Supporting Information

Endofungal Bacteria Boost Anthelminthic Host Protection with the Biosurfactant Symbiosin

Hannah Büttner,[a] Sacha Pidot,[b] Kirstin Scherlach,[a] Christian Hertweck,[a,c]

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Supplemental Experimental Procedures

Fungal strain culture conditions

Fungal strains (Table S 1) were kept on PDA agar (BD, Bacto) at room temperature or 4 °C. Sterile strains were kept on PDA agar containing 40 µg mL^{-1} ciprofloxacin. For cultivation in liquid media a small piece of aerial mycelia was brought into 20 mL of broth in a chicane flask or onto an agar plate and grown for 7-14 days until extraction.

Preparation of aposymbiotic fungal strains

A small piece of fungal aerial hyphae was continuously cultivated on PDA (BD, Bacto) supplemented with either ciprofloxacin (40 µg mL^{-1}) or kanamycin (50 µg ml^{-1}). The absence of the endosymbiont was verified by the phenotype, growth behavior, chromatographic profiles of the extracts and fluorescence microscopical methods.

Identification of endosymbionts with fluorescence staining

Endosymbionts in fungal hyphae were stained using Syto 9 Green stain (Invitrogen). 0.5 µL fluorescence stain were diluted in 1 mL 0.85 % NaCl solution and hyphae were incubated in the stain for 2 min, washed twice with pure 0.85 % NaCl solution and visualized by a Zeiss spinning disc microscope (Axio Observer microscope-platform equipped with Cell Observer SD).

Isolation of genomic DNA, 16S rDNA amplification and phylogenetic analysis.

For gDNA isolation the endosymbiont harboring fungal strain was cultivated in MM9 medium at 26 °C and orbital shaking at 120 rpm in a chicane flask. After approximately 5 days, turbid cultures were filtered twice through a 40 µm membrane (Corning cell strainer). The turbid flow-through was centrifuged at 12,000 × g for 15 min and gDNA was extracted from the resulting pellet using the MasterPure DNA Purification Kit (Epicentre) (Figure S2). gDNA was used to amplify 16S rDNA with the primers 8F (AGA GTT TGA TCC TGG CTC AG) and 1492R (CGG TTA CCT TGT TAC GAC TT) (Figure S3). The PCR was performed with Phusion High-Fidelity PCR Master Mix with HF Buffer (NEB) and the following PCR program: 95 °C for 10 s, 65 °C for 15 s, 72 °C for 2 min; 30 cycles. Phylogenetic analysis was performed as described elsewhere and the novel endosymbiont was included in a previously published phylogeny.[1] In short, 16S rDNA sequences of *Ca.* Mycoavidus spp., *Burkholderia* and control strains were used for a sequence alignment with Clustal Omega with default settings. Maximun likelihood phylogeny was created using IQ-tree 2 and Ultrafast bootstrapping analysis was performed.[2, 3] Wolbachia pipientis (16S rDNA accession number: AY833061.1) was used as outgroup.[4]

Genome assembly for *Candidatus* Mycoavidus sp. SF9855

DNA from the fungal/endosymbiont culture was extracted as described above and prepared for sequencing on the PacBio Sequel system according to the “Preparing gDNA Libraries Using the SMRTbell® Express Template Preparation Kit 2.0” protocol from PacBio. In brief, DNA was sheared to approximately 15 kb using g-TUBE shearing devices (Covaris) according to the aforementioned protocol. Following removal of single strand overhangs, damage repair, end repair, A-tailing and adapter ligation with Template Express prep kit v2.0 (PacBio), DNA was purified and size selected to >7kb using a BluePippin size selection system (Sage Science) on a 0.75% dye-free agarose cassettes with the “0.75% DF Marker S1 high-pass 6-10 kb vs3” definition file. Size selected fragments were recovered, concentrated and primer and polymerase were bound following the instructions from the Sample Setup calculator, as part of SMRT Link v9.0, using Binding Kit v3.0, sequencing primer v4 and loading at 10pM concentration. The library was sequenced on a single SMRTcell 1M v3 cell with data collected for 10 hrs. Assembly was performed using the Microbial Assembly protocol as implemented in SMRT Link v9.0 to yield a single contig bacterial genome of 2227690 bp.
Genome sequence of Ca. M. sp. SF9855 has been deposited to the NCBI database as part of BioProject PRJNA733818.

Completion and analysis of the *sym* biosynthetic gene cluster

In the previously published version of the *Ca*. M. necroximicus genome (GenBank: CP076444.1) parts of the symbiosin biosynthetic gene cluster were missing, due to a limited number of long reads overlapping a highly repetitive internal DNA region. Re-analysis of the initial CP076444.1 genome assembly revealed two assembled DNA regions (fragment 1.7 and 4) that contained sequence data potentially encoding the *sym*-biosynthetic gene cluster, but missing an ~4.5kb region in the middle of the cluster. A pair of primer (HBP53 + HBP54), each binding to one end of the two known fragments (fragment 1.7 and 4), were designed to amplify the

potentially missing region. A PCR (10 µL OneTaq Master Mix, 1 µL genomic DNA, 2 µL primer (10 µM), 2 µL DMSO, 5 µL H_2O; Program: 95 °C for 2 min; 95 °C for 20 s – 62 °C for 20 s – 68 °C for 6 min (30 ×); 72 °C for 5 min) amplified the expected ~5.5 kb fragment. Two PCR products (one of the expected ~5.5kb size and a second of ~2.5kb in size) were seen after running the reaction on an agarose gel (Figure S6) and both were gel extracted was repeated several times and the amplicon isolated from gel. The smaller fragment was later identified as a partial amplicon of the region of interest, due to partial internal homology with the primers. Sequencing primers were designed from the known parts of the fragments and later from sequencing results and individual Sanger sequencing reactions were performed to walk across the larger ~5.5kb fragment. Sequencing results were trimmed manually to exclude low quality sequences using Geneious Prime. Geneious Prime was also used to assemble the cleaned Sanger sequencing reads and to place the assembled fragment in the correct genomic context in the final assembly. The primers used are listed in Table S9. The newly generated sequence was included in the revised genome assembly of *Ca*. M. necroximicus (GenBank: CP076444.2).

NMR measurements, HRESI/MS measurements and structure elucidation

NMR data were recorded on a Brucker AVANCE III 600 MHz spectrometer with cryo probe in CD_3OD and DMSO-D_6.
All LC-HRESI/MS and LC-HRESI/MS/MS measurements were performed with a QExactive Hybrid-Quadrupol-Orbitrap (Thermo Fischer Scientific) with an electrospray ion source. The HPLC was fitted with an Accucore C18 column (2.1 × 100 mm, 2.6 µm). 3 µL of each sample were injected and eluted with a flow rate of 200 µL min^{-1} using solvent A (water + 0.1 % formic acid) and solvent B (acetonitrile + 0.1 % formic acid) with the following gradient: 5 % to 98 % solvent B in 10 min, constant 98 % solvent B for further 12 min with a change to 95 % solvent A after 22 min.
For LC-HRMS/MS measurements with hydrolyzed symbiosin (**3**) 1M NaOH was added previous to the measurement.

Extraction and isolation of symbiosin (3) and necroxime (2)

Liquid cultures were extracted with the same volume of ethyl acetate. Solid cultures were cut into small pieces, overlaid with ethyl acetate and left overnight for extraction. The organic phase was dried over sodium sulfate and evaporated *in vacuo*. Extracts were resolved in methanol and HPLC measurements were performed with an Alltech Eurosphere 100 C18, 5 µm, 250 × 4.5 mm column on a Shimadzu LC-10A HPLC system with PDA. The following solvent system was used: acetonitrile (ACN) and MilliQ water (supplemented with 0.1% trifluoroacetic acid) at a flow rate of 1 mL min^{-1}; gradient: 0–2 min 10% ACN, 2–22 min 10–100% ACN, 22–25 min 100–10% ACN, hold 5 min 10% ACN.
For isolation of **3** the crude extract of a five-week old 4 L solid culture of symbiotic *M. verticillata* NRRL 6337 was fractionated via size-exclusion with Sephadex LH-20 and the symbiosin-containing fraction was forwarded to a Shimadzu Prominence preparative HPLC system equipped with a diode array detector and a C18 Nucleosil, 2.1 × 250 mm, 100 Å, 5 µm column (Macherey Nagel). Chromatographic conditions were as follows: 50% ACN in MilliQ water with 0.1% TFA for 5 min followed by a linear gradient to 100% ACN in 30 min. Further purification was achieved with a second preparative isolation under the same parameters with a C18 Nucleodur HTec, 10 × 250 mm, 5 µm column (Macherey Nagel) and resulted in 8.8 mg pure compound.
For isolation of **2** the crude extract was fractionated via size-exclusion with Sephadex LH-20 and the **2**-containing fraction was forwarded to a Shimadzu Prominence preparative HPLC system equipped with a diode array detector and a Luna C18(2), 21.2 × 250 mm, 100 Å, 10 µm column (Phenomenex). Chromatographic conditions were as follows: 15% MeOH in MilliQ water with 0.1% TFA for 5 min followed by a linear gradient to 100% MeOH in 30 min.

Derivatization with Marfey's reagent and elucidation of amino acid configurations

The absolute configurations of the amino acids were determined with the method by Marfey, which includes the derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alanine-amide (L-FDAA). 200 µg of the peptide were hydrolyzed with 20 % DCl in D_2O supplemented with 0.05 % phenol overnight at 110 °C. The solvent was removed by reduced pressure and the remains resuspended in 100 µL 1 M $NaHCO_3$. 50 µL L-FDAA (10 mg mL^{-1} in acetone) were added to the reaction and the mix was heated at 50 °C for 1 h. 50 µL 2 M HCl were added and the reaction mixture was diluted with 200 µL 50 % (vol/vol) acetonitrile and MilliQ water. Standards of the amino acids were derivatized in the same way. The derivatives of Tyr and Trp were analyzed via analytical HPLC (Agilent Technologies 1100 Series) HPLC fitted with a Phenomenex Kinetex XB-C18 column (100 Å, 250 × 4.6 mm, 5 µm). 3 µL of each sample were injected and eluted with a flow rate of 500 µL min^{-1} using solvent A (MilliQ water + 0.1 % TFA) and solvent B (acetonitrile + 0.1 % TFA) with the gradient 30 % to 100 % solvent B in 30 min. Ser-, Thr- and Glu-derivates were analyzed with an LC-HRESI/MS QExactive Hybrid-Quadrupol-Orbitrap (Thermo Fischer Scientific) with an electrospray ion source and a Thermo Accucore C18 column (100 × 2.1 mm; 2.6 µm). 3 µL of each sample were injected and eluted with a flow rate of 200 µL min^{-1} using solvent A (MilliQ water + 0.1 % FA) and solvent B (acetonitrile + 0.1 % FA) with the gradient 10 % to 20 % solvent B in 20 min. Chromatographic traces were standardized on the elution time of L-FDAA and analysis of configuration was performed with the EIC of the compounds.

Derivatization as a Mosher ester and elucidation of fatty acid configuration

In order to analyse the configuration of the fatty acid of the isolated natural product, 200 µg of symbiosin (**3**) were hydrolyzed in 6 M HCl (450 µL). The reaction mixture was stirred at 105 °C for 16 h. The obtained hydrolysate was extracted 4 times with 1 mL chloroform. The organic extracts were combined, dried with sodium sulfate and evaporated to dryness. The following steps were carried out under an argon atmosphere. The residue was dissolved in 400 µL dry dichloromethane containing 0.2 mM dimethylaminopyridine, 5 µL *S*-MTPA-Cl was added and the reaction mixture was stirred for 4 h at room temperature. Reaction mixtures were quenched with 500 µL water and the organic layer was separated. The aqueous phase was extracted 3 times with 1 mL dichloromethane and the combined organic phases were dried with sodium sulfate and evaporated to dryness. 250 µg *R*-3-hydroxy myristic acid and 1 mg *R*,*S*-3-hydroxy myristic acid were derivatized in the same way. The samples were dissolved in methanol and analysed with an LC-HRESI/MS QExactive Hybrid-Quadrupol-Orbitrap (Thermo Fischer Scientific) with an electrospray ion source and a Thermo Accucore C18 column (100 × 2.1 mm; 2.6 µm). 5 µL were injected and eluted with a flow rate of 200 µL min^{-1} using solvent A (water + 0.1 % FA) and solvent B (acetonitrile + 0.1 % FA) with an isocratic method at 73 % solvent B for 20 min. Analysis of configuration was performed with the EIC of the compounds.

Analysis of NRPS-modules

Assignment of domain borders was automatically performed with Pfam and manually checked and adapted accordingly to previous studies.[5] For the prediction of A-domain specificities with the Stachelhaus code NRPSpredictor2 was used (Table S3).[6] Alignment of A-domain sequences of NRPS-modules responsible for symbiosin synthesis was performed using Mega7 and ClustalW, revealing a much lower similarity between the A-domain of the silent module 7 and the other modules (Table S4).[7, 8] Described core motives of catalytic importance for A-domains were manually assigned as shown in Figure S8. Conserved motives were analyzed and visualized using WebLogo (Figure S10).[9] Assigned A domains sequences were additionally used for homology modelling with SWISS-Model.[10] The crystal structure of the GrsA Phe A domain form gramicidin A (PDB code: 1amu) was used as a template for modeling of A domains from symbiosin-biosynthetic gene cluster.[11] Modeled A domains were analyzed with PyMOL.

