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Supporting Information

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Supporting Information

for

Post-translational Modification in Microviridin Biosynthesis

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General Experimental Procedures

Kanamycin sulfate (50 μg/mL), chloramphenicol (25 μg/mL), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (XGal, 30μg/ml) and Isopropyl β-D-1-thiogalactopyranoside (IPTG, 150 μM) were purchased from Fisher Scientific and used at the final concentrations listed. LB media and pET28b DNA were purchased from EMD chemicals (Gibbstown, NJ). *Escherichia coli* strains DH5α and BL21 (DE3) were purchased from Invitrogen (Carlsbad, CA). Plasmids were isolated from *E. coli* using the High pure plasmid isolation kit from Roche Applied Science (Indianapolis, IN). The QIAquick PCR purification kit, the ProofStart PCR kit and the pDrive cloning vector were purchased from Qiagen (Valencia, CA). *NdeI*, *XhoI* and pACYC DNA were purchased from New England Biolabs (Ipswich, MA). *SalI* and *MluI* were purchased from Promega (Madison, WI). *NotI*, Klenow fragment and T4 DNA ligase were purchased from Fermentas (Glen Burnie, MD). The iProof PCR kit was purchased from BioRad (Hercules, CA). Sequencing was done at the Greenwood Molecular Biology Facility (Honolulu, HI) using ABI Prism BigDye Terminator reactions.

Culture of *Planktothrix agardhii* CYA 126/8 wildtype. For mass culture the cyanobacterium was grown in 20L carboys containing 20L of Z+G medium adjusted to pH 7.0 before autoclaving. Irradiation used a 24h light cycle from cool white fluorescent tubes, 15 $\mu E/m^2 \times s^{-1}$. Cultures were harvested between 20 and 30 days post inoculation.

Culture of *Planktothrix agardhii* CYA 126/8 (DMV)-mutant. The culture was grown in 250 mL Erlenmeyer flasks containing 100 mL Z+G medium augmented with 1 μg/mL chloramphenicol. Wildtype material was produced on the same scale in a parallel fermentation under exactly the same conditions.

Analysis of (DMV)-mutant for microviridin K production. Cell mass was collected on a glass microfiber filter (Whatman International, Maidstone, England) and subsequently lyophilized overnight. The dried cell mass was extracted three times with 50% aq MeOH (6 mL) by sonication for 15 minutes in Branson 1510 Ultrasonic cleaner (Branson Ultrasonics, Danbury, CT) followed by shaking at 220 rpm at room temperature for 15 min. The cell mass was then pelleted by centrifugation and the cleared extract had the methanol removed under reduced pressure. The remaining water was removed by lyophilization. The crude extract was resuspended in 25% aq MeOH (1 mL) and applied to 2 g of YMC Gel ODS-A (120A, 70 mesh. YMC Inc., Wilmington,

NC) preconditioned with MeOH and then equilibrated in 25% aq MeOH. The column was washed with 15 mL 25% aq MeOH and then eluted with 15 mL 80% aq MeOH, followed by 22 mL 100% MeOH into pre-tared vials. The fractions were evaporated to dryness under reduced pressure. The 80% fraction was dissolved in 50% aq MeOH at a concentration of 1 mg/mL and 5 µL was injected onto the LC-TOFMSD containing an Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm, Agilent Technologies), pre-equilibrated with 90% H_2O: 10% ACN + 0.1% (*v/v*) formic acid. A linear gradient was employed ending in 20% H_2O: 80% ACN + 0.1% (*v/v*) formic acid over 40 min at a flow rate of 0.7 ml/min. The TIC was recorded in positive ESI mode with the following conditions: 350 °C drying gas temperature, 10 L/min drying gas, 40 psig nebulizer gas, fragmenter 225V, skimmer 60V, OctRFV 250V, capillary 3000V. The spectra were analyzed with the Analyst QS software v1.1 (Applied Biosystems).

Isolation of microviridin K (1). Freeze-dried cells were extracted in 50% aq MeOH (15 mL) by sonication for 15 min followed by shaking at RT for 15 min. The extraction was repeated three times and the extracts were combined. The methanol was removed under reduced pressure and the extract dried by lyophilization (extract mass: 270 mg). The dried extract was redissolved in 30% aq MeOH (10 mL) and loaded onto 6 g of YMC Gel ODS-A (120A, 70 mesh) that had been washed with MeOH and preconditioned with 30% aq MeOH. The column was then washed with 30% aq MeOH (36 mL), followed by 50% aq MeOH (40 mL), 70% aq MeOH (40 mL) and 100% MeOH (125 mL). The fractions were concentrated to dryness and redissolved in 50% aq MeOH at a concentration of 10 mg/ml. The 50% fraction was purified by HPLC using a LUNA-C18 column (10 µ, 10 x 250mm, Phenomenex, Torrence, CA) at a flow rate of 3 mL/min with a gradient of 30% ACN in water (0.05% TFA) for 10 min increasing to 50% ACN within 15 min and held there for 5 min. Elution was monitored at 240 nm. Microviridin K eluted at 12.3 min, while the sulfoxide of microviridin K eluted at 8.5 min. Microviridin K (5.3 mg, 0.2% w/w dry mass) and the sulfoxide (1.3 mg, 0.05% w/w dry mass) were obtained as a white solids.

