

**CHEMBIOCHEM**

## Supporting Information

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# CHEMBIOCHEM

## Supporting Information

for

### Post-translational Modification in Microviridin Biosynthesis

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## General Experimental Procedures

Kanamycin sulfate (50  $\mu\text{g}/\text{mL}$ ), chloramphenicol (25  $\mu\text{g}/\text{mL}$ ), 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (XGal, 30 $\mu\text{g}/\text{ml}$ ) and Isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG, 150  $\mu\text{M}$ ) 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 DH5 $\alpha$  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). *Nde*I, *Xho*I and pACYC DNA were purchased from New England Biolabs (Ipswich, MA). *Sal*I and *Mlu*I were purchased from Promega (Madison, WI). *Not*I, 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  $\mu\text{E}/\text{m}^2 \times \text{s}^{-1}$ . 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  $\mu\text{g}/\text{mL}$  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  $\mu$ L was injected onto the LC-TOFMSD containing an Eclipse XDB-C18 column (4.6 x 150 mm, 5  $\mu$ m, Agilent Technologies), pre-equilibrated with 90% H<sub>2</sub>O: 10% ACN + 0.1% (v/v) formic acid. A linear gradient was employed ending in 20% H<sub>2</sub>O: 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  $\mu$ , 10 x 250mm, Phenomenex, Torrance, 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]<sup>+</sup>: obs. 1770.7060, calc. 1770.7111,  $\Delta$  2.8 ppm error [ $\alpha$ ]<sub>D</sub><sup>25</sup> +3.0, c = 0.1, MeOH.

**Microviridin K sulfoxide.** [M+H]<sup>+</sup>: obs. 1786.6946, calc. 1786.7060,  $\Delta$  6.4 ppm error [ $\alpha$ ]<sub>D</sub><sup>25</sup> +20.6, c = 0.044, MeOH.

