Supporting Information

Functional dissection and module swapping of fungal cyclooligomer depsipeptide synthetases

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1. Strains, plasmids, and culture conditions

Escherichia coli XL1-Blue (Agilent) was used for routine cloning and pJET1.2 (Fermentas) was used as the cloning vector. *E. coli* cells were grown in Luria-Bertani (LB) medium at 250 rpm and 37°C. When necessary, 50 μg mL^{-1} ampicillin was added. *S. cerevisiae* BJ5464-NpgA (*MATα ura3-52 his3-Δ200 leu2- Δ1 trp1 pep4::HIS3 prb1 Δ1.6R can1 GAL*) was obtained from Dr. Nancy Da Silva at the University of California, Irvine. The strain was maintained on YPD (yeast extract/peptone/dextrose) agar plates at 30°C. The *E. coli/S. cerevisiae* shuttle vectors pXW55 and pXW06 were gifts from Dr. Yi Tang at the University of California, Los Angeles. pXW55 carries the ampicillin-resistance gene and *URA3* as the selection markers, and was used to express M1-M2 linker-containing or -lacking M23 of BbBEAS and BbBSLS. pXW06 carries the ampicillin-resistance gene and *TRP1* as the selection markers, and was used to express M1-M2 linker-containing or -lacking M1 of BbBEAS and BbBSLS.

Beauveria bassiana ATCC 7159 was obtained from the American Type Culture Collection and was grown in potato dextrose broth at 250 rpm and 28°C. The genomic DNA was extracted from the mycelia of the fungus and used as the template to amplify the *bbBeas* and *bbBsls* gene fragments.

2. DNA manipulations

Isolation of plasmid DNA from *E. coli* was conducted with a GeneJET™ Plasmid Miniprep Kit (Fermentas) by following the manufacturer's instructions. Gene ligations were performed with T4 DNA ligase (New England Biolabs).

3. Gene amplification and plasmid construction

The gene fragments *bbBeasM1 with M1-M2 linker*, *bbBeas-M23 with M1-M2 linker*, *bbBeas-M1 without M1-M2 linker*, *bbBeasM23 without M1-M2 linker*, *bbBeasM1 with partial M1-M2 linker*, and *bbBeasM23a with partial M1-M2 linker* were amplified by PCR from the genomic DNA of *B. bassiana* ATCC 7159 with Phusion® High-Fidelity DNA Polymerase (New England Biolabs) with specific primers (Table S1). Similarly, *bbBslsM1 with M1-M2 linker*, *bbBslsM23 with M1-M2 linker*, *bbBslsM1 without M1-M2 linker*, *bbBslsM23 without M1-M2 linker*, *bbBslsM1 with partial M1-M2 linker* and *bbBslsM23a with partial M1-M2 linker* were amplified from the template. These gene fragments were ligated into the cloning vector pJET1.2 (Fermentas) to yield twelve plasmids including pDY38, pDY39, pDY60, pDY61, pDY62, pDY66, pDY69, pDY72, pDY90, pDY91, pDY94 and pDY95. These plasmids were confirmed by digestion checks and gene sequencing.

The *bbBeasM1 with M1-M2 linker* and *bbBeasM1 without M1-M2 linker* inserts were excised from pDY39 and pDY94 with *Nde*I and *Pme*I and ligated into pXW06 between the same sites to generate pDY45 and pDY99, respectively. The *bbBeasM23 with M1-M2 linker* and *bbBeas-M23 without M1-M2 linker* inserts were excised from pDY38 and pDY91 with *Spe*I and *Pml*I and ligated into pXW55 between the same sites to yield pDY58 and pDY98. The *bbBslsM1 with M1-M2 linker* and *bbBslsM1 without M1-M2 linker* inserts were excised from pDY69 and pDY95 with *Nde*I and *Pme*I and ligated into pXW06 between the same sites to generate pDY73 and pDY102. The *bbBslsM23 with M1-M2 linker* and *bbBslsM23 without M1-M2 linker* inserts were excised from pDY72 and pDY90 with *Spe*I and *Pml*I and ligated into pXW55 between the same sites to generate pDY78 and pDY97.