Microviridin K (1). $[M+H]^+$: obs. 1770.7060, calc. 1770.7111, Δ 2.8 ppm error $[\alpha]_D^{25}$ +3.0, *c* = 0. 1, MeOH.

Microviridin K sulfoxide. $[M+H]^+$: obs. 1786.6946, calc. 1786.7060, Δ 6.4 ppm error $[\alpha]_D^{25}$ +20.6, *c* = 0.044, MeOH.

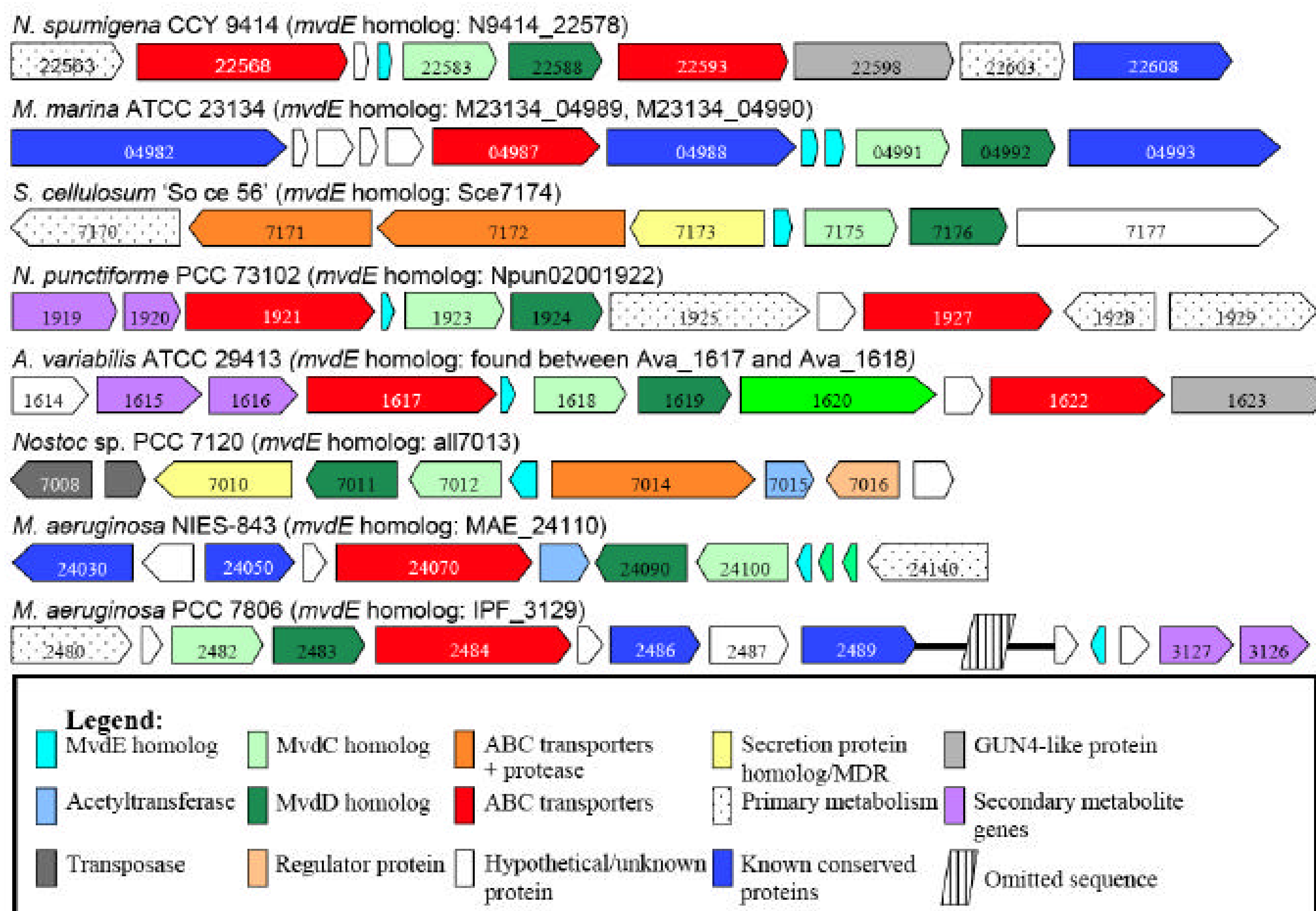


Figure S-1. Graphical representation of putative *mvd* biosynthetic clusters in publicly accessible databases obtained from the NCBI website by BLAST homology to MvdD. Block arrows represent ORFs. Genes not drawn to scale. Gene prefixes are as follows: *N. spumigena*, N9414_XXXX; *M. marina*, M23134_XXXXX; *S. cellulosum*, SceXXXX; *N. punctiforme*, Npun0200XXXX; *A. variabilis*, AvaXXXX; *Nostoc* sp. PCC 7120, allXXXX (transcribed to the left) or alrXXXX (transcribed to the right); *M. aeruginosa* NIES-843, MAE_XXXXX; *M. aeruginosa* PCC 7806, IPF_XXXX; where XXXX is the number seen in the above diagram.

Table S1. Masses of known microviridins.

Microviridin	**Monoisotopic mass**
Microviridin A	1728.7096
Microviridin B	1722.7566
Microviridin C	1738.7879
Microviridin D	1801.7294
Microviridin E	1664.7035
Microviridin F	1682.7141
Microviridin G	1805.7937
Microviridin H	1837.8199
Microviridin I	1764.7672
Microviridin J	1683.7682

Table S2. NMR data for Microviridin in CD_3OH.