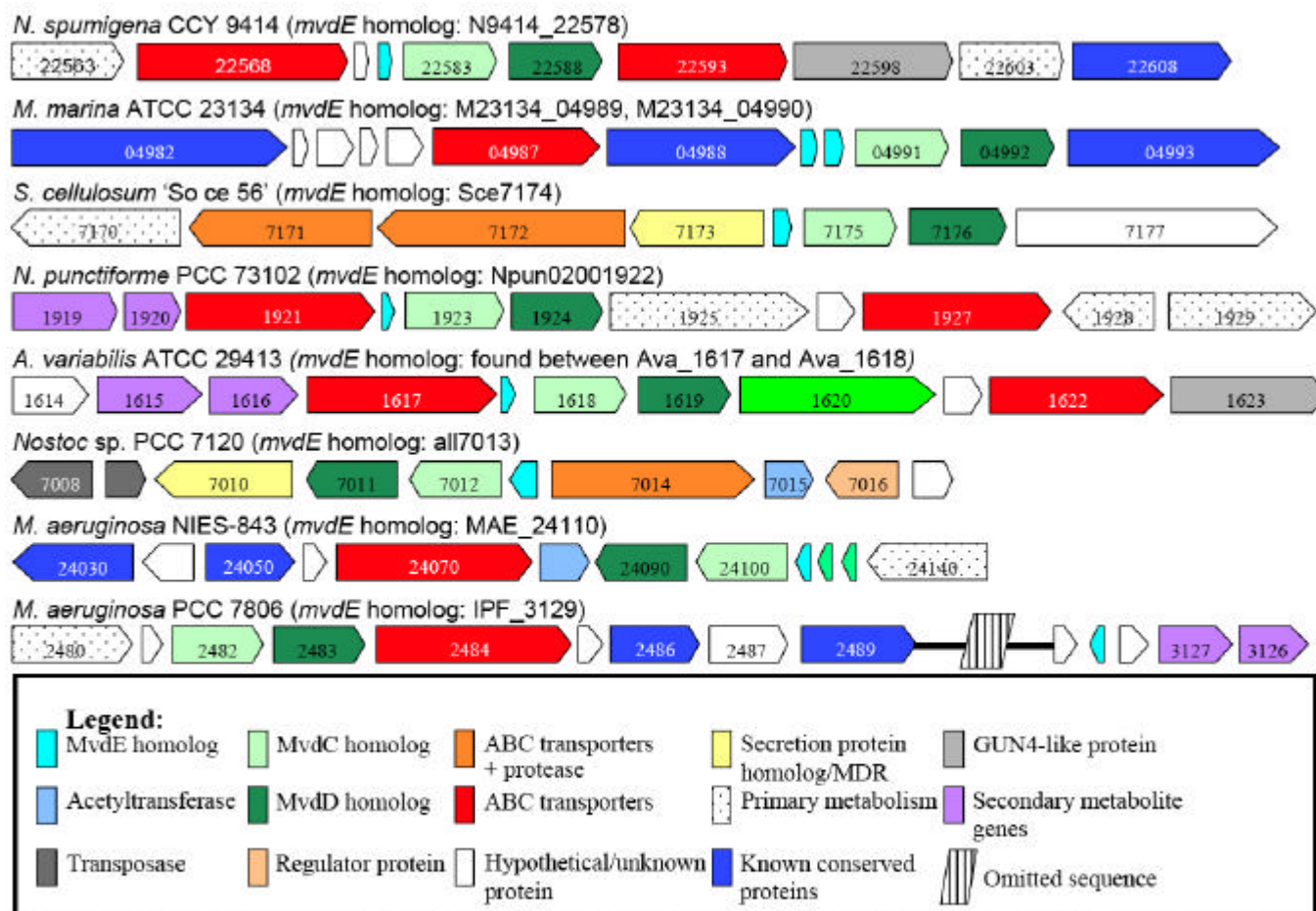


Figure S-1. Graphical representation of putative *mvd* biosynthetic clusters in publicly accessible databases obtained from the NCBI website by BLAST homology to MvdD. Block arrows represent ORFs. Genes not drawn to scale. Gene prefixes are as follows: *N. spumigena*, N9414\_XXXX; *M. marina*, M23134\_XXXXX; *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<sub>3</sub>OH.

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

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

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

1 10 20 30 40 50 60 70 80 90 100

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MvdE  NSKNVKSAPKAVPFFARFLAEQA---VEANNNSA--PYGNTHKYPDSWEEY
MvdF  NSKNIKYSTGSAVPPFFARFLSEQA---TETGOSTSDIPTIATFKWPSDWEES
N9414_22578  HTTATLANIEAVPFFARFLAAEAPPETPAPQPPEEQPLPPPITLKWPSDWEED
IPF_3129  HTNYPNSEQSKALPFFARFLSADQA---EAPTPDSPDSEPPVATWKPSPDWEES
Mae24110  MAYPNDQQGKALPFFARFLSVSKE---ESSIKSPSPEPTYGGTFKYPSDWEEDY
MdnA_NIES298  MAYPNDQQGKALPFFARFLSVSKE---ESSIKSPSPEPTFGTTLKYPSDWEEDY
MdnA_MRC  MAYPNDQQGKALPFFARFLSVSKE---ESSIKSPSPDHEI-STRKYPSDWEED
A117013  MPENRQEDLNAQAVPFFARFLEQNCELDLDEESEAVSGGKRGQTRKYPSDCEGNGVYTGKLRDEDIAYTLKYPSDNEONGGGEIVTLKFPDQDDQPVG
Npun1922a  MPTNTVKTVDVAVPFFARFLEEQA-----TEGTE---VPNTYKYPDLEDR
Ava_1618a  MSTNTVKTVDVAVPFFARFLEEQA-----TEGTPPSFPMTFKYPSDLEDR
Consensus  .....avPFFARFL..q.....T.KYPSDWEE#.....

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Figure S2. Alignments of MvdE and MvdF.



