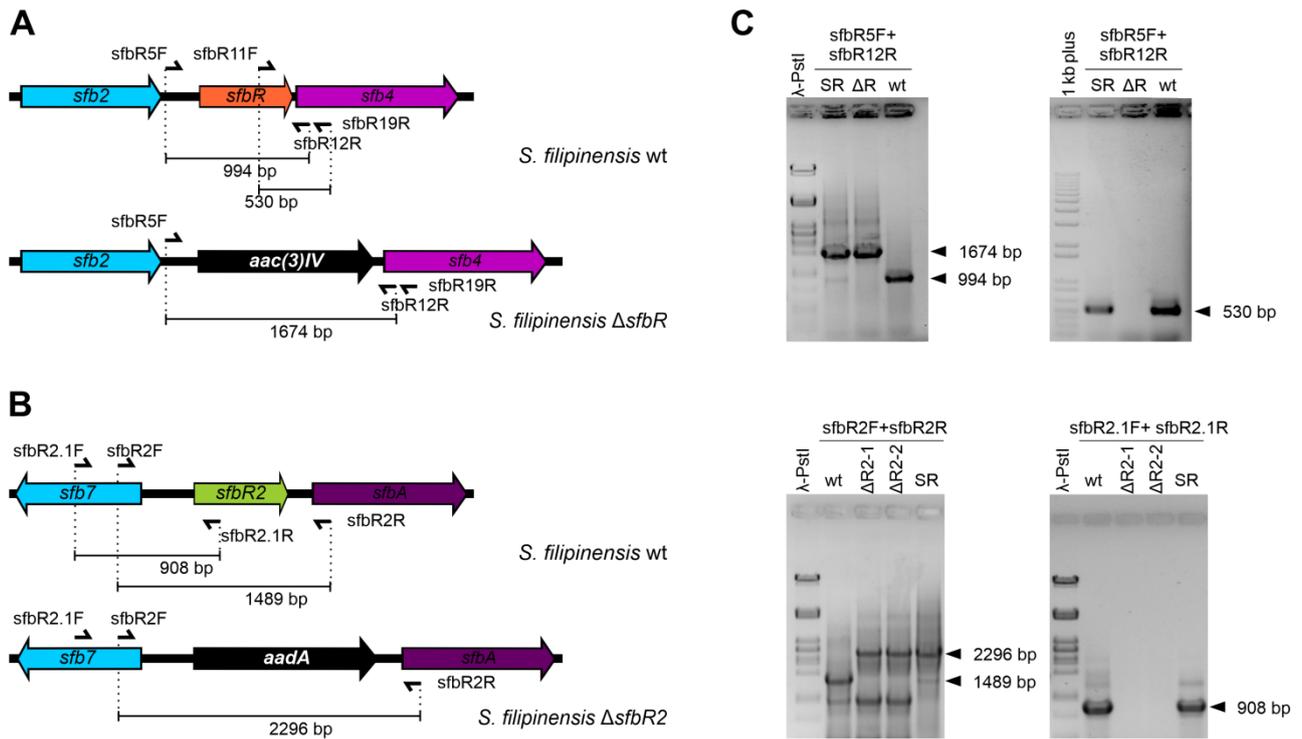
Fig S4. Construction of *sfbR* and *sfbR2* mutants. A) Predicted PCR fragment amplification of the parental strain and the Δ*sfbR* mutant. The primers used in the assay are indicated with arrowheads. The *acc(3)IV-oriT* cassette is indicated in black. B) Predicted PCR fragment amplification of the parental strain and the Δ*sfbR2* mutant. The primers used in the assay are indicated with arrowheads. The *aadA* cassette is indicated in black. C) PCR analyses of the wild type and the mutants. PCR fragments were verified by Sanger sequencing. SR: simple recombinant, ΔR: Δ*sfbR*; ΔR2: Δ*sfbR2*.
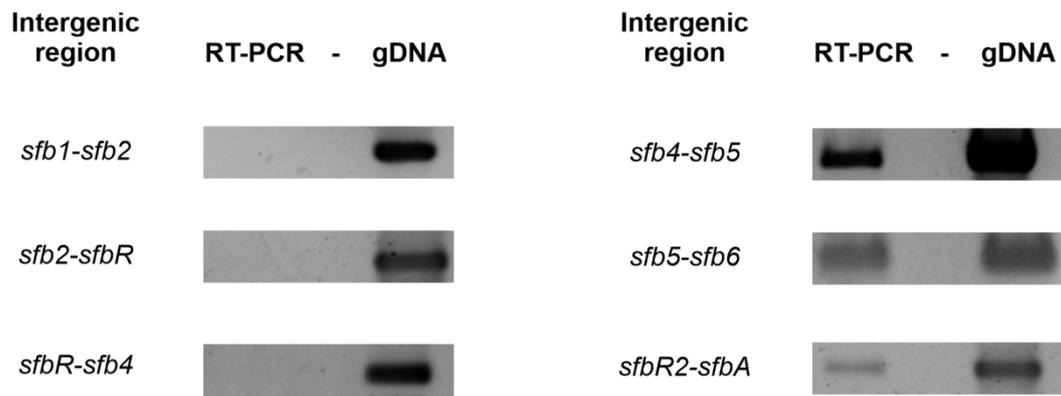
Fig S5. RT-PCR amplification of the *sfb* cluster intergenic regions. Total RNA was prepared after growth for 48 h in YEME medium without sucrose. In the PCR 40 amplification cycles were used to detect low abundant transcripts. Primers, designed to obtain a cDNA corresponding to unabated transcription between the two genes, are listed in Table 1.
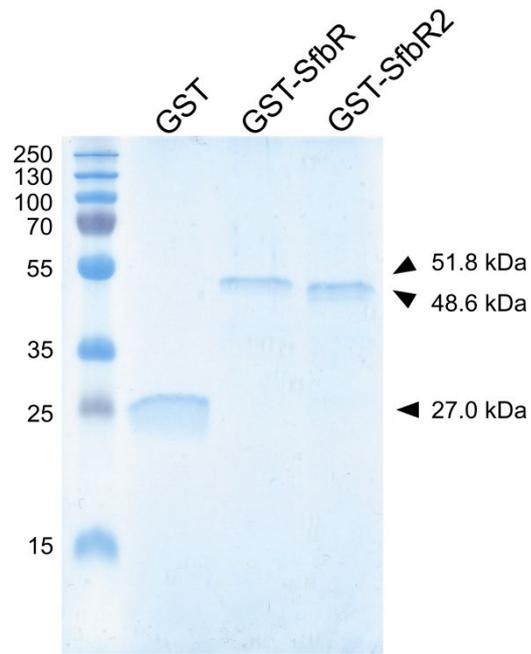
Figure S6. Purified GST fusion proteins. 2 µg purified GST, GST-SfbR and GST-SfbR2 proteins after affinity chormatography were analysed by SDS-PAGE. Left lane, molecular size markers (in kDa).