Unit	C/H No.	δ_H (J in Hz)	δ_C	key HMBC correlations	key ROESY correlations
Tyr(1)	1		174.4	Tyr(1) H-2, H-3a, 3b	
	2	4.55, *br*dd (8.4, 5.1)	55.32		
	3a	3.11, dd (14.1, 4.4)	37.5		
	3b	2.97, dd (14.1, 8.4)			
	4		128.97	Tyr(1) 3a, 3b	
	5/9	7.07, d (8.6)	131.5	Tyr(1) 3a, 3b	
	6/8	6.70, d (8.6)	116.3		
	7		157.1		
	NH	7.74, *br*s			
Glu(1)	1		173.59	Tyr(1) H-2, NH	
	2	4.16, ddd (11.6, 7.9, 3.2)	54.3		Tyr(1) NH
	3a	2.08, m	28.5		
	3b	1.93, m			
	4a	2.41, m	33.3		Lys 6-NH
	4b	2.04, m			Lys 6-NH
	5		174.73	Lys 6-NH	
	NH	7.36, *br*d (7.9)			
Glu(2)	1		172.99	Glu(1) NH	
	2	4.14, m	55.34		Glu(1) NH
	3a	2.02, m	26.1		
	3b	1.58, m			
	4a	2.09, m	30.9		
	4b	1.01, m			
	5		173.2	Ser H-3a	
	NH	6.75, *br*d (8.1)			
Trp	1		174.5	Trp H-2, H-3b	
	2	4.66, *br*dd (5.2, 5.2)	56.2		Glu(2) NH
	3a	3.46, dd (14.9, 5.2)	26.6		
	3b	3.29, dd (14.9, 5.2)			
	1’	7.28, s	125.0	Trp 3a, 3b	Trp 8’-NH

Unit	C/H No.	δ_H (J in Hz)	δ_C	key HMBC correlations	key ROESY correlations
	2’		110.2	Trp 3a, 3b, 8’-NH	
	3’		128.90	Trp 3a, 3b, 8’-NH	
	4’	7.55, *br* d (7.6)	119.1		
	5’	7.10, m	120.6		
	6’	7.14, m	123.1		
	7’	7.32, d (7.9)	113.1		
	8’		138.2	Trp 8’-NH	
	2-NH	7.08, *br* s			
	8’-NH	10.55, s			
Asp	1		174.6	Asp H-2, Trp 2-NH	
	2	4.27, *br* d (11.6)	54.5		Trp 2-NH
	3a	2.95, dd (18.4, 11.6)	34.9		
	3b	2.77, d (18.4)			
	4		171.6	Asp H-2, H-3a, 3b, Thr H-3	
	NH	9.19, *br* s			
Ser	1		172.95	Ser H-2	
	2	4.48, *br* dd (2.6, 1.5)	55.0		Asp NH
	3a	4.90, dd (11.9, 2.6)	63.0		
	3b	3.57, *br* d (11.9)			
	NH	6.76, *br* s			
Pro	1		172.7		
	2	3.50, m	62.3		
	3a	1.62, m	31.8		
	3b	1.50, m			
	4a	1.61, m	23.2		
	4b	1.49, m			
	5a	3.38, m	47.7		
	5b	3.27, m			
Tyr(2)	1		174.0	Tyr(2) H-3a, 3b	
	2	4.40, ddd (10.8, 6.8, 5.7)	54.1		Pro H-2
	3a	2.89, m	39.0		
	3b	2.85, m			
	4		127.8	Tyr(2) 3a, 3b	
	5/9	7.01, d (8.5)	131.5	Tyr(2) 3a, 3b	
	6/8	6.72, d (8.5)	116.5		
	7		157.8		
	NH	8.32, d (6.8)			
Lys	1		172.4	Lys H-2, Tyr(2) NH	
	2	4.11, m	55.41		Tyr(2) NH
	3a	1.75, m	32.3		
	3b	1.59, m			
	4a	1.38, m	23.6		
	5a	1.59, m	29.6		
	5b	1.38, m			
	6a	3.17, m	39.6		
	6b	3.12, m			
	2-NH	7.34, d (7.0)			
	6-NH	7.29, m			
Met	1		172.8	Met H-2, Lys 2-NH	
	2	4.61, ddd (9.5, 9.0, 4.4)	53.1		Lys 2-NH
	3a	2.26, m	31.8		
	3b	1.79, m			
	4a	2.52, ddd (13.3, 7.8, 5.2)	31.1	Met S-Me	