Antiproliferative and cytotoxic activity of symbiosin (3)

Cell assays were conducted with human umbilical vein endothelial cells HUVEC (ATCC CRL-1730) and human chronic myeloid leukaemia cells K-562 (DSM ACC 10) for antiproliferative effects and with human cervix carcinoma cells HeLa (DSM ACC 57) for cytotoxic effects as previously described.[12] Results are summed up in Table S7.

Antimicrobial activity of symbiosin (3)

Antimicrobial activity of symbiosin (**3**) was tested against *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* SG511, *Mycobacterium vaccae* IMET 10670, *Pseudomonas aeruginosa* K 799/61, *Escherichia coli* SG 458, *Staphylococcus aureus* 134/94 (MRSA), *Enterococcus faecialis* 1528 (VRE), *Sporobolomyces salmonicolor* SBUG 549, *Candida albicans* ATCC14053 and *Penicillium notatum* JP 36 (Table S8). Agar with the respective test organism was prepared and holes with 7 mm diameter were aseptically prepared. The test compound was dissolved to a concentration of 1 mg/mL in methanol, and 50 µL of this solution was transferred to each hole. Ciprofloxacin and amphotericin B served as positive controls. Depending on the growth rate of the test organisms, agar plates were incubated for several days and inhibition zones were measured.
For MIC data bacteria were cultivated in 96 well plates supplemented with different concentrations of **3** or ciprofloxacin and growth was determined by measuring the optical density at 600 nm.

Drop collapse assay

To test the effect of **3** on the water tension, a drop collapse assay was conducted. MilliQ water was stained with 0.001% crystal violet and used to create drops of 10 µL placed onto parafilm. Stained water and stained water with 10 % MeOH were used as a negative control. These drops have only a small contact point to the hydrophobic surface and form a sphere shape. Stained water containing 10 % MeOH and 0.1 % Tween80 was used as a positive control. Due to its lowered water tension the drops collapse and spread onto the hydrophobic surface. **3** was tested in a concentration of 1 mM in stained water with 10 % MeOH. The drop collapsed comparable to the positive control.

Nematode maintenance

Caenorhabditis elegans (wild type N2 (var. Bristol); *C. elegans* Genetics Centre (CGC, University of Minnesota, USA)) was used as a model nematode to test for anthelmintic effects as described before.[1] Nematodes were kept at 20 °C on NGM plates overgrown with *Escherichia coli* OP50. Every 5-7 days a small nematode containing agar piece was transferred onto fresh NGM/*E. coli* OP50-plates. *E. coli* OP50 was cultured in LB medium.

Aphelenchus avenae (Bastian, 1865; provided by Prof. Dr. Markus Künzler (ETH Zürich, Switzerland)) was maintained at 21 °C on a endosymbiont-free stain of *Mortierella verticillata* SF9854[1] grown on PDA plates or on a sporulation-deficient strain of *Botrytis cinerea* (BC-3) grown on MEA containing 100 µg mL^{-1} chloramphenicol.

C. elegans nematode bioassay

Liquid assays for the determination of anthelmintic effects were conducted as previously described.[13] Nematodes were washed from a plate with 15 ml K-medium and left at 4 °C for 30 min for settling. After one washing step *C. elegans* was resuspended in 12 mL K-medium and left at 4 °C until usage. E. coli were grown in 2 × 50 mL LB medium overnight with orbital shaking at 150 rpm, spun down and the pellet resuspended in K-medium. After two washing steps, cells were diluted to an OD_{600} of 1.2. 1.75 mL of the suspension was transferred into each well of a 6-well plate (Greiner Bio-One), combined with 50 µL test substance(es) dissolved in MeOH and supplemented with 200 µL of the prepared *C. elegans* suspension. Plates were shaken at 50 rpm and 20 °C for four days until OD_{600} was measured again. Measurements were performed with three biological replicates and two technical replicates each, if not stated differently. The potency verification of pure necroxime D (**2**) was performed with only one technical replicate. As controls, for every biological replicate measurements of wells containing only bacteria and 50 µL MeOH (without nematodes) and wells containing bacteria and 50 µL MeOH with test substances (without nematodes) were performed. Additionally, 50 µL MeOH containing the test substances (alone and in combination) were dissolved in 1.75 µL K-medium and measured as samples to exclude any distortion produced by precipitation. A positive control was achieved with the addition of 50 µL boric acid (stock: 0.9 M) and the negative control was performed with the addition of only 50 µL pure MeOH. Symbiosin (**3**) alone was tested with concentrations up to 100 µg mL^{-1}. Synergistic effects were tested with different concentrations of **2** (0.1 µg mL^{-1}, 0.3 µg mL^{-1}, 1 µg mL^{-1}, 3 µg mL^{-1}, 10 µg mL^{-1}, 30 µg mL^{-1}, 100 µg mL^{-1}) and supplemented with either 0.2 µg mL^{-1}, 2 µg mL^{-1} or 20 µg mL^{-1} of **3** or surfactin (**8**). For statistical evaluation and significance evaluation of the IC_{50} curves and mean comparison unpaired t-tests with GraphPad Prism 9.3.1 were performed.

A. avenae nematode bioassay

Nematodes were harvested from plates by Baermann-funneling overnight as described earlier.[1] In principle, nematode containing plates were cut in pieces and left in a closed funnel filled with K-medium overnight. Nematode-containing medium was released into 50 mL falcons and treated with 100 mM geneticin (G418), 20 µg mL^{-1} amphotericin and 25 µg mL^{-1} kanamycin for 2-3 h at 4 °C to sterilise the nematodes and eliminate fungal residues. Nematodes were washed twice with K-medium and stored at 4 °C for assays on the same day.
For chemical complementation assays *M. verticillata* SF9854 (necroxime- and symbiosin-negative) was inoculated in 24 well-plates containing 1 mL PDA and grown for two days at 25 °C, until the whole well was covered by the fungus. To test the protective effect of necroxime, symbiosin and the combination of necroxime and symbiosin each well was covered with 100 µL MeOH containing the test substances (20 µg mL^{-1} pure symbiosin; 5.7 µg mL^{-1}, 11.4 µg mL^{-1}, 22.8 µg mL^{-1} or 45.6 µg mL^{-1} necroxime D, for synergistic effects in combination with 2 µg mL^{-1} symbiosin) or pure MeOH as controls. After MeOH was dried under a sterile bench, 20 µl resuspended nematodes were transferred into each well. Co-cultures were incubated at 20 °C for 12-14 days until nematodes were harvested overnight in 5 mL K-medium. Nematodes were transferred onto water-agar in 6 well-plates, recovered overnight and analyzed using a Zeiss Axio Zoom.V16 Stereomicroscope (Zeiss, Oberkochen, Germany) and a magnitude of 12. The number of nematodes was counted manually on two frames for each well. Nematode numbers in MeOH control wells were set to 100 % and the numbers of the test wells were calculated in relation to the control. For statistical evaluation and significance evaluation multiple unpaired t-tests with GraphPad Prism 9.3.1 were performed. Each assay consisted of three wells for each condition (technical replicates; for 20 µg mL^{-1} pure symbiosin only two wells), which were individually analysed. The assay was repeated three times.

Supplemental tables

Table S 1. *M. verticillata* strains used in this study.

Strain	No.	Original isolation side
Mortierella verticillata	NRRL6337 (CBS 131.66)	Sandy forest soil, UK
Mortierella verticillata	NRRL6369 (CBS 100561)	Soil of Great Bear Lake, Canada
Mortierella verticillata	SF9852 (CBS 346.66)	Tundra soil, Alaska
Mortierella verticillata	SF9853 (CBS 220.58)	Soil under *Betula* sp., France
Mortierella verticillata	SF9854 (CBS 225.35)	Former West Germany
Mortierella verticillata	SF9855 (CBS 374.95)	Forest soil, China
Mortierella verticillata	SF9856 (CBS 315.52)	Forest soil, former West Germany

Table S 2. Media used in this study.

Medium	Ingredients per L or purchaser
PDA/PDB	Potato dextrose agar/broth (Roth)
MM9 medium	2 g amino acid mix, 10 g glycerol, 900 mL water, sterilization, add: appropriate antibiotics, 20 mL M9 salt A, 20 mL M9 salt B, 16.8 mL L-leucine solution (100 mM), 5 mL L-histidine solution (60 mM), each 10 mL of L-lysine (100 mM), L-tryptophan (40 mM), L-methionine solution (40 mM), 2 mL vitamin solution, 1 mL trace element solution
TSB	Tryptone soy broth (BD, Bacto), sterilization
MEP	30 g malt extract, 5 g peptone (BD, Bacto)
LB	Lysogeny broth (BD, Bacto), sterilization
BMSW	40 g marine broth (Roth), 20 g malt extract, 10 g glycerol
Czapex Dox	30 g saccharose, 0.5 g KCl, 2 g $NaNO_2$, 0.01 g $FeSO_4$, 1g K_2PO_4
MGY	10 g Glycerol, 1.25 g yeast extract (autolyzed yeast cells, BD, Bacto), 960 mL water, sterilization, add: 20 mL M9 salt A, 20 mL M9 salt B
CYE	Charcoal yeast extract medium; 10 g yeast extract (autolyzed yeast cells, BD, Bacto), 10 g ACES, 1 g potassium oxoglutamate, 2 g active charcoal, pH 6.9, sterilization, add: 0.25 g Fe-pyrophosphate (sterile filtered)
K-medium	3.1 g NaCl, 2.4 g KCl, sterilization
NGM	3 g NaCl, 2.5 g peptone (BD, Bacto), 17 g agar, sterilization, add (sterile): 5 mg cholesterol, 0.11 g $CaCl_2$, 0.25 g $MgSO_4$, 2.7 g KH_2PO_4, 0.89 g K_2HPO_4
MEA	Malt extract agar (Roth)
Water agar	7.5 g agar, sterilization, add 200 mM geneticin (G418), 50 µg mL^{-1} kanamycin
M9 salts A	350 g K_2HPO_4, 100 g KH_2PO_4, sterilization
M9 salts B	29.4 g Sodium citrate, 50 g $(NH_4)_2SO_4$, 5 g $MgSO_4$, sterilization
Amino acid mix	L-Amino acids in equal amounts: alanine, asparagine, cysteine, glutamate, isoleucine, serine, arginine, aspartate, glutamine, glycine, proline, threonine, valine
Vitamin solution	10 mg Folic acid, 6 mg biotin, 200 mg *p*-aminobenzoic acid, 1 g thiamine-HCl, 1.2 g pantothenic acid, 1 g riboflavin, 2.3 g nicotinic acid, 12 g pyridoxine HCl, 100 mg vitamin B_{12}
Trace element solution	40 mg $ZnCl_2$, 200 mg $FeCl_3 \times 6\ H_2O$, 10 mg $CuCl_2 \times 2\ H_2O$, 10 mg $MnCl_2 \times 4\ H_2O$, 10 mg $Na_2B_4O_7 \times 10\ H_2O$, 10 mg $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$

Table S 3. Specificity predictions of A-domains from *Ca.* Mycoavidus sp. SF9855 using NRPSpredictor2.[6]

Position in NRP	Module structure	A-domain PFAM score	Signatures/Stachelhaus code	Most probable amino acid (score in %)
1	$C_{Starter}$ – A – T	133.5	LALAFDASLQSSDCLVGGEYNVYGPTECTVDATL / DAQDLGVVD-	Gln (90)
2	C_{Dual} – A – T	190.8	LATHFDFSVWEGNQIFGGEVNMYGITETTVHVTY / DFWNIGMVH-	Thr (90)
3	$^{L}C_{L}$ – A – T	89.5	RWFTFVDSVTEGVVVCSGELNFYGSSEVNGDVTF / VDTVVSFGD-	β-Ala (50)
4	C_{Dual} – A – T	150.9	LAQVFDVSAADMSLILGGEFNAYGPTEVSVCATA / DVASIGAVC-	Trp (70)
5	C_{Dual} – A – T	198.2	RWMTFDVSVWEWHFICSGEYNLYGPTEAAIDVTA / DVWHISLID-	Ser (90)
6	$^{L}C_{L}$ – A – T – E	147.2	LAQAFDASVSEMTLILAGEFNAYGPTEASVCATA / DASTIAAVC-	Tyr (90)
-	$^{D}C_{L}$ – A – T – TE	*11.5*	*LHGSSDASMYEQDNYLSGDAHPFGP----VGARM / DAYDYSPVG-*	*Lys (40)*

Table S 4. Percent identity matrix of A-domains of *Ca.* Mycoavidus sp. SF9855 and *Ca.* Mycoavidus necroximicus using Clustal2.1. *Unfunctional module, closest amino acid prediction by NRPSpredictor2.[6]