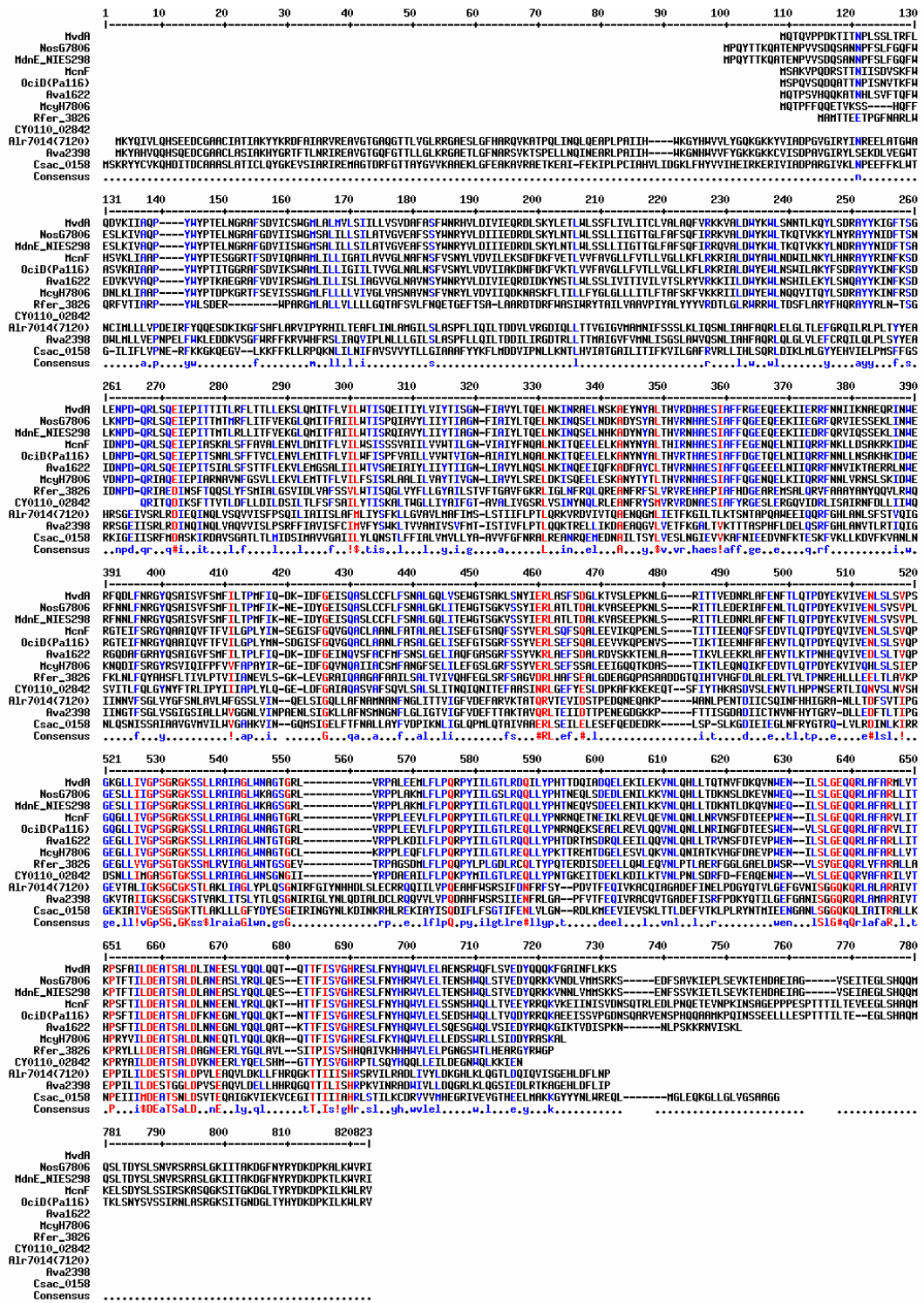


Figure S3. Alignments of MvdA.