The *bbBslsM23a with partial M1-M2 linker* insert was excised from pDY62 with *Spe*I and ligated into pDY60 at the same site to generate pDY68. Then the *bbBeasM1-bbBslsM23a* insert

was excised from pDY68 with *Nde*I and ligated into the pDY42 (harboring the entire *bbBsls* gene) vector treated with *Nde*I to replace a fragment to afford pDY70. The *bbBeasM23a with partial M1-M2 linker* insert was excised from pDY66 with *Spe*I and ligated into pDY61 at the *Nhe*I site to generate pDY67. The *bbBslsM1-bbBeasM23a* insert was then excised from pDY67 with *Nde*I and ligated into the pDY37 (harboring the entire *bbBeas* gene) vector treated with *Nde*I to replace a fragment to generate pDY71.

The plasmids constructed in this work are shown in Table S2.

4. Transformation of *S. cerevisiae* BJ5464-NpgA and fermentation of the engineered strains

Plasmids were introduced into *S. cerevisiae* BJ5464-NpgA using the lithium acetate method. Correct transformants were selected by appropriate autotrophy. The engineered *S. cerevisiae* strain carrying pDY37, pDY42, pDY70 or pDY71 was fermented in a SC-Ura dropout medium (6.7 g L^{-1} yeast nitrogen base; 20 g L^{-1} glucose; 0.77 g L^{-1} -Ura DO supplement) at 30°C with shaking at 250 rpm. When the OD_{600} reached 0.6, equal volume of YP medium (10 g L^{-1} yeast extract; 20 g L^{-1} peptone) was added. The cultures were maintained under the same conditions for an additional 72 h to produce the cyclooligomer depsipeptides (CODs). Strains carrying a pXW55 derived plasmid and a pXW06 derived plasmid were fermented in a SC-Ura-Trp dropout medium (6.7 g L^{-1} yeast nitrogen base; 20 g L^{-1} glucose; 0.72 g L^{-1} -Trp/-Ura DO supplement) at 30°C with shaking at 250 rpm until the OD_{600} reached 0.6, and then equal volume of YP medium was added. The fermentation broths were cultured under the same conditions for an additional 72 h to produce the corresponding CODs.