Unit	C/H No.	δ_H (J in Hz)	δ_C	key HMBC correlations	key ROESY correlations
	4b	2.41, m			
	S-Me	2.06, s			
	NH	8.84, d (9.0)	15.4		
Thr	1		172.6	Thr H-2, Met NH	
	2	4.57, dd (8.7, 1.2)	57.5		Met NH
	3	5.47, qd (6.6, 1.2)	72.8		
	4	1.34, d (6.6)	18.4		
	NH	8.04, d (8.7)			
Asn	1		173.64	Asn H-2, H-3a, 3b, Thr H-2, NH	
	2	4.91, ddd (7.6, 6.9, 6.2)	51.4		Thr NH
	3a	2.84, dd (15.6, 6.2)	37.6		
	3b	2.74, dd (15.6, 6.9)			
	4		175.1	Asn H-2, H-3a, 3b	
	2-NH	8.23, d (7.6)			
	4-NH_2	7.64, s			
		6.94, s			
Gly	1		171.8	Gly H-2a, 2b, Asn H-2, NH	
	2a	3.97, dd (16.9, 6.1)	43.6		Asn NH
	2b	3.83, dd (16.9, 5.4)			Asn NH
	NH	8.40, dd (6.1, 5.4)			
Tyr(3)	1		174.7	Tyr(3) H-2, H-3a, 3b, Gly H-2a, 2b, NH	
	2	4.44, ddd (9.0, 6.6, 5.9)	57.3		Gly H-2
	3a	3.08, dd (14.1, 5.9)	37.5		
	3b	2.85, dd (14.1, 9.0)			
	4		129.03	Tyr(3) 3a, 3b	
	5/9	7.05, d (8.6)	131.2	Tyr(3) 3a, 3b	
	6/8	6.71, d (8.6)	116.3		
	7		157.2		
Ac	NH	8.21, d (6.6)			
	1		174.0	Ac H-2, Tyr(3) H-2, NH	
	2	1.94, s	22.5		Tyr(3) NH

```
             1       10        20        30        40        50        60        70        80        90       100
             |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
        MvdE  MSKNVKVSAPKAVPFFARFLAEQA----VEANNSNSA--PYGNTMKYPSDWEEY
        MvdF  MSKNIKVSTGSAVPFFARFLSEQD----TETGDSTSTDIPTIWTFKWPSDWEDS
 N9414_22578   MTTATLANIEAVPFFARFLAAEEPPETPAPQPEEQPLPPPIFTLKWPSDWEDC
    IPF_3129 MTMNYPNSEQSKAIPFFARFLSADQD-EAPTPDSPPDSEPAPVWTWKWPSDWEDS
    Mae24110   MAYPNDQQGKALPFFARFLSVSKE---ESSIKSPSPEPTYGGTFKYPSDWEDY
MdnA_NIES298   MAYPNDQQGKALPFFARFLSVSKE---ESSIKSPSPEPTFGTTLKYPSDWEEY
    MdnA_MRC   MAYPNDQQGKALPFFARFLSVSKE---ESSIKSPSPDHEI-STRKYPSDWEEW
     A117013 MPENRQEDLNAQAVPFFARFLEGQNCEDLTDEESEAVSGGKRGQTRKYPSDCEDGNGVTGKLRDEDIAVTLKYPSDNEDNGGGEIVTLKFPSDDDDQPVG
   Npun1922a MPTNTVKTVDVVAVPFFARFLEEQA--------TEGTE---VPWTYKFPSDLEDR
   Ava_1618a MSTNTVKTVNVAVVPFFARFLEEQA--------TEGTDPPSFPWTFKYPSDLEDQ
   Consensus ............avPFFARFL..q....................T.KyPSDwE#..............................................
```

Figure S2. Alignments of MvdE and MvdF.