		***Ca.* Mycoavidus sp. SF9855**							***Ca.* Mycoavidus necroximicus**						
		Gln	Thr	β-Ala	Trp	Ser	Tyr	***Lys****	Gln	Thr	β-Ala	Trp	Ser	Tyr	***Val****
***Ca.* M. sp. SF9855**	Gln	-	67.96	65.50	71.60	68.16	70.25	***43.56***	96.95	68.57	66.12	73.87	68.16	69.96	***40.66***
	Thr	67.96	-	64.50	70.76	71.52	67.97	***43.70***	68.57	96.79	65.52	68.51	70.10	67.69	***40.49***
	β-Ala	65.50	64.50	-	65.15	68.43	65.22	***42.22***	65.30	64.30	96.56	64.95	67.62	63.92	***39.47***
	Trp	71.60	70.76	65.15	-	71.37	82.75	***42.22***	71.60	71.98	65.98	92.43	73.42	82.82	***38.67***
	Ser	68.16	71.52	68.43	71.37	-	71.25	***44.94***	67.76	70.30	68.43	70.76	94.14	69.94	***40.04***
	Tyr	70.25	67.97	65.22	82.75	71.25	-	***43.95***	70.04	68.17	64.39	83.37	71.66	95.89	***40.92***
	Lys*	***43.56***	***43.70***	***42.22***	***42.22***	***44.94***	***43.95***	**-**	***43.32***	***44.20***	***43.46***	***43.46***	***44.69***	***44.44***	***96.36***
***Ca.* M. nec.**	Gln	96.95	68.57	65.30	71.60	67.76	70.04	***43.32***	-	67.96	66.32	73.46	67.55	69.75	***40.46***
	Thr	68.57	96.79	64.30	71.98	70.30	68.17	***44.20***	67.96	-	65.31	69.33	71.11	67.89	***40.90***
	β-Ala	66.12	65.52	96.56	65.98	68.43	64.39	***43.46***	66.32	65.31	-	65.57	68.23	65.15	***40.49***
	Trp	73.87	68.51	64.95	92.43	70.76	83.37	***43.46***	73.46	69.33	65.57	-	72.19	84.05	***39.71***
	Ser	68.16	70.10	67.62	73.42	94.14	71.66	***44.69***	67.55	71.11	68.23	72.19	-	70.55	***40.04***
	Tyr	69.96	67.69	63.92	82.82	69.94	95.89	***44.44***	69.75	67.89	65.15	84.05	70.55	-	***41.37***
	Val*	***40.66***	***40.49***	***39.47***	***38.67***	***40.04***	***40.92***	***96.36***	***40.46***	***40.90***	***40.49***	***39.71***	***40.04***	***41.37***	**-**

Table S 5. Genes flanking the biosynthetic gene cluster of symbiosin (3) in *Ca.* Mycoavidus sp. SF9855.

Locus tag	Length [bp]	Closest orthologous protein (HHpred or swissprot BLAST) [Species]	Accession number	Identity/ similarity
SF9855v2_01413	1278	Protein adenylyltransferase FICD [*Cricetulus griseus*]	A0A061I403.1	31%/44%
SF9855v2_01412	423	Biopolymer transport protein exbD1 [*Xanthomonas campestris* pv. *campestris* str. B100]	B0RLE7.1	36%/57%
SF9855v2_01411	723	Biopolymer transport protein exbD1 [*Xanthomonas campestris* pv. *campestris* str. B100]	B0RLE7.1	42%/61%
SF9855v2_01410	459	TonB C-terminal domain [*Escherichia coli*]	1XX3_A	17%/24%
SF9855v2_01409	282	uncharacterized protein [*Desulfitobacterium hafniense*]	3IPF_B	10%/16%
SF9855v2_01408	849	Glutamate racemase [*Ralstonia solanacearum* GMI1000]	Q8XY07.1	60%/72%
SF9855v2_01407	480	Bacterioferritin Cytochrome b-557.5 [*Azotobacter vinelandii*]	P22759.2	68%/78%
SF9855v2_01406	576	UPF0114 protein PM1258 [*Pasteurella multocida subsp. multocida* str. Pm70]	P57929.1	55%/74%
SF9855v2_01404	138	Citrate/malate transporter [*Bacillus subtilis subsp. subtilis* str. 168]	P94363.1	68%/90%
SF9855v2_01403	822	Alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1 [*Gallus gallus*]	Q92183.1	32%/50%
SF9855v2_01402	744	Peptidoglycan endopeptidase RipA [*Mycobacterium tuberculosis*]	4Q4G_X	11%/3%
SF9855v2_01401	1545	Protein adenylyltransferase SelO [*Paraburkholderia xenovorans* LB400]	Q13YZ6.1	63%/76%
SF9855v2_01400	156	Protein PTHB1 [*Homo sapiens*]	4YD8_B	25%/30%
SF9855v2_01399	24195	Nonribosomal peptide synthetase mpbA (Malpibaldin synthetase) [Mortierella alpina]	P0DUV4.1	52%/66%
SF9855v2_01398	2628	Uncharacterized WD repeat-containing protein alr3466 [*Nostoc* sp. PCC 7120]	Q8YRI1.1	39%/54%
SF9855v2_01397	105	VPP_BPHC1 Probable terminase [*Haemophilus phage* HP1]	P51718	15%/2%
SF9855v2_01396	4329	Dimodular non-ribosomal peptide synthetase [*Bacillus subtilis* subsp. *subtilis* str. 168]	P45745.4	42%/61%
SF9855v2_01395	1464	Uncharacterized monooxygenase Mb0916 [*Mycobacterium tuberculosis* variant bovis AF2122/97]	P64746.1	41%/60%

SF9855v2_01394	423	Probable tail fiber assembly protein [*Enterobacteria phage* SfV]	O22005.2	47%/60%
SF9855v2_01393	243	Antiholin [*Enterobacteria phage* P21]	P27360.1	38%/17%
SF9855v2_01392	519	Lysozyme muramidase [*Acinetobacter baumannii*]	6ET6_A	27%/35%
SF9855v2_01391	462	VG55_BPN15 Protein gp55 OS [*Escherichia phage* N15 OX]	O64363	19%/17%
SF9855v2_01390	5331	Uncharacterized WD repeat-containing protein alr3466 [*Nostoc* sp. PCC 7120]	Q8YRI1.1	37%/53%

Table S 6. Genes in front of or associated with the biosynthetic gene cluster of symbiosin (3) in *Ca.* Mycoavidus necroximicus.

Locus tag	Length [bp]	Closest orthologous protein (HHpred or swissprot BLAST) [Species]	Accession number	Identity/similarity
Mnec_01371	258	Bacterioferritin-associated ferredoxin [*Serratia marcescens*]	O68934.1	26%/56%
Mnec_01370	849	Glutamate racemase [*Ralstonia solanacearum* GMI1000]	Q8XY07.1	60%/73%
Mnec_01369	480	Bacterioferritin [*Azotobacter vinelandii*]	P22759.2	66%/78%
Mnec_01368	576	UPF0114 protein PM1258 [*Pasteurella multocida subsp. multocida* str. Pm70]	P57929.1	59%/80%
Mnec_01367	309	Prophage integrase IntR [*Escherichia coli* K-12]	P76056.1	40%/56%
Mnec_01366	537	Unknown function [*Bacteroides caccae* ATCC 43185]	3NO2_A	17%/12%
Mnec_01365	7176	tRNA nuclease CdiA [*Escherichia coli* O157:H7 str. EC869]	B3BM48.1	27%/43%
Mnec_01364	1059	Puromycin-sensitive aminopeptidase [*Caenorhabditis elegans*]	Q4TT88.1	29%/53%
Mnec_01363	951	HTH-type transcriptional activator AmpR [*Citrobacter freundii*]	P12529.3	31%/50%
Mnec_01362	1383	Citrate/malate transporter [*Bacillus subtilis subsp. subtilis* str. 168]	P94363.1	50%/70%
Mnec_01361	408	Ribulose bisphosphate carboxylase large chain [*Nitrobacter vulgaris*]	Q59613.1	26%/41%
Mnec_01360	288	DNA topoisomerase II-binding protein 1 [*Mus musculus*]	Q6ZQF0.2	46%/60%

Mnec_01359	1545	Protein adenylyltransferase SelO [*Paraburkholderia xenovorans* LB400]	Q13YZ6.1	63%/76%
Mnec_01358	228	Protein SPIRRIG [*Arabidopsis thaliana*]	F4HZB2.1	55%/59%
Mnec_01357	24438	Nonribosomal peptide synthetase mpbA (Malpibaldin synthetase) [*Mortierella alpina*]	P0DUV4.1	52%/67%
Mnec_01356	219	Mitotic checkpoint serine/threonine-protein kinase BUB1 beta [*Homo sapiens*]	5LCW_S	16%/11%
Mnec_01355	2616	Uncharacterized WD repeat-containing protein alr3466 [*Nostoc* sp. PCC 7120]	Q8YRI1.1	40%/55%
Mnec_01354	366	Tn5 transposase [*Escherichia coli*]	1MUS_A	13%/-%
Mnec_01353	2388	Nonribosomal peptide synthetase mpcA (Malpicyclin synthetase) [*Mortierella alpina*]	P0DUV3.1	34%/54%
Mnec_01352	1326	Tn5 transposase [*Escherichia coli*]	Q46731.1	26%/43%
Mnec_01351	3576	Bacitracin synthase 1 [*Bacillus licheniformis*]	O68006.1	23%/45%
Mnec_01350	1236	Mycinamicin biosynthesis protein G [*Micromonospora griseorubida*]	Q59523.1	34%/50%
Mnec_01349	615	Hypothetical protein Ta1170/Ta1414 [*Thermoplasma acidophilum*]	1PAV_A	11%/18%
Mnec_01348	264	Vesicle-associated membrane protein 2	5W5C_A	16%/43%

Table S 7. Biological activity of symbiosin (3) against human cell lines.

Compound	Antiproliferative Effect [µM]		Cytotoxicity [µM]
	HUVEC GI_{50}	K-562 GI_{50}	HeLa CC_{50}
symbiosin	50.9	37.5	> 51

Table S 8. Bioactivity of symbiosin (3) against microbes.

	Inhibitory zone symbiosin (**3**) [mm]	Inhibitory zone ciprofloxacin [mm]	Inhibitory zone amphotericin [mm]	MIC [µM]
Concentration	1 g mL^{-1}	5 µg mL^{-1}	10 µg mL^{-1}	
Bacillus subtilis ATCC 6633	11/13 P	30	-	-
Staphylococcus aureus SG511	11 P	19	-	-
Mycobacterium vaccae IMET 10670	18	23 p	-	6.49
Mycobacterium smegmatis SG987	13 p	21 p	-	6.49
Mycobacterium aurum SB66	13 p	25/35 p	-	12.98
Mycobacterium fortuitum	12 p	21/33 p	-	25.97
Pseudomonas aeruginosa K 799/61	0	26	-	-
Escherichia coli SG 458	0	24/32 p	-	-
Staphylococcus aureus 134/94 (MRSA)	0	0	-	-
Enterococcus faecialis 1528 (VRE)	12	17	-	-
Sporobolomyces salmonicolor SBUG 549	0	-	18 p	-
Candida albicans ATCC14053	0	-	21	-
Penicillium notatum JP 36	0	-	18 p	-

Table S 9. Primers used in this study.

Primer name	Sequence	Forward/ reverse	Function
HBP53	ACA AGA CGG GAG ATC TGG CC	F	Amplification of missing parts of the symbiosin BGC between fragment 1.7 and fragment 4
HBP54	GCC AGA ATA TGC GTG AGC CG	R	Amplification of missing parts of the symbiosin BGC between fragment 1.7 and fragment 4
HBP62	GGC AGC GTA TTG ATA AAC AGC	R	Sequencing of missing part amplified with HBP53 and HBP54
HBP60	CAA CCG GCA CGA CAT TCT GC	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP71	CGA TCG CTG AAC AAT TGG	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP74	TGC TGC GCT TTG TGA TTG C	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP61	CAT TGA ACG ATC GGC TGC	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP76	CAC ATA CGC GAG ATG ACG	R	Sequencing of missing part amplified with HBP53 and HBP54
HBP64	ATC CAC CAG TTG TTC GAA GC	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP63	TAC TGG CGA TCC TGA AGG	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP81	CCA GTA GTT CAG ACC AGA T	R	Sequencing of missing part amplified with HBP53 and HBP54

HBP70	GGA TTC CGG ATT TGA AGG	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP67	TTC TCG GAC AAG GAT GAC G	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP69	GCT TTC GTA TCG AGC TGG	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP79	ATC TGG TCT GAA CTA CTG G	F	Sequencing of missing part amplified with HBP53 and HBP54
HBP83	CAA AGA AGC CGA TCA ACG	R	Sequencing of missing part amplified with HBP53 and HBP54
HBP80	CGT ATC ACA TCC CGT TCG	F	Sequencing of missing part amplified with HBP53 and HBP54

Supplemental Figures

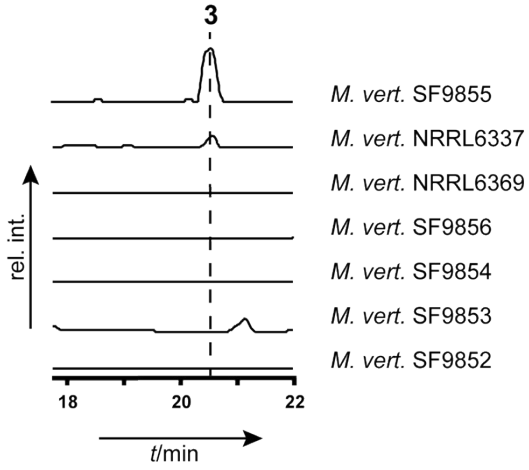


Figure S 1. Producer of symbiosin (3). Growth on PDA induced the production of symbiosin in the two *M. verticillata* strains NRRL6337 and SF9855, whereas for the other tested strains no regarding HPLC signal could be detected.