5. Extraction and analysis of the products in the engineered *S. cerevisiae* strains

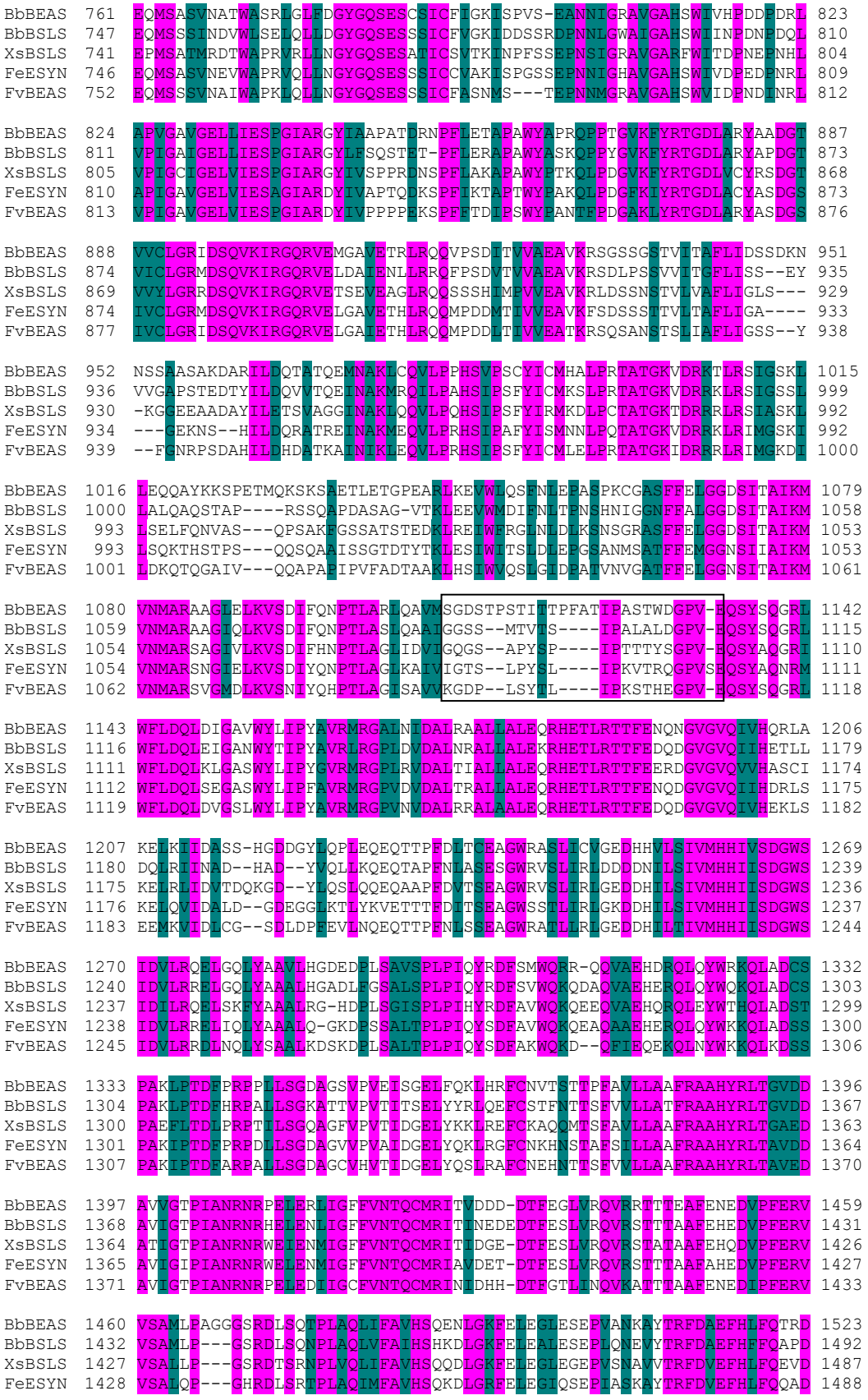
A typical procedure used to analyze the products in the engineered *S. cerevisiae* BJ5464-NpgA strains is given below. 100 mL of fermentation broth was centrifuged at 2,737 ×g for 5 min to separate the supernatant and the cells. The supernatant was extracted three times with 100 mL of ethyl acetate, and the cells were extracted twice with 50 mL of methanol. The extracts were then combined and evaporated *in vacuo* to dryness. The residue was re-dissolved in 500 μL of methanol and analyzed on an Agilent 6130 Single Quad LC-MS (C18, 5 μm, 4.6 mm × 250 mm column). A linear gradient of acetonitrile-water (containing 0.1% formic acid) was programmed from 80% to 100% over 20 min at a flow rate of 1 mL min^{-1} and the metabolites were monitored at 210 nm. Standard curves based on the linear relationship between the amounts and the peak areas were established for beauvericin, beauvericin A, beauvericin B, beauvericin C and bassianolide for quantitative measurement of their production in different engineered strains.