1 10 20 30 40 50 60 70 80 90 100 110 120 130

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MvdB MDLVTKRLRLVFPKLPVQAHTIGD-IELGKVLGVTVLSQHIQDFEYD-NLEPIANLKSPLQGEHMGSLIIVQ-QRENTLIGHVMLKIIPDPTDPTGSALEIGVEIAPSYRRQGYGSE
MdnD_NIE5298 MKALEKLVTERLVLVPLKLELLKRAITIGD-DELATLGVKVAADDAEQLIQL-AFDPIANLILQFLPLQNEHMGSLIIVQ-QRENTLIGHVMLKIIPDPTDPTGSALEIGVEIAPSYRRQGYGSE
BBI14905_02675 MSTLITERRLALTPVSLPLLEAVIRGDRAREALSGARFPGHAPGRALVERAFSSPLDRRDDPFAFLIGTRLHVTRGQEEVYVYGSVNLNRPDPA-----TGTVVEIGVEGKSSQGGVYATE
LIn2009 HMFELYSKRLRLIPLNAKYLQLLIEHE-MDLRIEFSLHLEGGIDDELKQ-ALDYRSKVLDEEYTAQIHALIVC-KEQCRTGGIDLKGHPME-----RQEVLYYVYVYPSFGQRYHTE
LM0f2365_2005 HDNLETRDLILINYLLEHQAITINGT-EALKEKSGYHVSPPDPIGDIFF-YLPPYLENKKDQRHKI4---TRLVILKEEN-KITGEIGGQGNPDE-----TGTEIGVSYVDPYQKGYHSE
LM0f7858_2103 HDNLETRDLILINYLLEHQAITINGT-EALKEKSGYHVSPPDPIGDIFF-YLPPYLENKKDQRHKI4---TRLVILKEEN-KITGEIGGQGNPDE-----TGTEIGVSYVDPYQKGYHSE
CA17_08040 MYTLETEMLLLVPYQLSYEATITIGD-EKLSVGSFSVAQEPNGVEFFF-YLPPYLENKKDQRHKI4---TRLVILKEEN-KITGEIGGQGNPDE-----TGTEIGVSYVDPYQKGYHSE
BSU12040 MYTLETEMLLLVPYQLSYEATITIGD-EKLSVGSFSVAQEPNGVEFFF-YLPPYLENKKDQRHKI4---TRLVILKEEN-KITGEIGGQGNPDE-----TGTEIGVSYVDPYQKGYHSE
CNC3423 ATLRWDTDRLLHPYTLLEVKSILVYGS-MDELLKLDNPKTKPTKTKD-ILPIARLLEKANKPSGF---ETADLTKDNNQVTDGTFASEPME-----KGEVYVYCLVEDERKGFQFPE
StrOp_3908 HAEVGRSRLSCPVASTLATELLEDPYRD-EDIDRLLEYEMLPEYTHLITDIDVDPAFPAARARSNEDEP---HDHRYVTDGSPYVIGSLDPRYSHMGQGTGPTGEVYVYDPRYGGHGYATE
VF1328 MHADQDLLELVYKTSRLKHELDSEVYVTEQASQVYLNKIVALLTPVYDQGLPPYFDIETIKEAELHKKSNES-QLFVYSAKNTDIIQLFLFLSDGNK-----EVLGYLLDGEYHMKGYGSE
Alr7015 MNLRLPDLADLNLRLHMDQPHYIADSPDNDN-GHEVELARSDPAREQLIVEIDSRIFGIQIIPDPAQDSHYHGVDGVENLRRI-----DIDIG---EETDLKGGYVGTQ
Consensus .....l.t.r.l.l.....l.....a.....g.....l.....g.....u.....p.....l.....u.....i6.v.g.p.....ge.ei6g.....y.gk6y. #

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131 140 150 160 170 180 190 200 206