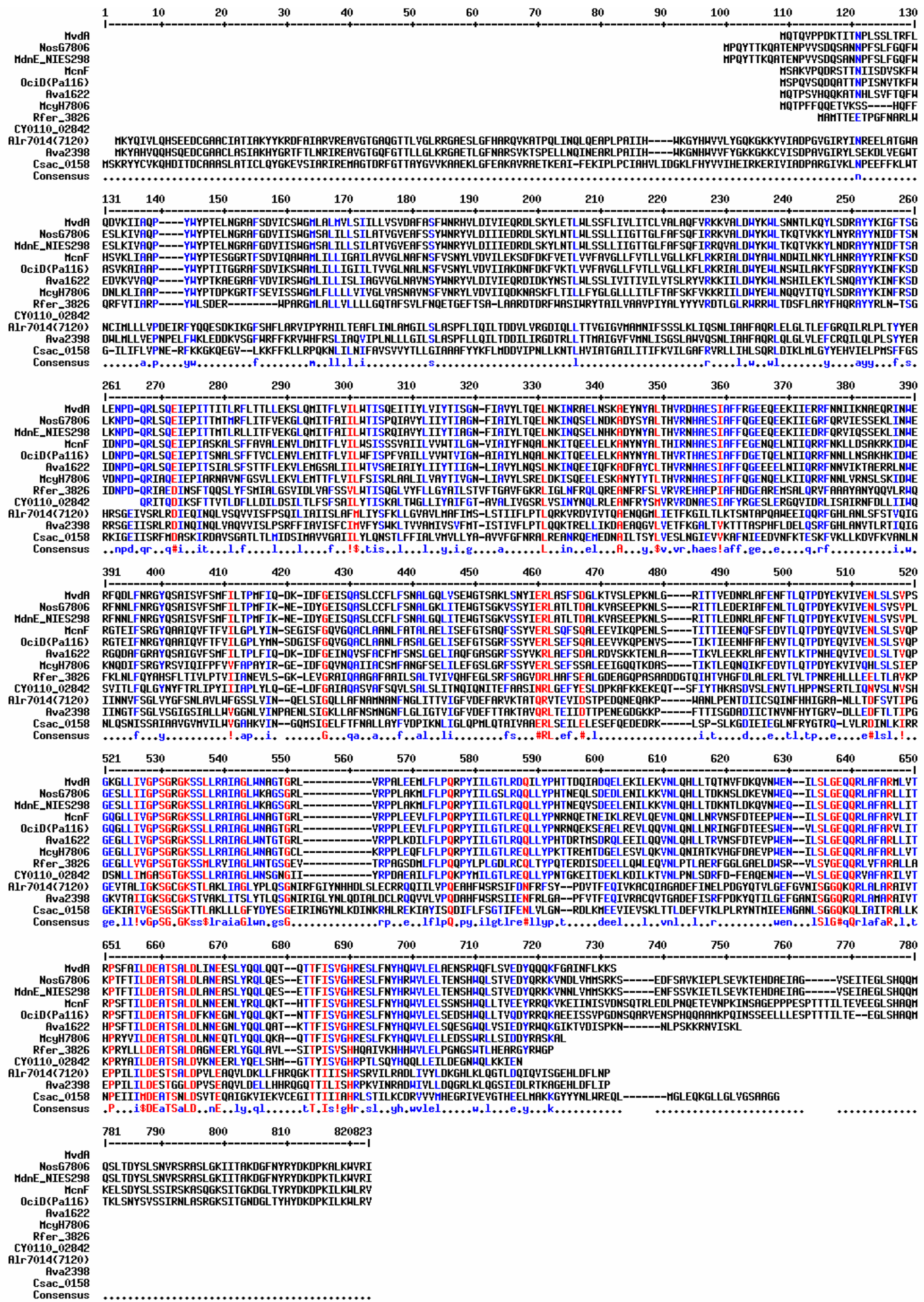


Figure S3. Alignments of MvdA.

Figure S4. Alignments of MvdB.

Figure S5. Alignments of MvdC.

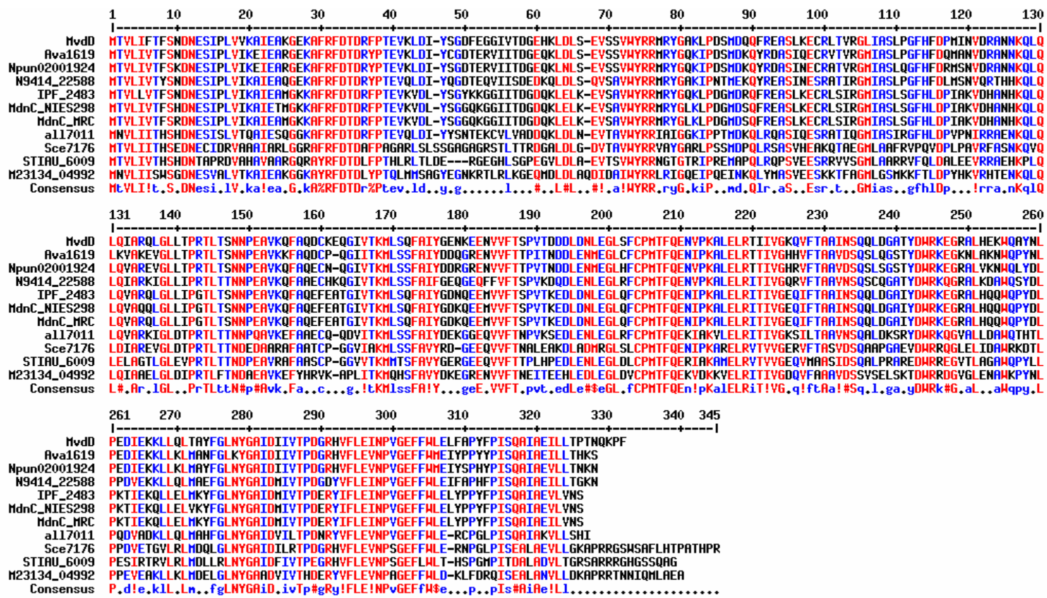


Figure S6. Alignments of MvdD.

Molecular Biology procedures for homologous recombination and protein expression

1. Homologous recombination construct assembly.

A PCR reaction was performed using isolated genomic DNA from *P. agardhii* utilizing the primers MVassembleLeftFwd2 (ATTAAATGTG*ACGCGT*C-CCTAGCCAATGGTCAAGTATTAGTTTG) and MVassembleLeftRev2 (ACAATG-CAT*GTCGAC*CAGTTAAGTTCCTTTTGCAATATTGA-CCTG) with the iProof PCR Kit according to the manufacturer's supplied protocol using a modified cycling program: 98 °C-30 s; 98 °C-10 s, 57 °C-30 s, 72 °C-1 min (5 cycles); 98 °C-10 s, 66 °C-30 s, 72 °C-1 min (30 cycles); 72 °C-10 min. The italics indicate *MluI* and *SalI* restriction sites, while underlined letters indicate base pairs added for restriction efficiency. The PCR product was isolated with the QIAquick PCR purification kit according to the supplied protocol. The DNA was then restricted with *MluI* and *SalI* according to the manufacturer's supplied protocol, followed by reisolation of the DNA with the QIAquick PCR purification kit. The PCR product was then ligated into a similarly digested pDrive vector with T4 DNA ligase according to the supplied protocol. The ligation mixture was then trans-

formed into chemically competent *E. coli* DH5α using standard protocols. The transformation was plated out on LB agar containing kanamycin and blue/white screening was performed with XGal/IPTG. Colonies containing inserts were grown overnight in LB medium containing kanamycin and the plasmid pDMV-Left2-1 was isolated. The insert was confirmed by sequencing using vector specific primers.