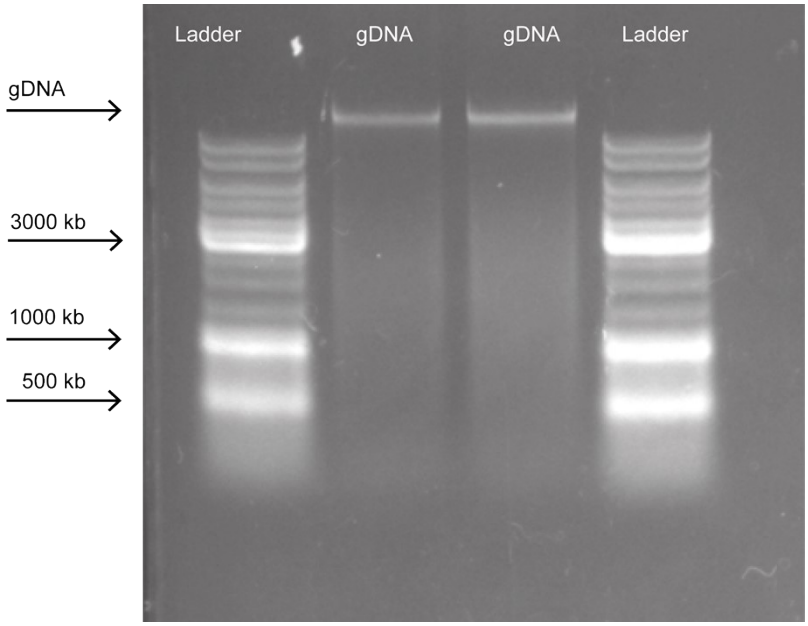


Figure S 2. Gel picture of genomic DNA isolated from *Ca*. Mycoavidus SF9855 for amplification of 16 rDNA and genome sequencing.

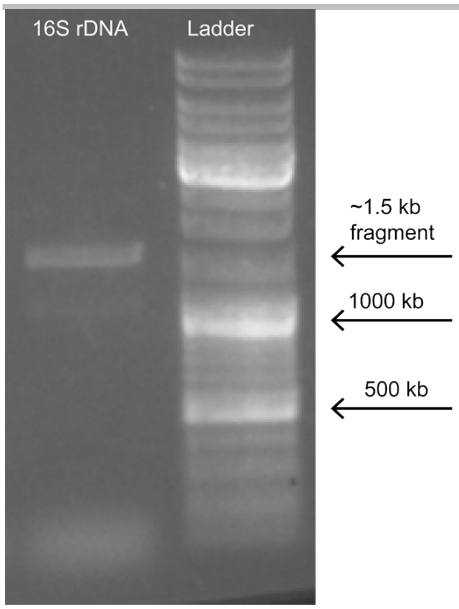


Figure S 3. Gel picture of PCR-amplified 16S rDNA of *Ca*. Mycoavidus SF9855 of *M. verticillata* SF9855. 16S rDNA was amplified with the primers 8F (AGA GTT TGA TCC TGG CTC AG) and 1492R (CGG TTA CCT TGT TAC GAC TT).

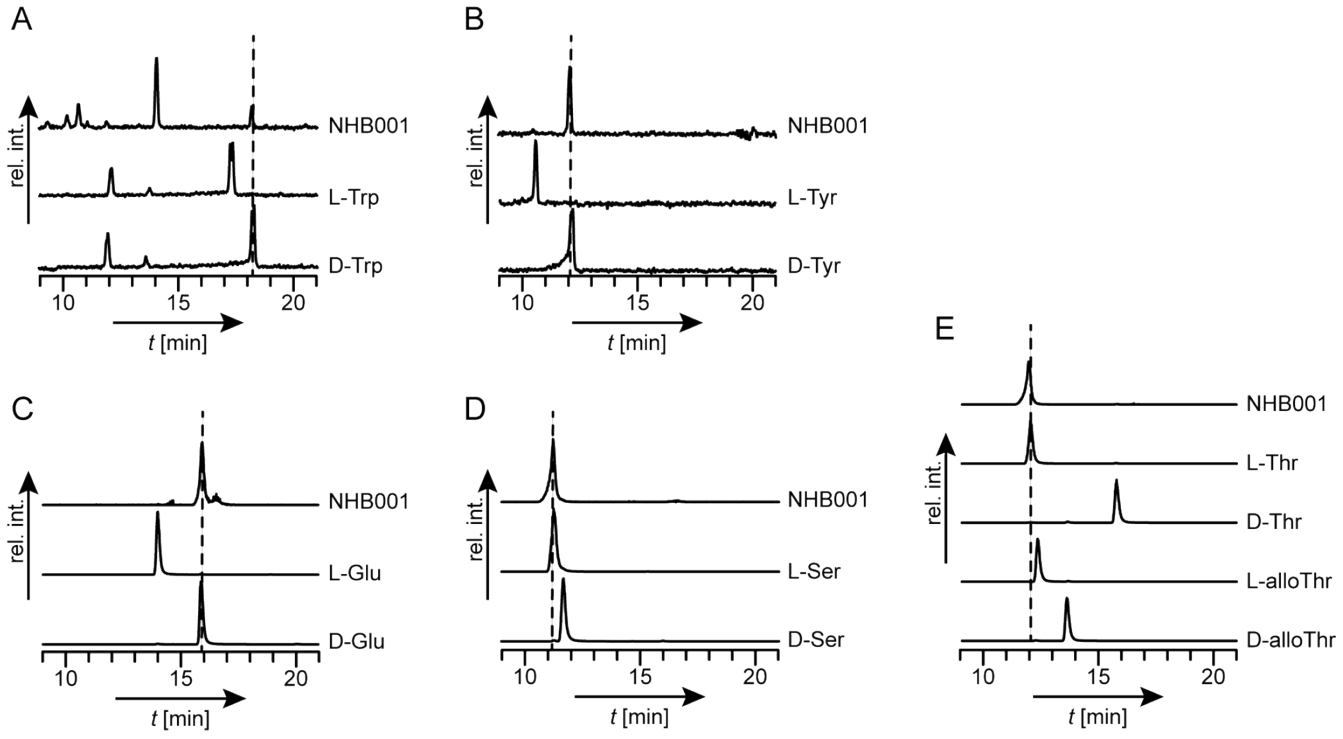


Figure S 4. EIC traces or HPLC-profiles of derivatized amino acids for determination of absolute configuration of symbiosin (3) by Marfey's reagent. A) HPLC-profile for the determination of the configuration of tryptophan. B) HPLC-profile for the determination of the configuration of tyrosine. C) EIC trace for the determination of the configuration of glutamine. D) EIC trace for the determination of the configuration of serine. E) EIC trace for the determination of the configuration of threonine.

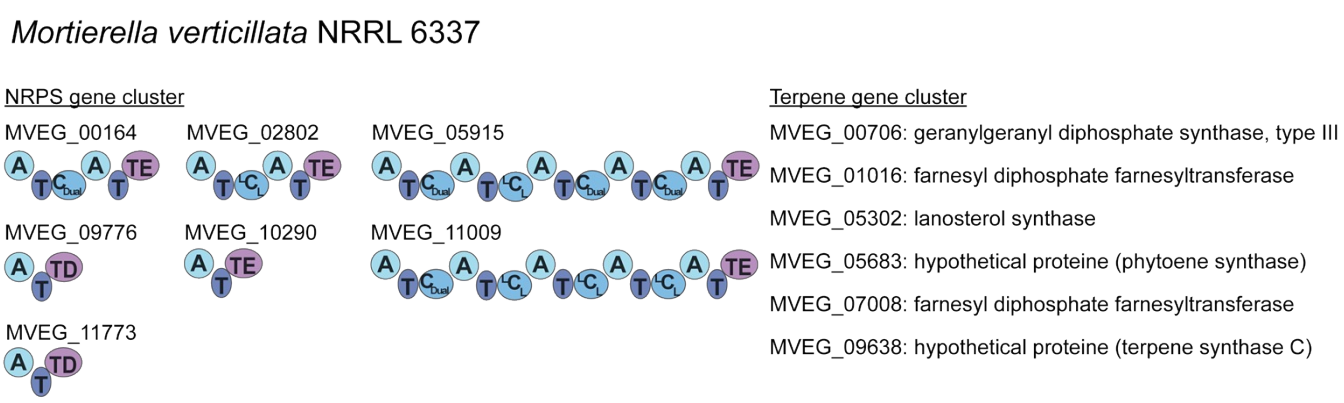


Figure S 5. Secondary metabolite gene clusters of *Mortierella verticillata* NRRL 6337. A: Adenylation domain, T: thiolation domain, C: condensation domain, TE: thioesterase domain, TD: terminal reductase domain.

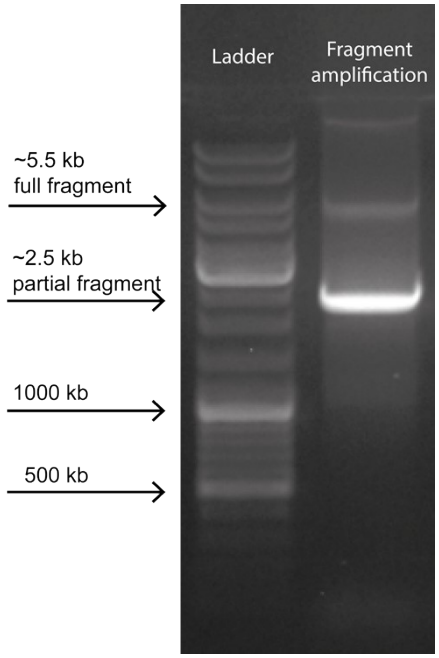


Figure S 6. Gel picture of the amplified region (Primer HBP53 and HBP54) for sequencing of missing regions in genome sequence of *Ca.* M. necroximicus.