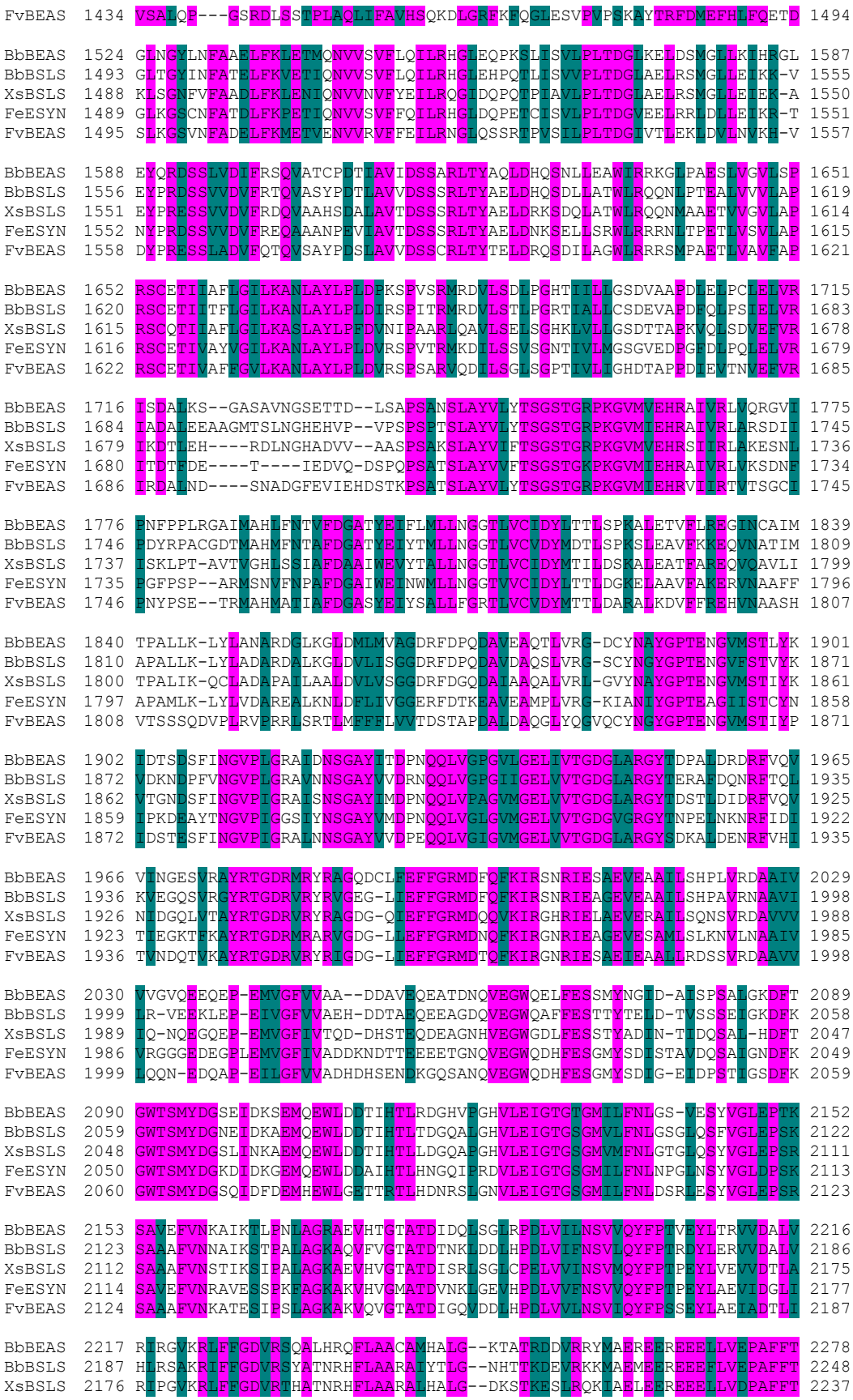
Table S1 Primers used in this work. The restriction sites are in bold.

Primer	Sequence	Restriction sites
BEAS-M1-5	aa**CATatg**gagccgctcaaaaatgt	NdeI
BEAS-M1-short-3	aa**GTTTAAAC**tcacatcacggcctgaagacgag	PmeI
BEAS-M23-short-5	aa**ACTAGT**tcttactctcaaggtcgatt	SpeI
BEAS-M23-3	aa**CACGTG**tcacaaagccgagtttagac	PmlI
BSLS-M1-5	aa**CATatg**gagccacccaacaacgc	NdeI
BSLS-M1-short-3	aa**GTTTAAAC**tcaaatagctgcctggaggctgg	PmeI
BSLS-M23-short-5	aa**ACTAGT**tcatactcgcaaggtcgact	SpeI
BSLS-M23-3	aa**CACGTG**tcataaagacgcattcaaagcc	PmlI
BSLS-M1-long-3	aa**GTTTAAAC**tcactgttccacaggaccatccaa	PmeI
BSLS-M23-long-5	aa**ACTAGT**ggtggcagctcaatgaccgt	SpeI
BEAS-M1-long-3	aa**GTTTAAAC**tcactgctcaacgggtccgtccca	PmeI
BEAS-M23-long-5	aa**ACTAGT**agcggcgattctacgccctc	SpeI
20120725BEAS-M1-3	aa**ACTAGT**gatggtcgagggcgtagaat	SpeI
20120725BSLS-M23a-5	aa**ACTAGT**atcccggcgctagctttgga	SpeI
20120725BSLS-M23a-3	aa**ACTAGT**gtg**catatg**ggatgcttgaaagt	NdeI, SpeI
20120725BSLS-M1-3	atccaaa**gctagc**gccgggat	NheI
20120725BEAS-M23a-5	aa**ACTAGT**ttgggacggacccgttgagca	SpeI
20120725BEAS-M23a-3	aa**ACTAGT**ttg**catatg**cgtggctggata	NdeI, SpeI