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MvdB ATKYVLDHAFSOPAYEKYTAGCDARONIGSKRLLEKIGHQHTKLA-N---GQVLMELSRSS
MdnD_NIE5298 ATKAMHDTLSDPGHQTAGCDARONISKVLEKIGHDLIE--A-R---EKVLMLKLSYVC
Sce5912 GSRAYLHNTLQDPGVRYRZIATPPAHRASLRVIEKLGHRPAGTLD-HOLLGELLVFERYPE
BBI14905_02675 TITITANLHNPVUSITLADIEKONFASHWLEKAGHLYKEIE-SLYYARFL
LIn2009 ALISHDIALEQEPVYKRIARCYEQNESIQVKNHNFVHKEKDTTEKQRVHMHYEPIKDT
LM0f2365_2005 ALIGHIANLEEQPATHRIFARSYEQNASIQVKNHNFVHKEKDTTEKQRVHMHYEPIKDT
LM0f7858_2103 ALIGHIANLEEQPATHRIFARSYEQNASIQVKNHNFVHKEKDTTEKQRVHMHYEPIKDT
CA17_08040 GARAFALMSLVEE-IKKIKAKLYLHKKSHLKLQVKTGEGYVTDG-RVYFEMKPHHTLQSEKSRQDKAAH
BSU12040 AAQLINHALSRETYSRITARCEHNLGSQKTEKLEQLN-----HKSREYKHIVYTK
CNC3423 ALSAINDHIFDKNSYVKAQDLLENKPSARILQVQWKEI-----DRIADHSYNEFKPVS
StrOp_3908 RVYIAYVYSPALGRIRIAGCYADNLRSVALLKYVRRQDQGVNCSHAELEGAQDGYVTLHAEEMSKQP
VF1328 LGLGLDYLHEHPSIERLVGVQDNKISAKLEKVEFTKEE-----KHPIVYFYELEHHT
Alr7015 MRLALARCFADPLVYRILYDPLASNTRVHFYERLQFKFIEHRCFGDDCFYRLNREDNHCKSEVYV
Consensus .....u.....q.p.!..i.a.....dn.as.r.lek.g.....v.....e.....

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Figure S4. Alignments of MvdB.

1 10 20 30 40 50 60 70 80 90 100 110 120 130

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MvdC HSLSRVYLLITHSQDFIDIRVEALIKKGVQPFRTDQDFLNVALTNFQKSNDSQALVEYNGNSISTKVQAVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
Npun02001923 HKATYLLFNSYLLKSKRYNHLSDRYVLLTYISVDFIDIRVEALIKKGVQPFRTDQDFLNVALTNFQKSNDSQALVEYNGNSISTKVQAVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
Ava1618 NMLSRDYLITHSQDFIDIRVEALIKKGVQPFRTDQDFLNVALTNFQKSNDSQALVEYNGNSISTKVQAVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
MdnB_NIE5298 NMLSGDYLITHSQDFIDIRVEALIKKGVQPFRTDQDFLNVALTNFQKSNDSQALVEYNGNSISTKVQAVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
HdnB_HRC MKESPKVYLLITHSQDFIDIRVEALIKKGVQPFRTDQDFLNVALTNFQKSNDSQALVEYNGNSISTKVQAVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
IPF_2482 MKESPKVYLLITHSQDFIDIRVEALIKKGVQPFRTDQDFLNVALTNFQKSNDSQALVEYNGNSISTKVQAVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
a117012 MKESPKVYLLITHSQDFIDIRVEALIKKGVQPFRTDQDFLNVALTNFQKSNDSQALVEYNGNSISTKVQAVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
Sce7175 MREKHPVYTTASASVLLVSHSQDFIDIRVEALIKKGVQPFRTDQDFLNVALTNFQKSNDSQALVEYNGNSISTKVQAVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
M23134_04991 MKLLITHSQDFIDIRVQALRAGTAPRLRITNDLYLEPEIKYHFLHLEAGTDPKVFYEIETDGTDTDEVEGVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
Nui_0610 MSETSPITLITAKLDTHA-DAVISELKARGQPFRLMDDDFITEYKTYLSDGSRVTFEDIDGSRVPHQVHAHARRVTEPDE-QELDPAQACAKRESKATLNGFH
Sarc_3330 HGGRTVAVFTGELDV-TADRVISELHRRGVVPRCDPAFPAEHTLGRFGAGTGH-LRVGRVLDVADVACARRRPTITVPEVPEPES-QAVHARRVTEPDE-QELDPAQACAKRESKATLNGFH
Consensus .....!l.t.hsqd.f.tidr.va.al.r6.pfR1#d.z.p.#.l.a.f.....e.....i...#l.avH.Rr.w.p.l.e.....r..c.res.a.g.l.g

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131 140 150 170 180 190 200 210 220 230 240 250 260