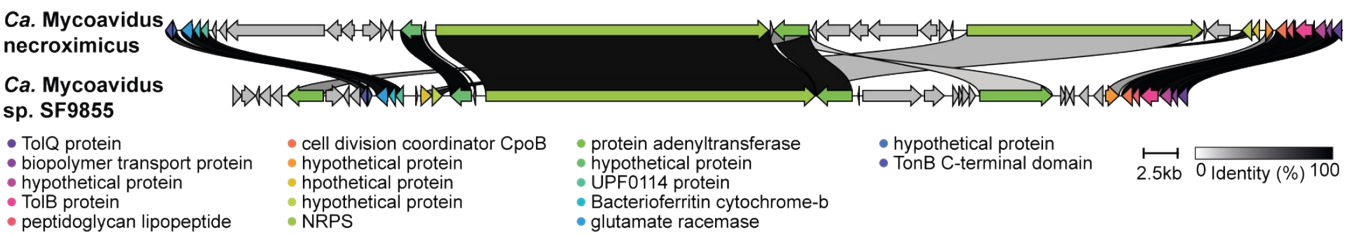
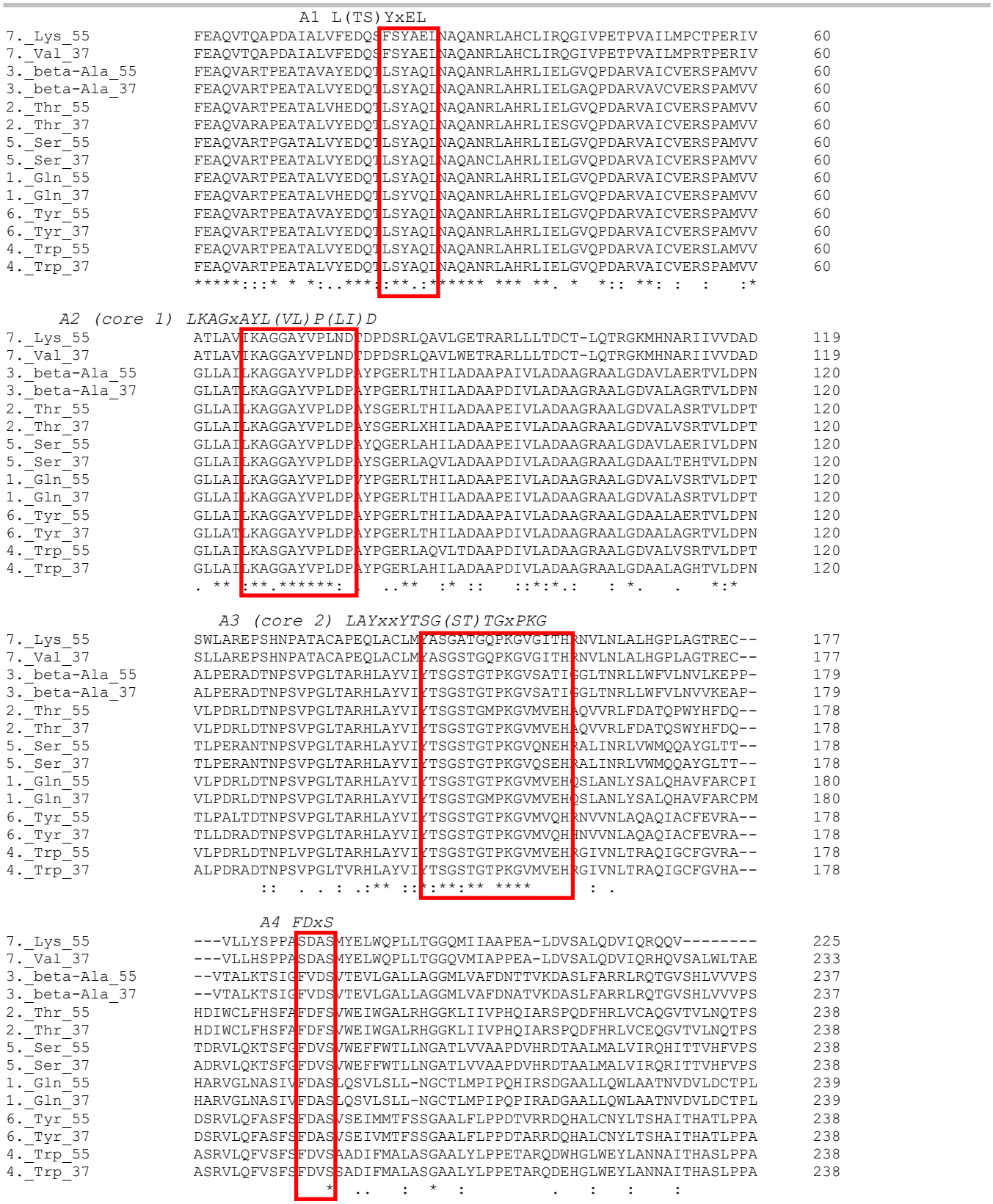
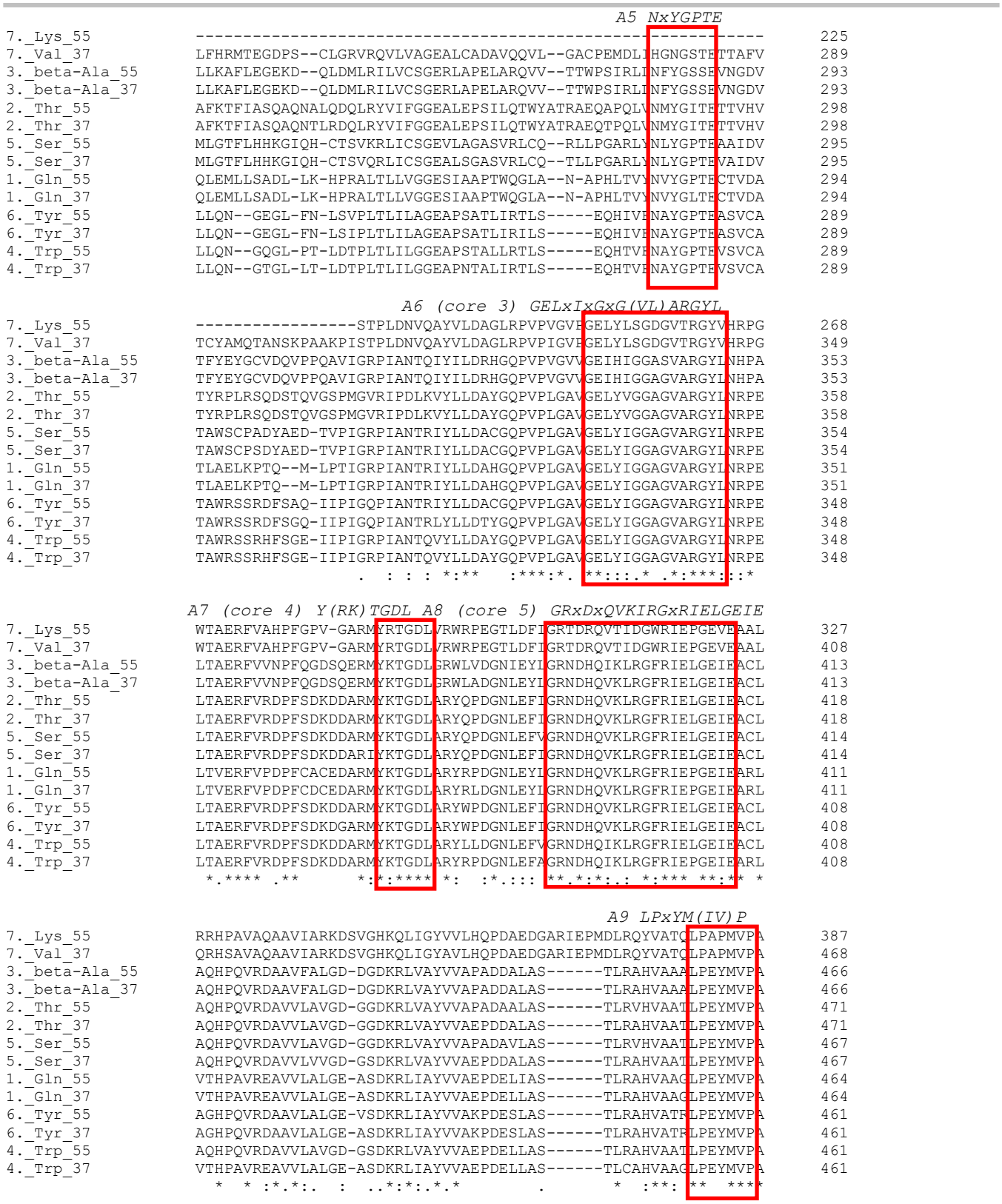


Figure S 7. Comparison of symbiosin (3) biosynthetic gene cluster and surrounding genes of *Ca.* M. sp. SF9855 and *Ca.* M. necroximicus.


A1 L(TS)YxEL
7._Lys_55 FEAQVTQAPDAIALVFEDQSFSYAELNAQANRLAHCLIRQGIVPETPVAILMPCTPERIV 60
7._Val_37 FEAQVTQAPDAIALVFEDQSFSYAELNAQANRLAHCLIRQGIVPETPVAILMPRTPERIV 60
3._beta-Ala_55 FEAQVARTPEATAVAYEDQTLSYAQLNAQANRLAHRLIELGVQPDARVAICVERSPAMVV 60
3._beta-Ala_37 FEAQVARTPEATALVYEDQTLSYAQLNAQANRLAHRLIELGAQPDARVAVCVERSPAMVV 60
2._Thr_55 FEAQVARTPEATALVHEDQTLSYAQLNAQANRLAHRLIELGVQPDARVAICVERSPAMVV 60
2._Thr_37 FEAQVARAPEATALVYEDQTLSYAQLNAQANRLAHRLIESGVQPDARVAICVERSPAMVV 60
5._Ser_55 FEAQVARTPGATALVYEDQTLSYAQLNAQANRLAHRLIELGVQPDARVAICVERSPAMVV 60
5._Ser_37 FEAQVARTPEATALVYEDQTLSYAQLNAQANCLAHRLIELGVQPDARVAICVERSPAMVV 60
1._Gln_55 FEAQVARTPEATALVYEDQTLSYAQLNAQANRLAHRLIELGVQPDARVAICVERSPAMVV 60
1._Gln_37 FEAQVARTPEATALVHEDQTLSYVQLNAQANRLAHRLIELGVQPDARVAICVERSPAMVV 60
6._Tyr_55 FEAQVARTPEATAVAYEDQTLSYAQLNAQANRLAHRLIELGVQPDARVAICVERSPAMVV 60
6._Tyr_37 FEAQVARTPEATALVYEDQTLSYAQLNAQANRLAHRLIELGVQPDARVAICVERSPAMVV 60
4._Trp_55 FEAQVARTPEATALVYEDQTLSYAQLNAQANRLAHRLIELGVQPDARVAICVERSLAMVV 60
4._Trp_37 FEAQVARTPEATALVYEDQTLSYAQLNAQANRLAHRLIELGVQPDARVAICVERSPAMVV 60
A2 (core 1) LKAGxAYL(VL)P(LI)D
7._Lys_55 ATLAVIKAGGAYVPLNDTDPDSRLQAVLGETRARLLLTDCT-LQTRGKMHNARIIVVDAD 119
7._Val_37 ATLAVIKAGGAYVPLNDTDPDSRLQAVLWETRARLLLTDCT-LQTRGKMHNARIIVVDAD 119
3._beta-Ala_55 GLLAILKAGGAYVPLDPAYPGERLTHILADAAPAIVLADAAGRAALGDAVLAERTVLDPN 120
3._beta-Ala_37 GLLATLKAGGAYVPLDPAYPGERLTHILADAAPDIVLADAAGRAALGDVALAGRTVLDPN 120
2._Thr_55 GLLAILKAGGAYVPLDPAYSGERLTHILADAAPEIVLADAAGRAALGDVALASRTVLDPT 120
2._Thr_37 GLLAILKAGGAYVPLDPAYSGERLXHILADAAPEIVLADAAGRAALGDVALVSRTVLDPT 120
5._Ser_55 GLLAILKAGGAYVPLDPAYQGERLAHILADAAPEIVLADAAGRAALGDAVLAERIVLDPN 120
5._Ser_37 GLLAILKAGGAYVPLDPAYSGERLAQVLADAAPDIVLADAAGRAALGDAALTEHTVLDPN 120
1._Gln_55 GLLAILKAGGAYVPLDPVYPGERLTHILADAAPEIVLADAAGRAALGDVALVSRTVLDPT 120
1._Gln_37 GLLAILKAGGAYVPLDPAYPGERLTHILADAAPEIVLADAAGRAALGDVALASRTVLDPT 120
6._Tyr_55 GLLAILKAGGAYVPLDPAYPGERLTHILADAAPAIVLADAAGRAALGDAALAERTVLDPN 120
6._Tyr_37 GLLATLKAGGAYVPLDPAYPGERLTHILADAAPDIVLADAAGRAALGDAALAGRTVLDPN 120
4._Trp_55 GLLAILKASGAYVPLDPAYPGERLAQVLTDAAPDIVLADAAGRAALGDVALVSRTVLDPT 120
4._Trp_37 GLLAILKAGGAYVPLDPAYPGERLAHILADAAPDIVLADAAGRAALGDAALAGHTVLDPN 120
A3 (core 2) LAYxxYTSG(ST)TGxPKG
7._Lys_55 SWLAREPSHNPATACAPEQLACLMYASGATGQPKGVGITHRNVLNLALHGPLAGTREC-- 177
7._Val_37 SLLAREPSHNPATACAPEQLACLMYASGSTGQPKGVGITHRNVLNLALHGPLAGTREC-- 177
3._beta-Ala_55 ALPERADTNPSVPGLTARHLAYVIYTSGSTGTPKGVSATIGGLTNRLLWFVLNVLKEPP- 179
3._beta-Ala_37 ALPERADTNPSVPGLTARHLAYVIYTSGSTGTPKGVSATIGGLTNRLLWFVLNVVKEAP- 179
2._Thr_55 VLPDRLDTNPSVPGLTARHLAYVIYTSGSTGMPKGVMVEHAQVVRLFDATQPWYHFDQ-- 178
2._Thr_37 VLPDRLDTNPSVPGLTARHLAYVIYTSGSTGTPKGVMVEHAQVVRLFDATQSWYHFDQ-- 178
5._Ser_55 TLPERANTNPSVPGLTARHLAYVIYTSGSTGTPKGVQNEHRALINRLVWMQQAYGLTT-- 178
5._Ser_37 TLPERANTNPSVPGLTARHLAYVIYTSGSTGTPKGVQSEHRALINRLVWMQQAYGLTT-- 178
1._Gln_55 VLPDRLDTNPSVPGLTARHLAYVIYTSGSTGTPKGVMVEHQSLANLYSALQHAVFARCPI 180
1._Gln_37 VLPDRLDTNPSVPGLTARHLAYVIYTSGSTGMPKGVMVEHQSLANLYSALQHAVFARCPM 180
6._Tyr_55 TLPALTDTNPSVPGLTARHLAYVIYTSGSTGTPKGVMVQHRNVVNLAQAQIACFEVRA-- 178
6._Tyr_37 TLLDRADTNPSVPGLTARHLAYVIYTSGSTGTPKGVMVQHNVVNLAQAQIACFEVRA-- 178
4._Trp_55 VLPDRLDTNPLVPGLTARHLAYVIYTSGSTGTPKGVMVEHRGIVNLTRAQIGCFGVRA-- 178
4._Trp_37 ALPDRADTNPSVPGLTVRHLAYVIYTSGSTGTPKGVMVEHRGIVNLTRAQIGCFGVHA-- 178
A4 FDxS
7._Lys_55 ---VLLYSPPASDASMYELWQPLLTGGQMIIAAPEA-LDVSALQDVIQRQQV-------- 225
7._Val_37 ---VLLHSPPASDASMYELWQPLLTGGQVMIAPPEA-LDVSALQDVIQRHQVSALWLTAE 233
3._beta-Ala_55 --VTALKTSIGFVDSVTEVLGALLAGGMLVAFDNTTVKDASLFARRLRQTGVSHLVVVPS 237
3._beta-Ala_37 --VTALKTSIGFVDSVTEVLGALLAGGMLVAFDNATVKDASLFARRLRQTGVSHLVVVPS 237
2._Thr_55 HDIWCLFHSFAFDFSVWEIWGALRHGGKLIIVPHQIARSPQDFHRLVCAQGVTVLNQTPS 238
2._Thr_37 HDIWCLFHSFAFDFSVWEIWGALRHGGKLIIVPHQIARSPQDFHRLVCEQGVTVLNQTPS 238
5._Ser_55 TDRVLQKTSFGFDVSVWEFFWTLLNGATLVVAAPDVHRDTAALMALVIRQHITTVHFVPS 238
5._Ser_37 ADRVLQKTSFGFDVSVWEFFWTLLNGATLVVAAPDVHRDTAALMALVIRQRITTVHFVPS 238
1._Gln_55 HARVGLNASIVFDASLQSVLSLL-NGCTLMPIPQHIRSDGAALLQWLAATNVDVLDCTPL 239
1._Gln_37 HARVGLNASIVFDASLQSVLSLL-NGCTLMPIPQPIRADGAALLQWLAATNVDVLDCTPL 239
6._Tyr_55 DSRVLQFASFSFDASVSEIMMTFSSGAALFLPPDTVRRDQHALCNYLTSHAITHATLPPA 238
6._Tyr_37 DSRVLQFASFSFDASVSEIVMTFSSGAALFLPPDTARRDQHALCNYLTSHAITHATLPPA 238
4._Trp_55 ASRVLQFVSFSFDVSAADIFMALASGAALYLPPETARQDWHGLWEYLANNAITHASLPPA 238
4._Trp_37 ASRVLQFVSFSFDVSSADIFMALASGAALYLPPETARQDEHGLWEYLANNAITHASLPPA 238