A PCR was performed using isolated genomic DNA from *P. agardhii* by utilizing the primers MVassembleRightFwd2 (ATGTGTATA*CTCGAG*GTCAGAA-CAAGACACAGAAACTGGTGA) and MVassembleRightRev2 (ATAAAATT*GC-GGCCGC*GTCGATTAGATCAAGGTGACTCCAC) with the iProof PCR Kit according to the manufacturer's supplied protocol using a modified cycling program: 98 °C-30 s; 98 °C-10 s, 57 °C-30 s, 72 °C-1 min (5 cycles); 98 °C-10 s, 66 °C-30 s, 72 °C-1 min (30 cycles); 72 °C-10 min. The italics indicate *XhoI* and *NotI* restriction sites, while underlined letters indicate base pairs added for restriction efficiency. The PCR product was isolated with the QIAquick PCR purification kit. The DNA was then restricted with *XhoI* and *NotI* (Fermentas) according to the manufacturer's supplied protocol, followed by reisolation of the DNA with the QIAquick PCR purification kit. The PCR product was then ligated into a similarly digested pDMVLeft2-1 with T4 DNA ligase according to the supplied protocol. The ligation mixture was then transformed into chemically competent *E. coli* DH5α using standard protocol. The transformation was plated out on LB agar containing kanamycin. Colonies containing the appropriate insert were found by colony PCR using vector specific primers. Colonies giving a band of appropriate size were grown in overnight in LB medium containing kanamycin and the plasmid pDMVLRassem-1 was isolated. The insert was confirmed by sequencing by using vector specific primers.

Plasmid pDMVLRassem-1 was restricted with appropriate units of *XhoI* followed by DNA isolation with the QIAquick PCR purification kit. The linearized vector was blunted with Klenow fragment according to the manufacturer's supplied protocol. The blunted DNA was ligated to the chloramphenicol resistance cassette from pACYC184, which was removed by restriction with *BsaAI* followed by aga-

rose gel purification and isolation, using T4 DNA ligase. The ligation mixture was then transformed into chemically competent *E. coli* DH5α using standard protocols.[1] The transformation was plated out on LB agar containing kanamycin and chloramphenicol. Colonies were grown overnight in LB medium containing kanamycin and chloramphenicol and the plasmid pDMVLRKO-1 was isolated. The orientation of the Cm^R cassette was established by sequencing to have the 5'-end of the Cm^R cassette toward the MVRight fragment. PDMVLRKO-1 was linearized with *PstI* and used in transformation of *P. agardhii* as previously described.[2]

Primers used for detection of homologous recombination:

MVKOOckFwd (TGGGGAAATTTAGTGATTCAACAAGCAGAAAATA)-289bp from construct

MVKOOckRev (CCAATTAACGGCTCCTATTTTG TTACTTTG)-362bp from construct

2. Creation of MvdC and MvdD expression constructs.

The ProofStart PCR kit was used to amplify the genes *mvdC* and *mvdD* from genomic DNA isolated from *P. agardhii* NIVA-CYA 126/8 using the primers MicvicGluTransIfwd (GCTAGTCGCGC*ATATG*TCTTTGTCTCGTGATGTTGTTTTATTAATTAC) and MicvicGluTransIrev1 (ATATTAACTAGC*CTCGAG*GGGAATTAGGGTATCGGCGATC) for *mvdC* and MicvicGluTransIIfwd (CCCGATGTACAGTC*ATATG*ACGGTTTTAATTTTCACTTTCAGCAAC) and MicvicGluTransII-rev1 (TTACTATCGACT*CTCGAG*AAATGGTTTCTGGTTTGTA GGAGTGAG) for *mvdD* per the manufacturer's supplied protocol. The italicized bases indicate the *NdeI* and *XhoI* restriction sites while the underlined bases are random bases added to enhance restriction efficiency of the PCR product. The PCR program used was as follows: 95 °C-3 min; 95 °C-30 s, 55 °C-30 s, 72 °C-1 min (5 cycles); 95 °C-3 min; 95 °C-30 s, 66 °C-30 s, 72 °C-1 min (35 cycles). The PCR product was isolated with the QIAquick PCR purification kit followed by restriction with *NdeI* and *XhoI*. The DNA was reisolated using the QIAquick PCR purification kit and ligated into a similarly digested pET28b vector with T4 DNA ligase. The liga-

tion mixture was transformed into *E. coli* DH5α cells and the transformation mixture was grown on LB agar plates supplemented with kanamycin. Clones containing plasmid were grown overnight in LB media plus kanamycin and the plasmids isolated yielding pEGluTrI-1 and pEGluTrII-2 for *mvdC* and *mvdD* respectively. The insert sequence was confirmed by sequencing with vector specific primers.

Procedures for Protein Expression and Isolation

Plasmid pEGluTrI-1 was transformed into *E. coli* BL21 (DE3) cells and plated on LB agar supplemented with kanamycin. A single colony was inoculated into 50 ml LB media containing kanamycin and grown overnight at 37 °C with shaking. 5 L of LB media containing kanamycin was inoculated with 50 mL overnight culture and grown at 30 °C for 3 h followed by 22 °C for 0.5 h. The culture was then induced with IPTG (final concentration 0.1 mM) and grown an additional 4 h at 22 °C. The cells were collected by centrifugation, the supernatant was decanted off and the cell pellet was stored at –20 °C until use.