Table S2 Plasmids constructed in this work.

Plasmid	Description
pDY38	*bbBeasM23 with M1-M2 linker* in pJET1.2
pDY39	*bbBeasM1 with M1-M2 linker* in pJET1.2
pDY45	*bbBeasM1 with M1-M2 linker* in pXW06
pDY58	*bbBeasM23 with M1-M2 linker* in pXW55
pDY60	*bbBeasM1 with partial M1-M2 linker* in pJET1.2
pDY61	*bbBslsM1 with partial M1-M2 linker* in pJET1.2
pDY62	*bbBslsM23a with partial M1-M2 linker* in pJET1.2
pDY66	*bbBeasM23a with partial M1-M2 linker* in pJET1.2
pDY67	*bbBslsM1-bbBeasM23a* in pJET1.2
pDY68	*bbBeasM1-bbBslsM23a* in pJET1.2
pDY69	*bbBslsM1 with M1-M2 linker* in pJET1.2
pDY70	*bbBeasM1-bbBslsM23* in pXW55
pDY71	*bbBslsM1-bbBeasM23* in pXW55
pDY72	*bbBslsM23 with M1-M2 linker* in pJET1.2
pDY73	*bbBslsM1 with M1-M2 linker* in pXW06
pDY78	*bbBslsM23 with M1-M2 linker* in pXW55
pDY90	*bbBslsM23 without M1-M2 linker* in pJET1.2
pDY91	*bbBeasM23 without M1-M2 linker* in pJET1.2
pDY94	*bbBeasM1 without M1-M2 linker* in pJET1.2
pDY95	*bbBslsM1 without M1-M2 linker* in pJET1.2
pDY97	*bbBslsM23 without M1-M2 linker* in pXW55
pDY98	*bbBeasM23 without M1-M2 linker* in pXW55
pDY99	*bbBeasM1 without M1-M2 linker* in pXW06
pDY102	*bbBslsM1 without M1-M2 linker* in pXW06





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XsBSLS  2919 KEVGPVGTCRASKGISIPLQGSADSR--ITQATVFATACALMLSKETKSRDVTFGRIVSGRQGL 2980
FeESYN  2916 QEMPASKAVHLSEVVNVPAQAIRNSTN--TQATVFNTACALVLAKESGSQDVVFGRIVSGRQGL 2977
FvBEAS  2912 GNDATCKALHLSKIVNIPSQVLRGSSNIITQATVFNAACALVLSRESDSKDVVFGRIVSGRQGL 2975

BbBEAS  3033 PVSWQNIVGPCTNAVPVRARIIDDDDDNHRQMLRDMQDQYLLSLPFETLDFDEVRRSCTNWPAT 3096
BbBSLS  2992 PVRWQNIIGPCTNAVPVRA-VVDAHG-NHQQMLRDLQEQYLLSLPYETIGFDEIKRSCTDWPDS 3053
XsBSLS  2981 PITCQHVVGPCTNTIPVRL-CMDENP-NLRELLRKMQDQYLDSLPFETLGFDDLKKNCTEWSDE 3042
FeESYN  2978 PVVWQDIIGPCTNAVPVHARVDDGNP---QRIIRDLRDQYLRTLPFESLGFEEIKRNCTDWPEE 3038
FvBEAS  2976 PVEYQDIVGPCTNAVPVRAHIESSDY---NQLLHDIQDQYLLSLPHETIGFSDLKRNCTDWPEA 3036