A5 NxYGPTE
7._Lys_55 225
7._Val_37 289
3._beta-Ala_55 293
3._beta-Ala_37 293
2._Thr_55 298
2._Thr_37 298
5._Ser_55 295
5._Ser_37 295
1._Gln_55 294
1._Gln_37 294
6._Tyr_55 289
6._Tyr_37 289
4._Trp_55 289
4._Trp_37 289
A6 (core 3) GELxIxGxG(VL)ARGYL
7._Lys_55 268
7._Val_37 349
3._beta-Ala_55 353
3._beta-Ala_37 353
2._Thr_55 358
2._Thr_37 358
5._Ser_55 354
5._Ser_37 354
1._Gln_55 351
1._Gln_37 351
6._Tyr_55 348
6._Tyr_37 348
4._Trp_55 348
4._Trp_37 348
A7 (core 4) Y(RK)TGDL A8 (core 5) GRxDxQVKIRGxRIELGEIE
7._Lys_55 327
7._Val_37 408
3._beta-Ala_55 413
3._beta-Ala_37 413
2._Thr_55 418
2._Thr_37 418
5._Ser_55 414
5._Ser_37 414
1._Gln_55 411
1._Gln_37 411
6._Tyr_55 408
6._Tyr_37 408
4._Trp_55 408
4._Trp_37 408
A9 LPxYM(IV)P
7._Lys_55 387
7._Val_37 468
3._beta-Ala_55 466
3._beta-Ala_37 466
2._Thr_55 471
2._Thr_37 471
5._Ser_55 467
5._Ser_37 467
1._Gln_55 464
1._Gln_37 464
6._Tyr_55 461
6._Tyr_37 461
4._Trp_55 461
4._Trp_37 461

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                    A10 NGK(VL)DR
7._Lys_55           AVRLLDSLPLMFNGKLDHKALSAWD---        412
7._Val_37           AVTLLDNLPLMFNGKLDHKALSAWDSTS        496
3._beta-Ala_55      AFVRLDAWPLTFNGKLDRRALPVPDADA        494
3._beta-Ala_37      AFVRLDAWPLTFNGKLDRRALPAPDADA        494
2._Thr_55           AFVQLNALPLTFNGKLDRRALPAPDADA        499
2._Thr_37           AFVQLDALPLTFNGKLDRRALPAPDADA        499
5._Ser_55           AFVQLNALPLTFNGKLDRRALPAPDADA        495
5._Ser_37           AFVQLDALPLTFNGKLDRRALPAPDADA        495
1._Gln_55           AFVQLDAWPLTFNGKLDRRALPVPDADA        492
1._Gln_37           AFVRLDAWPLTFSGKLDRRALPAPDADA        492
6._Tyr_55           AFMRLDAFPLTFNGKLDRRALPTPEF--        487
6._Tyr_37           AFMRLDAFPLTFNGKLDRRALPTPEFIS        489
4._Trp_55           AFVRLDAWPLTFNGKLDRRALPAPDADA        489
4._Trp_37           AFVQLDAWPLTFNGKLDRRALPAPDADA        489
                    *.  *:  **  .****::** . :
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Figure S 8. Sequence alignment of A-domains from NRPS-modules of *Ca*. M. sp. SF9855 (55) and *Ca*. M. necroximicus (37) responsible for production of symbiosin (3).

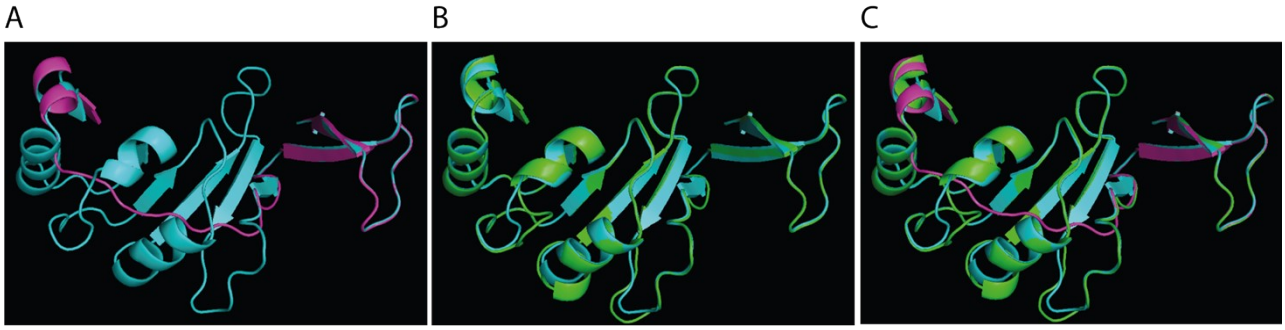

Figure S 9. Comparison of A domain subdomains. A) Structure alignment of the flavodoxin-like subdomain from the crystal structure of GrsA Phe A domain (cyan) with comparison to the respective part of the homology model from the *Ca*. M. sp. SF9855 derived A domain of module 7 of the NRPS associated with symbiosin (pink). B) Structure alignment of the flavodoxin-like subdomain from the crystal structure of GrsA Phe A domain (cyan) with comparison to the respective part of the homology model from the *Ca*. M. necroximicus derived A domain of module 7 of the NRPS associated with symbiosin (green). C) Structure alignment of the flavodoxin-like subdomain from the crystal structure of GrsA Phe A domain (cyan) with comparison to the respective part of the homology model from the *Ca*. M. sp. SF9855 (pink) and the *Ca*. M. necroximicus (green) derived A domain of module 7 of the NRPS associated with symbiosin.

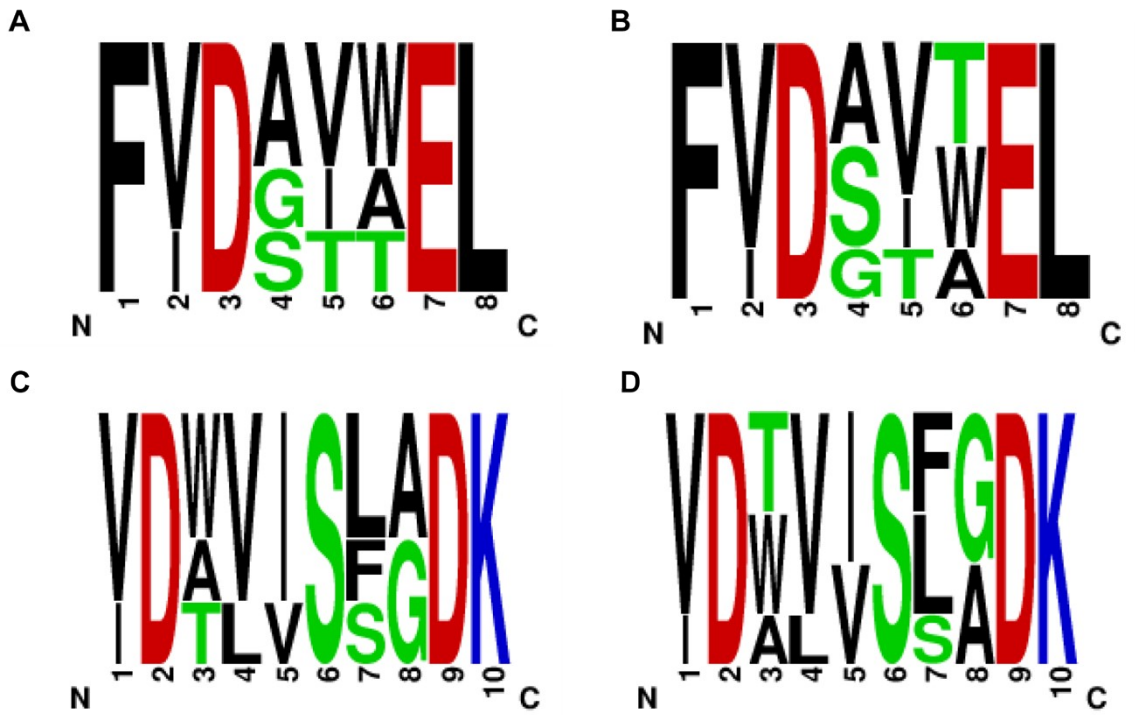

Figure S 10. WebLogos[9] of A domain regions specific for β-Ala. A) WebLogo of the A4 core sequence of A domains created from previously known β-Ala incorporating enzymes. B) WebLogo of the A4 core sequence of A domains created from previously known β-Ala incorporating enzymes including the domain responsible for β-Ala incorporation in symbiosin. C) WebLogo of the Stachelhaus-code created from previously known β-Ala incorporating A domains. Adapted from [14]. D) WebLogo of the Stachelhaus-code created from previously known β-Ala incorporating A domains including the domain responsible for β-Ala incorporation in symbiosin.

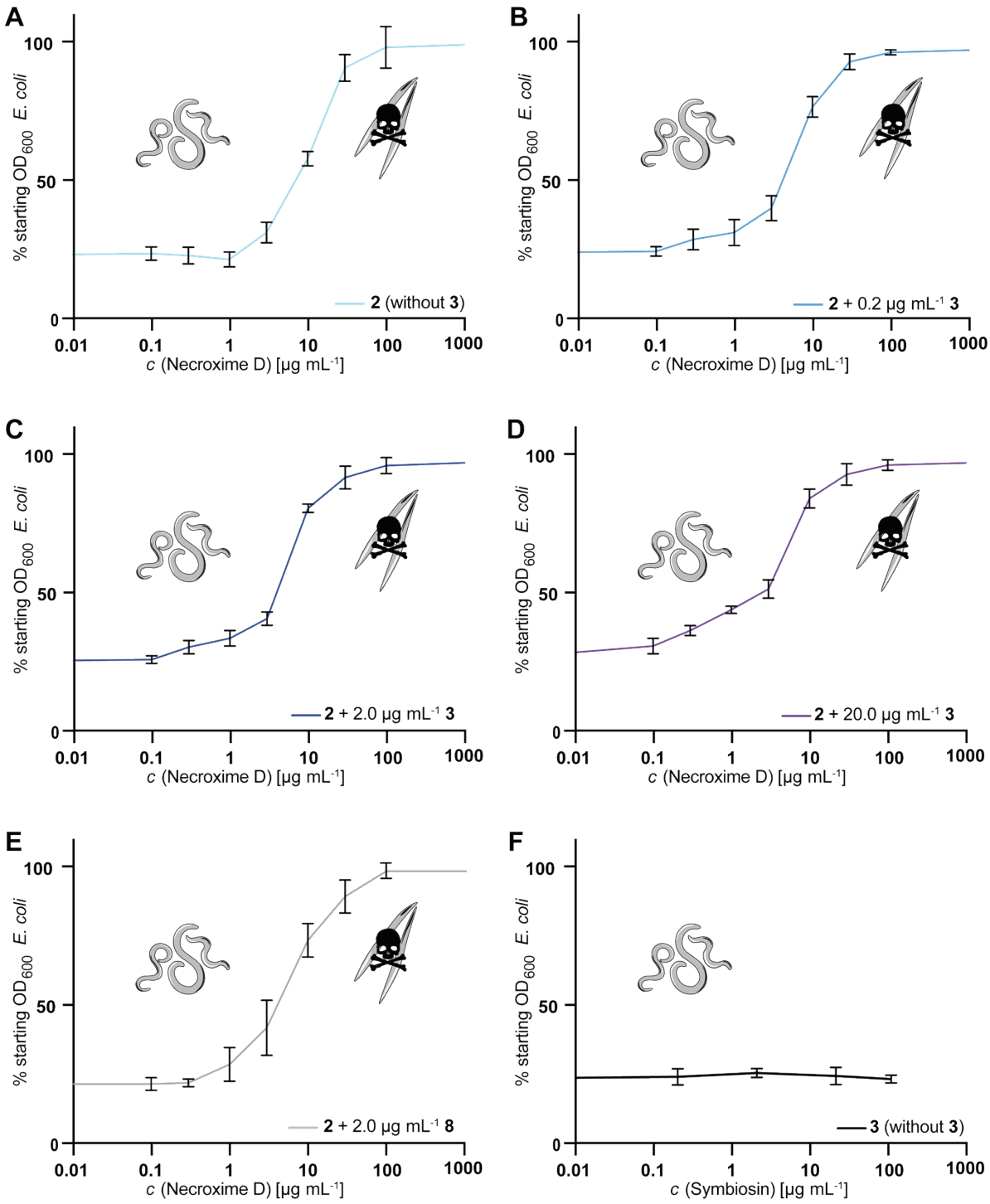

Figure S 11. Single curves of synergistic effects between necroxime D (2) and symbiosin (3) or surfactin (8). Nematode viability measurements in presence of different necroxime D concentrations, different symbiosin concentrations and necroxime D combined with 0.2 µg mL^{-1}, 2 µg mL^{-1} and 20 µg mL^{-1} **3** or **8**.