Plasmid pEGluTrII-2 was transformed into *E. coli* BL21 (DE3) cells and plated on LB agar supplemented with kanamycin. A single colony was inoculated into 50 ml LB media containing kanamycin and grown overnight at 37 °C with shaking. 5 L of LB media containing kanamycin was inoculated with 50 mL overnight culture and grown at 30 °C for 3 h followed by 22 °C for 0.5 h and 15 °C for 1 h. The culture was then induced with IPTG (final concentration 0.1 mM) and grown an additional 16 h at 15 °C. The cells were collected by centrifugation, the supernantant was decanted off and the cell pellet was stored at –20 °C until use.

Both MvdC and MvdD were prepared as follows: The frozen cells were defrosted on ice and resuspended in 30 ml Lysis buffer [50 mM Tris, 300 mM NaCl, 5 mM imidazole, pH 8.0]. The cells were lysed by 30 sec intervals of sonication on ice. The cell debris was pelleted by centrifugation (10,000 g, 30 min, 4 °C) followed by a second round of centrifugation (15,000 g, 15 min, 4 °C). The cleared lysate was incubated with Ni-NTA agarose (0.125 ml), which had previously been washed with 1.25 ml 20 mM Tris, pH 7.5, for 1 h at 4 °C with gentle rocking. The

Ni-agarose was loaded onto a BioRad disposable Poly-prep column and drained. The resin was washed with 1.25 ml Lysis buffer with 5 mM β-mercaptoethanol (BME) followed by 3 ml Buffer 2 [50 mM Tris, 300 mM NaCl, 25 mM imidazole, 5 mM BME, pH 8.0] and 3 ml Buffer 3 [50 mM Tris, 500 mM NaCl, 68 mM imidazole, 5 mM BME, pH 8.0]. The protein was eluted with 0.5 ml Buffer 4 [50 mM Tris, 300 mM NaCl, 250 mM imidazole, 5 mM BME, pH 8.0]. Fractions were checked by SDS-PAGE gel and those containing protein were desalted into Buffer 5 [50 mM Tris, 50 mM NaCl, 5 mM BME, 10% glycerol (w/v), pH 8.0] using Econo-Pac 10DG desalting columns (BioRad) according to the manufacturer's supplied protocol. Protein concentrations were determined using the Bradford protein assay using BSA as a standard.

Refrences:

[1] Sambrook, J.; Fritsch, E. F.; Maniatis, J. *Molecular Cloning: A Laboratory Handbook*

[2] Christiansen, G.; Fastner, J.; Erhard, M.; Börner, T.; Dittmann, E. *J. Bacteriol*. **2003**. *185*, 564-572.

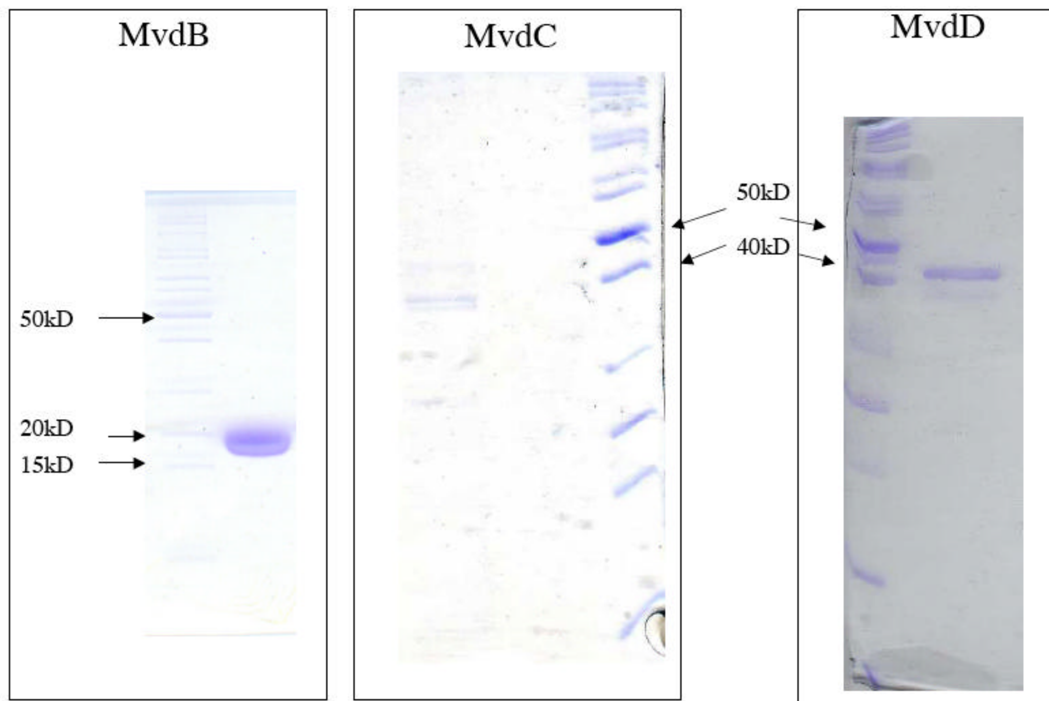
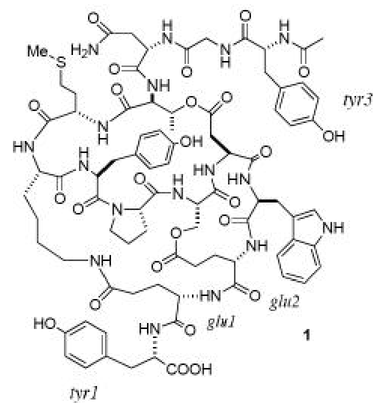
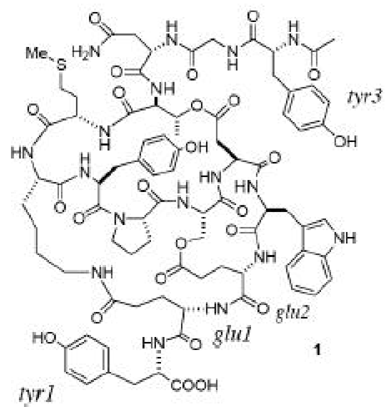