BbBEAS  3097 ANNYACCVTYHDFSYHPESEMEQQRVEMGVLARKDALLKEEPVYDLGIAGEVEPDGVHLQVTVV 3160
BbBSLS  3054 ARNYGCCVTYQNFEYHPESEVDQQRVEMGILAKKAELIKEEPLYNVAIAGEVEPDGVHLQVTVV 3117
XsBSLS  3043 TTTYGCCIVYQNFNFHPESQVEEQRIQIGVLSREDQLANEASIHDLVLAGSPEPDGQHLHVTVV 3106
FeESYN  3039 LTNFSVCVTYHNFEYHPESEVDNQKVEMGVLAKYVELSENEPLYDLAIAGEVEADGVNLKVTVV 3102
FvBEAS  3037 ITNFSCCITYHNFEYHPESQFEQQRVEMGVLTKFVNIEMDEPLYDLAIAGEVEPDGAGLKVTVI 3100

BbBEAS  3161 AKTRLFSEERAAYLMEEVCRLFESLNSAL                                    3189
BbBSLS  3118 VDSQLFSQEGATHLMEQVCNTFQALNASL                                    3146
XsBSLS  3107 ANRQLCDDSRVKHMLEELCEMIETLSKALRDPYISDTNPANPESSLVAPVEIPRTPTNGFAPDI 3170
FeESYN  3103 AKARLYNEARIRHVLEEVCKTFNGLNEAL                                    3131
FvBEAS  3101 AKTQLFGRKRVEHLLEEVSKTFEGLNSSL                                    3129

BbBEAS  3190                                                                  3189
BbBSLS  3147                                                                  3146
XsBSLS  3171 SSESRIHILGVGNLGKFVAHALRKRHPQLPITLLFHRADLVSEWDAAVKAITCVTSGVSDKSTG 3234
FeESYN  3132                                                                  3131
FvBEAS  3130                                                                  3129

BbBEAS  3190                                                                  3189
BbBSLS  3147                                                                  3146
XsBSLS  3235 FDVEFLSNSTGHKTLIKHLIVATRTYMTVSAVDLVKRRLESTSSILFLQNGMGKNRHRAMNDVK 3298
ESYN    3132                                                                  3131
FvBEAS  3130                                                                  3129

BbBEAS  3190                                                                  3189
BbBSLS  3147                                                                  3146
XsBSLS  3299 SRPSYWAGVCSCGVYPTSPFSIVHAGRGPIVLGRVLEEANDSPESTSAASVAQDFMIQRLLETP 3362
FeESYN  3132                                                                  3131
FvBEAS  3130                                                                  3129

BbBEAS  3190                                                                  3189
BbBSLS  3147                                                                  3146
XsBSLS  3363 DLEATLTTPNDIKEVQLQKLIVNSMINPLTVVFRCNNGQLFGQPARLALMRTLLDEAGEIVRAI 3426
FeESYN  3132                                                                  3131
FvBEAS  3130                                                                  3129

BbBEAS  3190                                                                  3189
BbBSLS  3147                                                                  3146
XsBSLS  3427 LPQESQKSGNSQLTNENLLAAVLRVAEMTGECSSSMLQDVQAGRRTEIDHINGYLALQGKRLGL 3490
FeESYN  3132                                                                  3131
FvBEAS  3130                                                                  3129

BbBEAS  3190                                                                  3189
BbBSLS  3147                                                                  3146
XsBSLS  3491 PYSNIETLVNMVKQGRAIVNGDIQSLFKMSGIKPMDLGKVLKAGSSNGAVAAYGKR         3546
FeESYN  3132                                                                  3131
FvBEAS  3130                                                                  3129
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Fig. S1 Amino acid sequence alignment of five fungal CODSs. BbBEAS: GenBank accession number ACI30655; BbBSLS: GenBank accession number ACR78148; XsBSLS: GenBank accession number ABR28366; FvESYN: GenBank accession number Q00869; FvBEAS: GenBank accession number AGC31490. The intermodular M1-M2 linker is boxed.