Spectra of the new compound

Table S 10. NMR data of symbiosin (3) in CD_3OD.

***Overlapping signals**

	Position	δ_C [ppm]	δ_H [ppm]; Signal (*J* [Hz])
Fatty acid	C=O	174.9	-
	2	26.6	1.43; 2 H m*
	3	69.7	3.87; 1 H m*
	4	44.5	2.23; 1 H m
			2.33; 1 H m*
	5-11	30.7	1.24; 14 H m*
	12	33.1	1.26; 2 H m*
	13	23.7	1.29; 2 H m*
	14	14.4	0.88; 3 H t (*7.1*)
Gln	C=O	175.9	-
	2	55.5	3.89; 1 H m*
	3	27.1	1.74; 1 H m
			1.86; 1 H m
	4	32.3	2.02; 1 H m
			2.17; 1 H m*
	5	176.9	-
	NH_2	-	-
	NH	-	8.25; 1 H d (*4.7*)
Thr	C=O	171.0	-
	2	57.5	4.60; 1 H m
	3	71.9	5.44; 1 H m
	4	16.6	1.18; 3 H d (*6.3*)
	NH	-	8.94; 1 H d (*10.1*)
β-Ala	C=O	174.6	-
	2	35.2	2.87; 1 H m
			3.06; 1 H m
	3	38.3	1.42; 2 H m*
	NH	-	7.56; 1 H d (*6.9*)
Trp	C=O	174.9	-
	2	54.9	4.69; 1 H m
	3	29.0	3.09; 1 H m*
			3.25; 1 H m
	NH	-	10.35; 1 H s
	4	125.1	7.07; 1 H s
	5	109.6	-
	6	137.9	-
	7	119.4	7.47; 1 H d (*7.9*)
	8	119.9	7.00; 1 H dd (*8.0*)
	9	122.6	7.07; 1 H dd (*8.2*)
	10	112.5	7.33; 1 H d (*8.2*)
	11	128.8	-
	NH	-	7.85; 1 H d (*8.0*)
Ser	C=O	172.8	-
	2	59.7	4.06; 1 H m
	3	62.4	3.66; 2 H m*
	NH	-	8.74; 1 H d (*5.0*)
Tyr	C=O	171.6	-
	2	57.0	3.80; 1 H m
	3	38.5	3.05; 1 H m*
			3.64; 1 H m*
	4	157.1	-
	5	131.6*	6.97; 2 H d (*8.4*)
	6	116.1*	6.68; 2 H d (*8.5*)
	7	130.4	-
	NH	-	8.22; 1 H d (*7.3*)

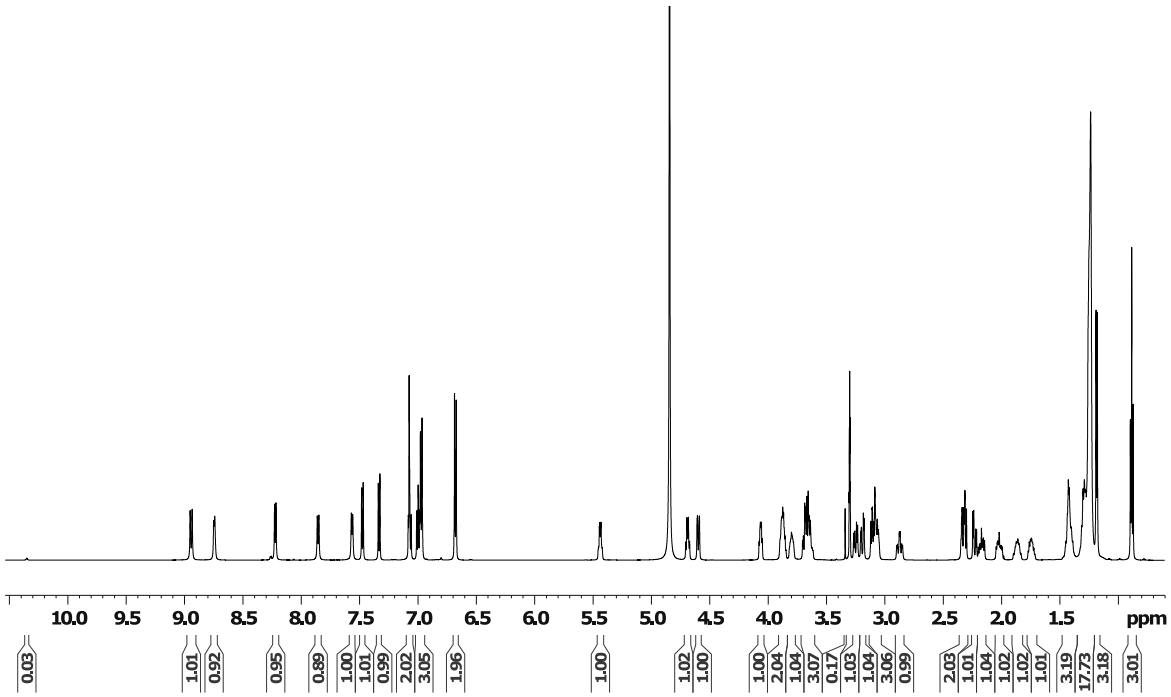

Figure S 12. ^{1}H NMR spectrum of symbiosin (3) in CD_3OD.

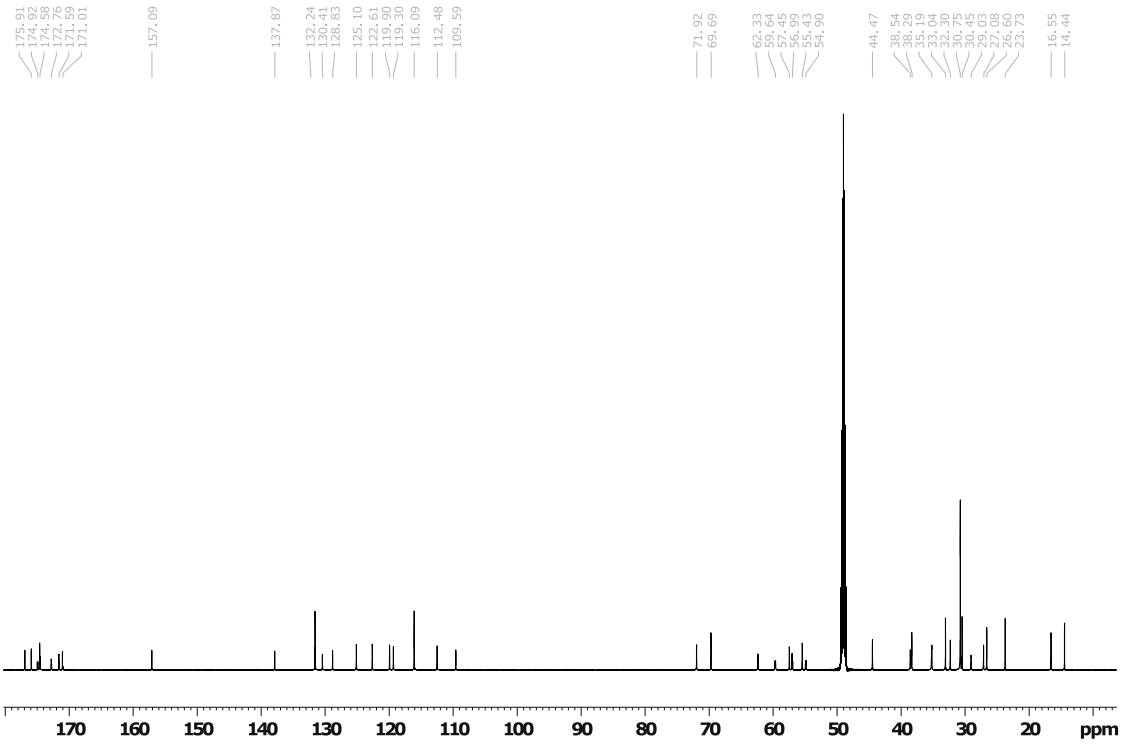

Figure S 13. ^{13}C NMR spectrum of symbiosin (3) in CD_3OD.

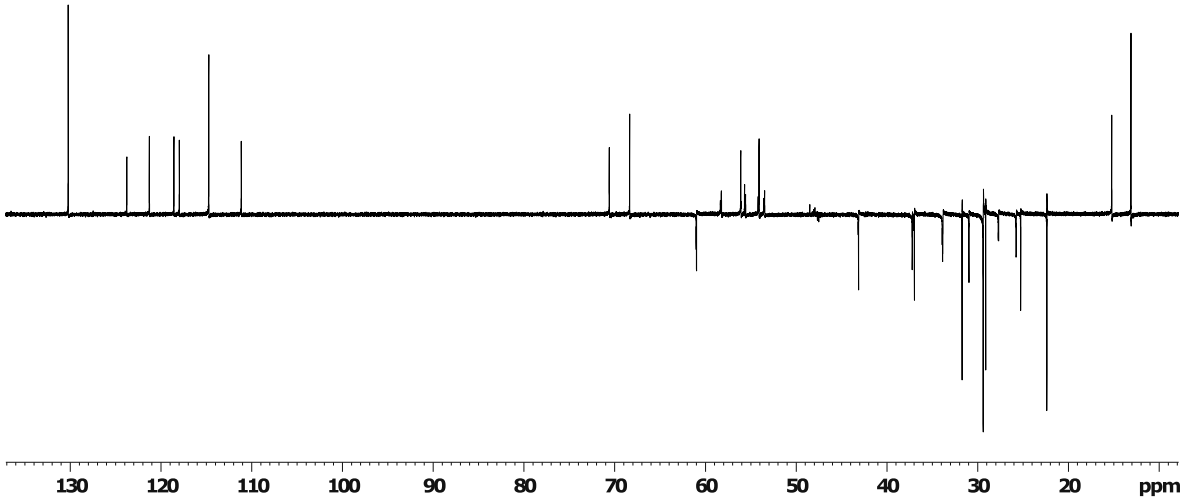

Figure S 14. ^{13}C DEPT135 spectrum of symbiosin (3) in CD_3OD.