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MvdC DSLRDNHVDHQAIEAKKLLQLRLASEVGLTIPQTILINKAAREFFQVNGKMYSKLLTALSRESHES-TSFFLYSLVKEEYLDQRESLYCPHYQEQIFKQQLRVYVNGKVFVGLNADHY
Npun02001923 DSLRDNHVDHQAIEAKKLLQLRLASEVGLTIPQTILINKAAREFFQVNGKMYSKLLTALSRESHES-TSFFLYSLVKEEYLDQRESLYCPHYQEQIFKQQLRVYVNGKVFVGLNADHY
Ava1618 DSLRDNHVDHQAIEAKKLLQLRLASEVGLTIPQTILINKAAREFFQVNGKMYSKLLTALSRESHES-TSFFLYSLVKEEYLDQRESLYCPHYQEQIFKQQLRVYVNGKVFVGLNADHY
MdnB_NIE5298 DSLRDNHVDHQAIEAKKLLQLRLASEVGLTIPQTILINKAAREFFQVNGKMYSKLLTALSRESHES-TSFFLYSLVKEEYLDQRESLYCPHYQEQIFKQQLRVYVNGKVFVGLNADHY
HdnB_HRC DSLRDNHVDHQAIEAKKLLQLRLASEVGLTIPQTILINKAAREFFQVNGKMYSKLLTALSRESHES-TSFFLYSLVKEEYLDQRESLYCPHYQEQIFKQQLRVYVNGKVFVGLNADHY
IPF_2482 DSLRDNHVDHQAIEAKKLLQLRLASEVGLTIPQTILINKAAREFFQVNGKMYSKLLTALSRESHES-TSFFLYSLVKEEYLDQRESLYCPHYQEQIFKQQLRVYVNGKVFVGLNADHY
a117012 DSLRDNHVDHQAIEAKKLLQLRLASEVGLTIPQTILINKAAREFFQVNGKMYSKLLTALSRESHES-TSFFLYSLVKEEYLDQRESLYCPHYQEQIFKQQLRVYVNGKVFVGLNADHY
Sce7175 RGLPARRINEPHAREQAQDLRARRASVGLRVPPETLVYVNPKEAREFFQVNGKMYSKLLTALSRESHES-TSFFLYSLVKEEYLDQRESLYCPHYQEQIFKQQLRVYVNGKVFVGLNADHY
M23134_04991 LLLDHAPLQLEHISRSQKLYQLKLYKQLVAPRALITNEARALLSFEYKQ-DITLKHADDTGEM-GRSARQHYMYTPHVEEFSLYQCPHTQEKIKKCLRYVYVGGVFTGADTSQS
Nui_0610 SCFHDRPYNMPESNRYSORKFPQLCLASCLGRVPRITLINDPGEARRADDTGSLCKAKGAYSTPMGSYIFRSQVGDQDFADHADRDLCTLLQYIEKAYELRVYVGGVFTGADTSQS
Sarc_3330 AVL---PMLNMPEDIRAREHKLQLATARRVAVPEITLNDPQARRAV-ERAVPYLYKPLCGVLD---GGRVYAGVPDARLDA---VRYTALHFRQPKAYEVATYVDRIFARRIDLS
Consensus ..l..a.H.#.le.i..a.nkL.QL...R.evGf.IP.TI.TN.p..ar.ff.q.g.....K.lt.....sm.....ff.gt..v..edl..ae.lrycpmvfQe.lpkA.EIrvr.l.g..f.gal#a.y

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261 270 280 290 300 310 320 330 340 350 359

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MvdC EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
Npun02001923 EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
Ava1618 EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
MdnB_NIE5298 EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
HdnB_HRC EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
IPF_2482 EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
a117012 EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
Sce7175 EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
M23134_04991 EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
Nui_0610 EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
Sarc_3330 EDSTVDRHPVSEVIGNEPDLPRDVEARLGRALLRALGLVFGVGLIRTDPGEHYVLEVPNGEGMGLERDLPISTRIADTLIP
Consensus .....DR.....e.....u.....L.p.v..l..f#..l.g.L.g.D.f.I.P.g#yVLE.NP.G#HGLERDL.PI.a.RiD.L.....