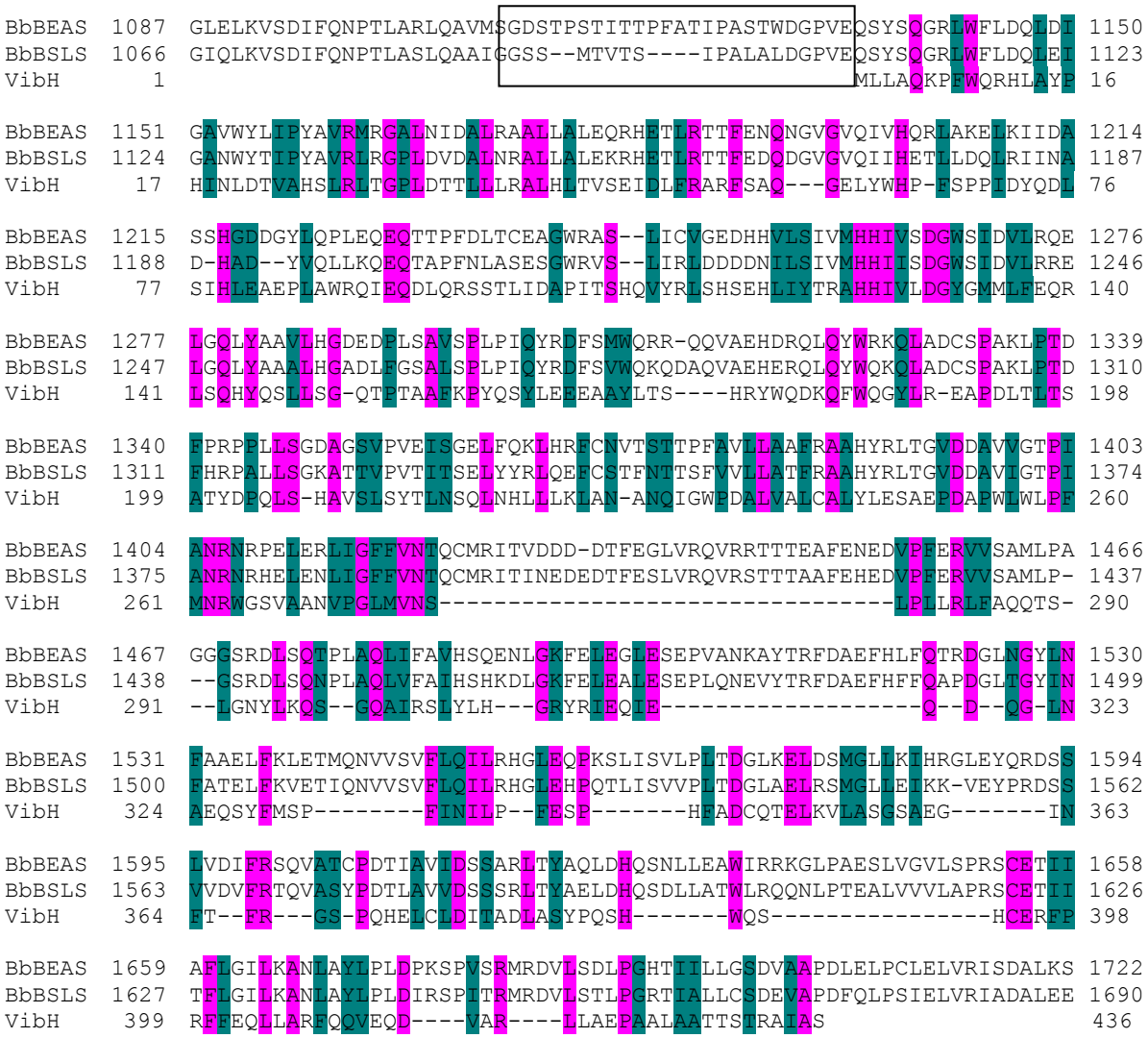

Fig. S2 Amino acid sequence alignment of BbBEAS, BbBSLS and a stand-alone NRPS condensation domain VibH (GenBank accession number AAD48879). The intermodular M1-M2 linker is boxed.