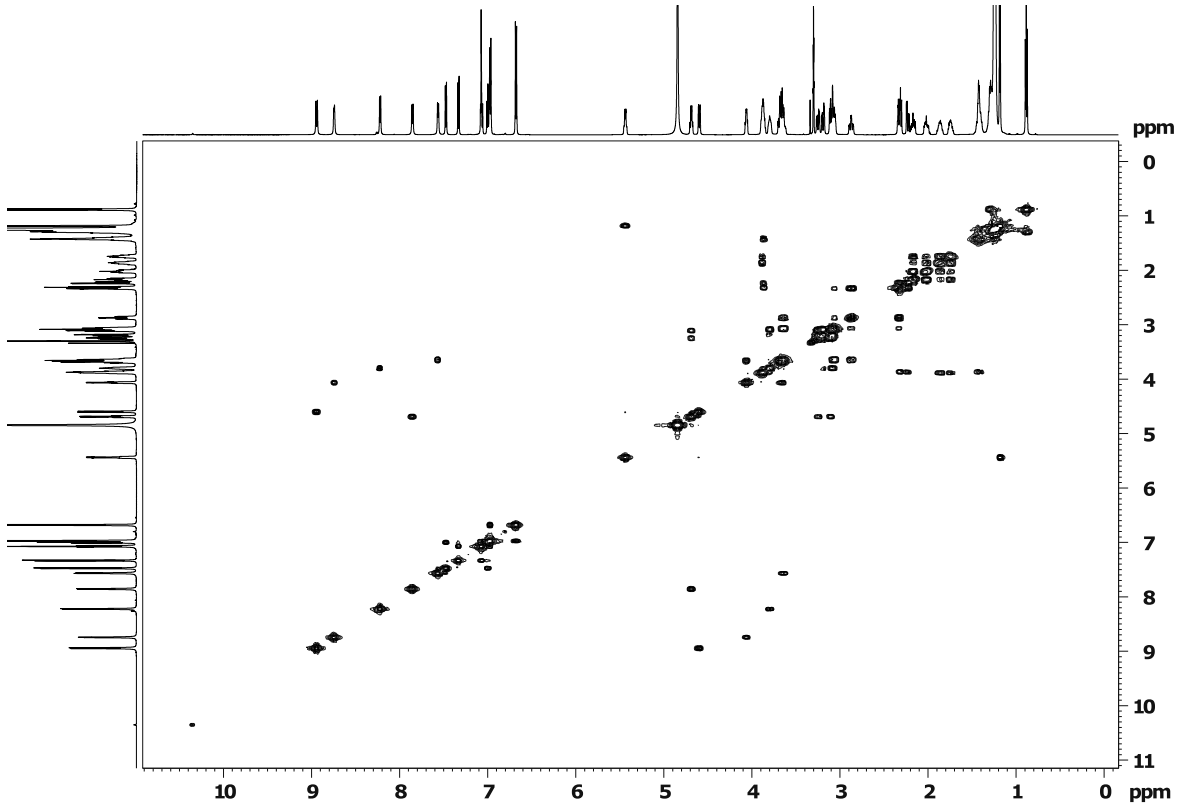

Figure S 15. ^{1}H-^{1}H COSY spectrum of symbiosin (3) in CD_3OD.

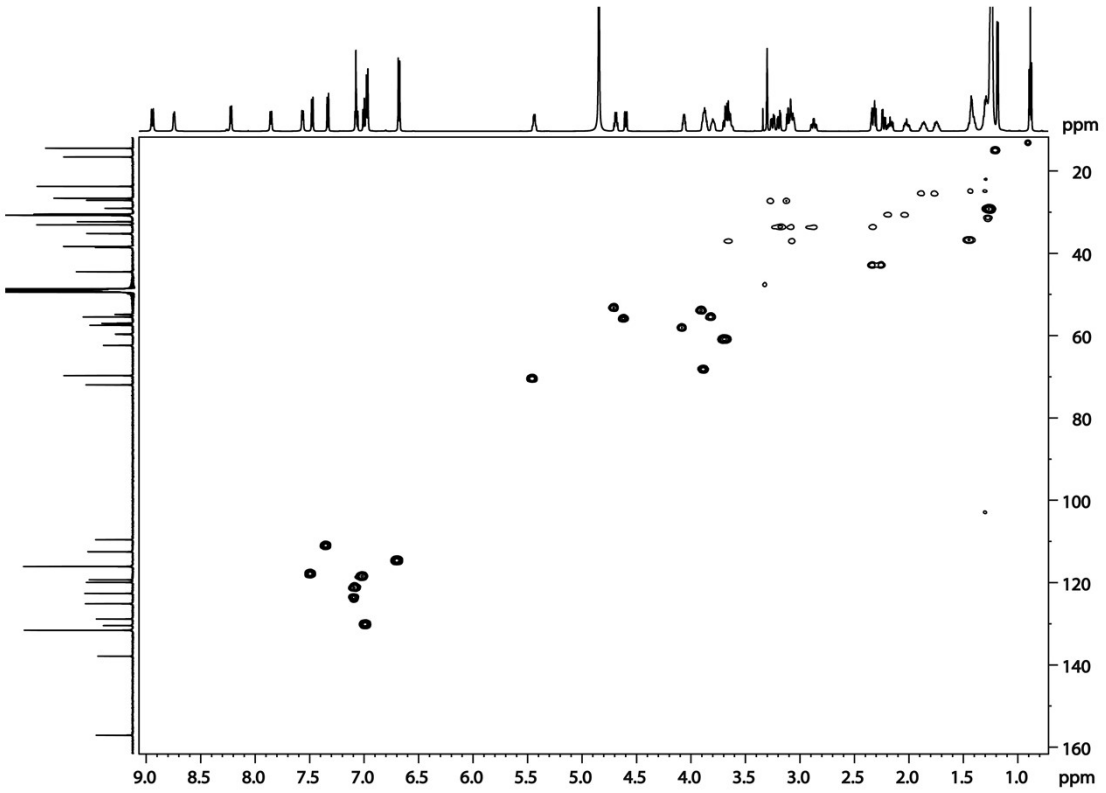

Figure S 16. ^{1}H-^{13}C HSQC spectrum of symbiosin in (3) CD_3OD.

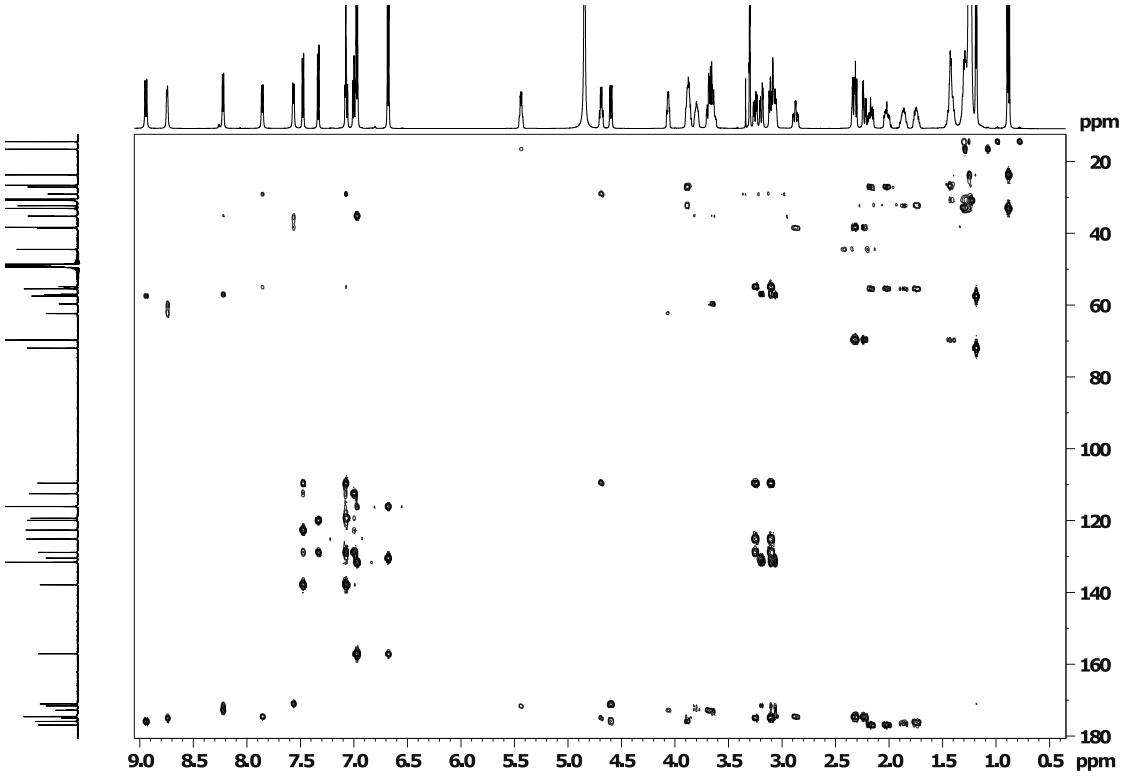

Figure S 17. ^{1}H-^{13}C HMBC spectrum of symbiosin (3) in CD_3OD.

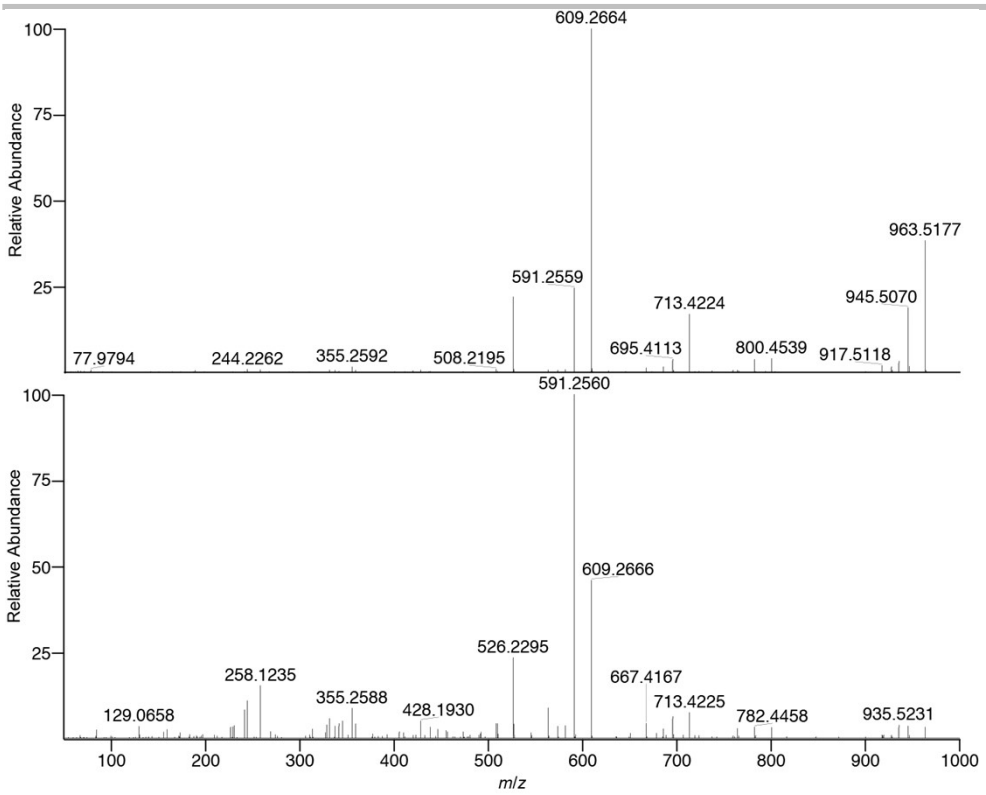

Figure S 18. LC-HRESI/MS/MS fragmentation of symbiosin (3) using different energies (top: hcd 15, bottom: hcd 22).

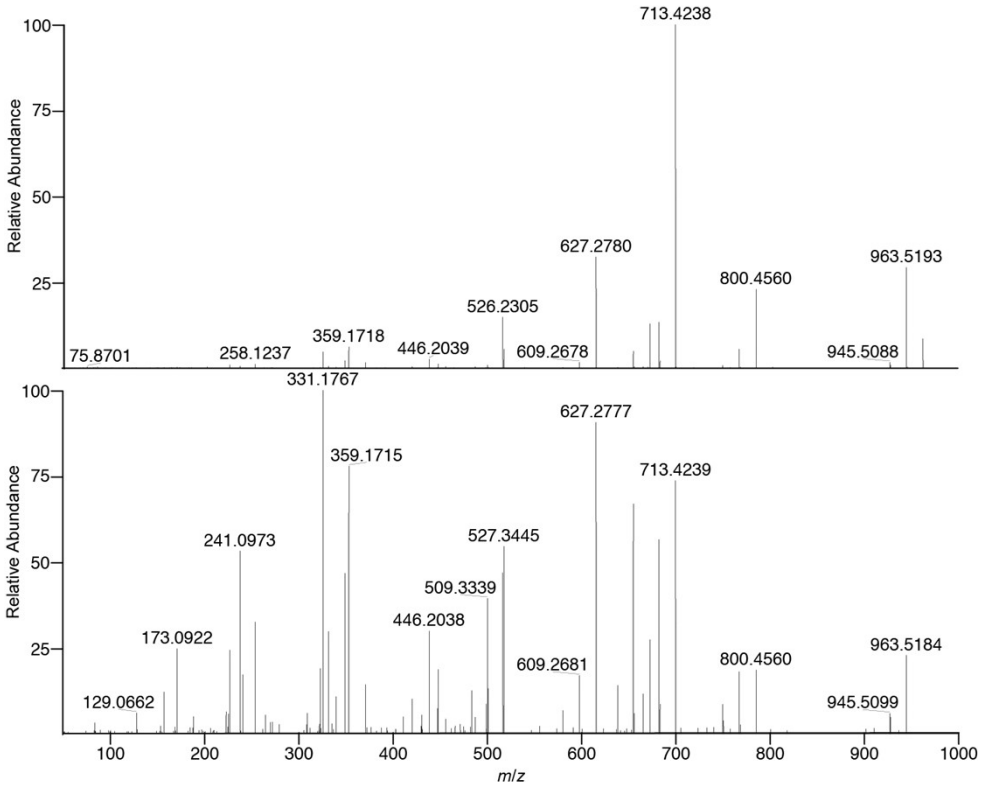

Figure S 19. LC-HRESI/MS/MS fragmentation of symbiosin (3) after hydrolysis using different energies (top: hcd 15, bottom: hcd 22).

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Author Contributions

H. B. and C. H. conceived the study. H. B. did the project administration and designed the experiments. H. B. and S.J. P. conducted the experiments. H. B., S.J. P. and K. S. analysed and interpreted the data. H. B. and C. H. wrote the manuscript.