Fig. S-7. Protein gels for MvdB, MvdC and MvdD. Fermentas Pager Ruler Unstained Protein Ladder as ref..

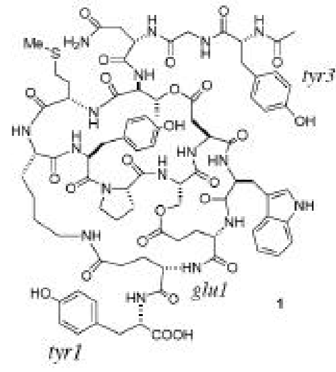


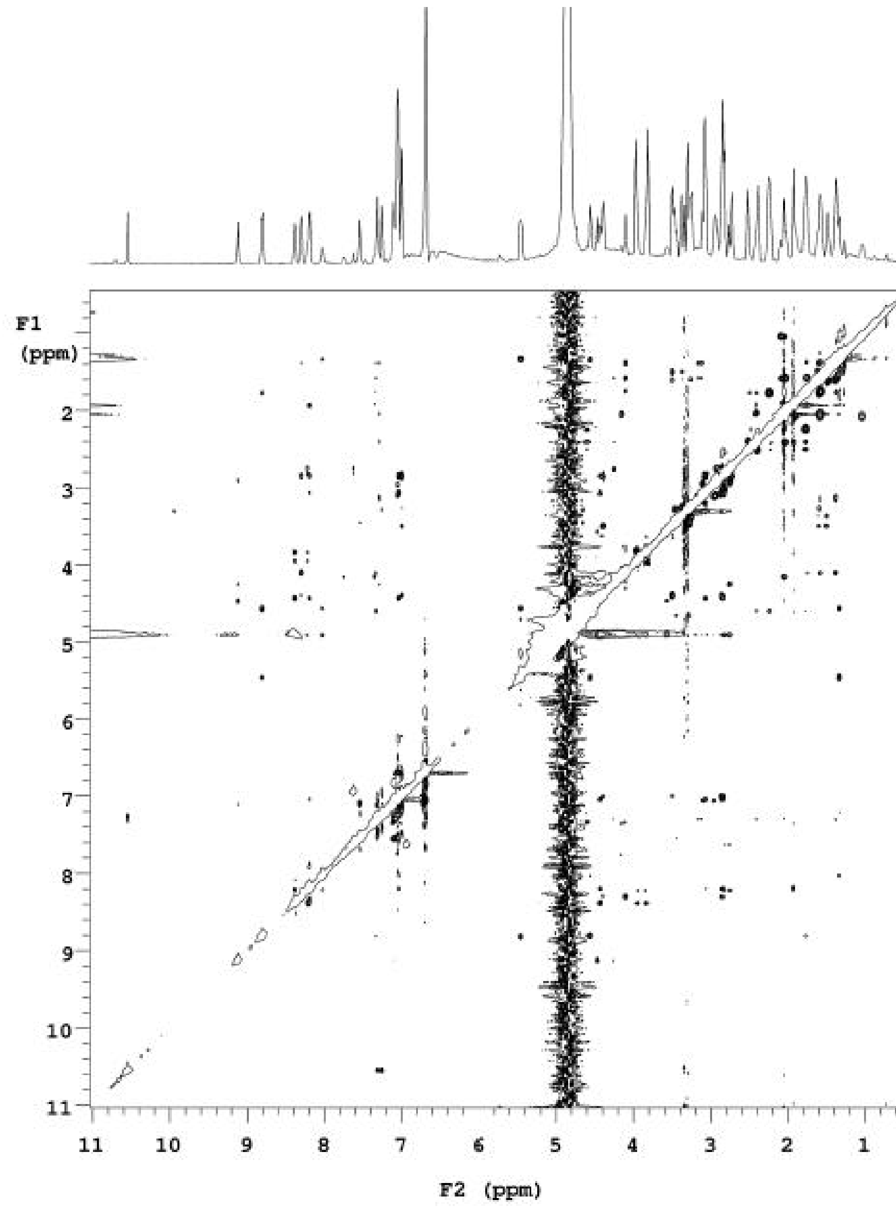
10 9 8 7 6 5 4 3 2 1 ppm

1H-NMR spectrum of microviridin K in MeOH-d3.



220 200 180 160 140 120 100 80 60 40 20 ppm


Me
S
H2N
NH
O
OH
tyr3
HN
NH
COOH
glu1
tyr1
1


F1
(ppm)
2
3
4
5
6
7
8
9
10
11
11 10 9 8 7 6 5 4 3 2 1
F2 (ppm)