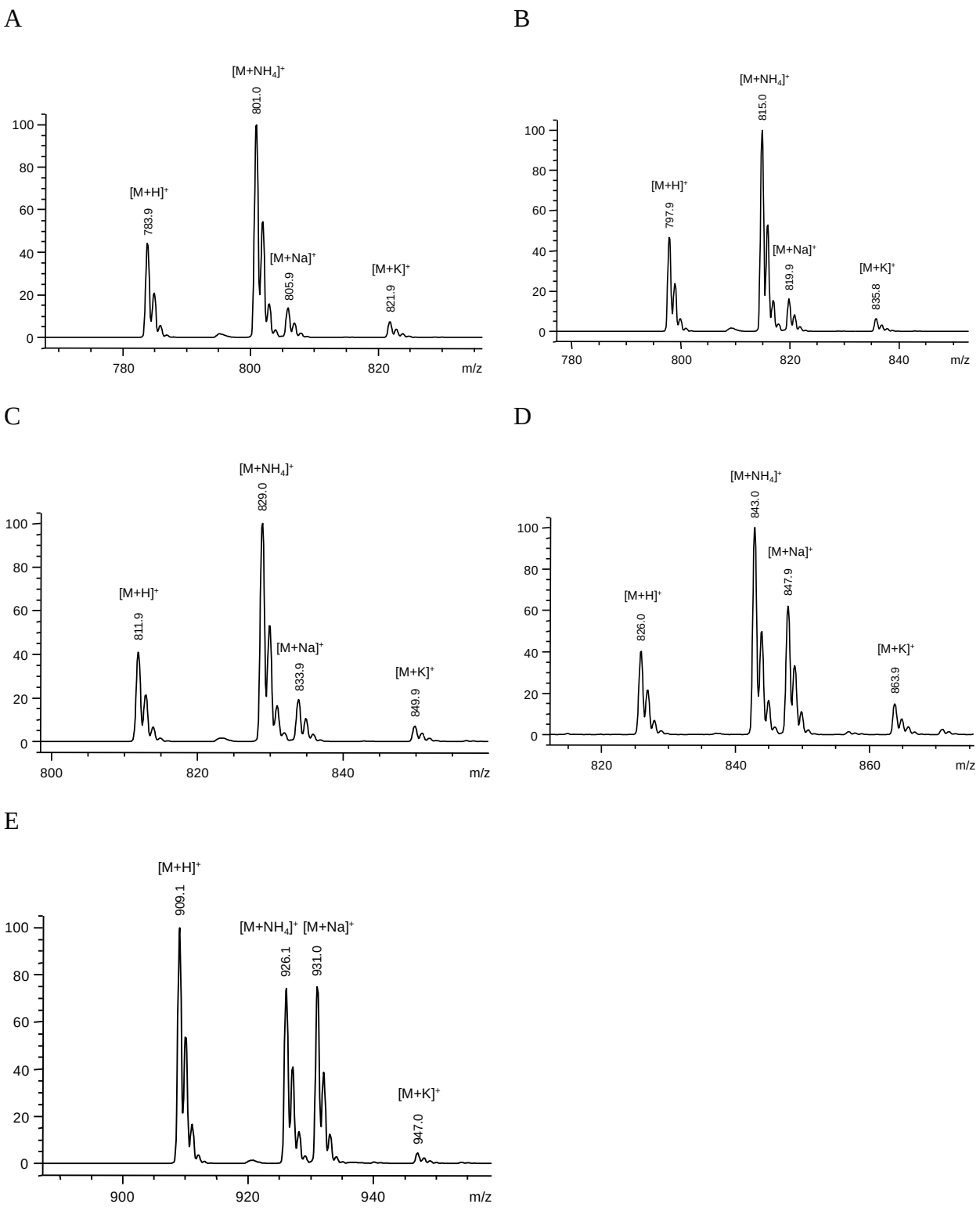

Fig. S3 ESI-MS spectra of beauvericin (A), beauvericin A (B), beauvericin B (C), beauvericin C (D) and bassianolide (E).