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Figure S5. Alignments of MvdC.

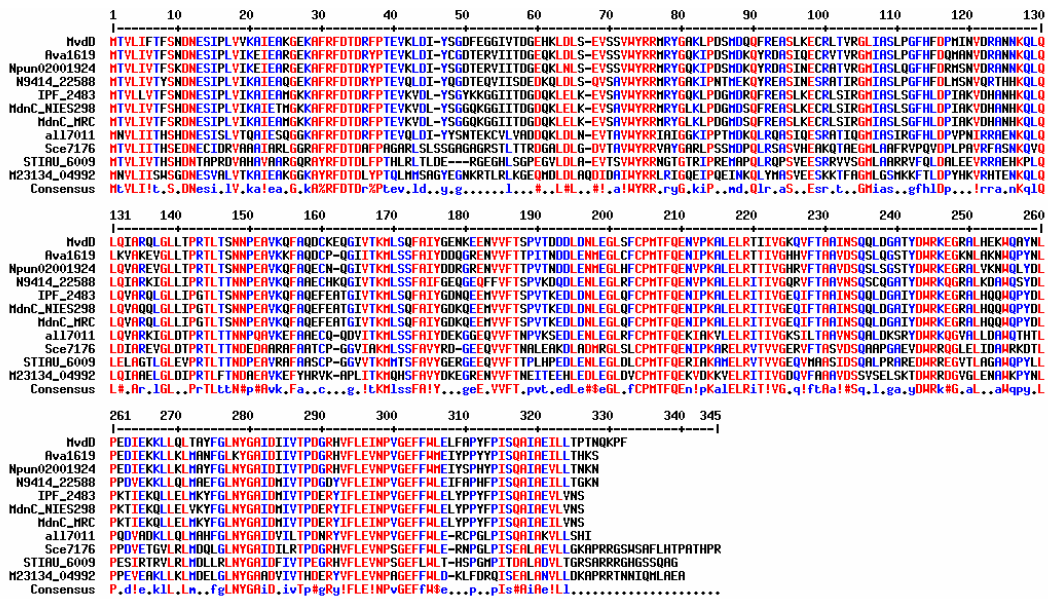


Figure S6. Alignments of MvdD.

## 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 (ATTAATGTGACGCGTC-CCTAGCCAATGGTCAAGTATTAGTTTG) and MVassembleLeftRev2 (ACAATG-CATGTCGACCAGTTAAGTTCCTTTTGAATATTGA-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 *Sall* 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 *Sall* according to the manufacturer's supplied protocol, followed by reisoalation 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* DH5 $\alpha$  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 (ATGTGTATACTCGAGGTCAGAACAAGACACAGAACTGGTGA) and MVassembleRightRev2 (ATAAAAATTGC-GGCCGCGTCGATTAGATCAAGGTGACTCCAC) 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 *Xho*I and *Not*I 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 *Xho*I and *Not*I (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* DH5 $\alpha$  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 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 *Xho*I 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 *Bsa*AI followed by aga-

rose gel purification and isolation, using T4 DNA ligase. The ligation mixture was then transformed into chemically competent *E. coli* DH5 $\alpha$  using standard protocols.<sup>1</sup> 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<sup>R</sup> cassette was established by sequencing to have the 5'-end of the Cm<sup>R</sup> cassette toward the MVRright fragment. PDMVLRKO-1 was linearized with *Pst*I and used in transformation of *P. agardhii* as previously described.<sup>2</sup>

Primers used for detection of homologous recombination:

MVKOOckFwd (TGGGGAAATTTAGTGATTCAACAAGCAGAAAATA)-289bp  
from construct

MVKOOckRev (CCAATTAACGGCTCCTATTTTG TTA CTTTG)-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 MicvicGluTranslfwd (GCTAGTCGCGCATATGTCTTTGTCTCGTGATGTTGTTT-TATTAATTAC) and MicvicGluTranslrev1 (ATATTAAGTAGCCTCGAGGGGAAT-TAGGGTATCGGCGATC) for *mvdC* and MicvicGluTransllfwd (CCCGATGTAC-AGTCATATGACGGTTTTAATTTTCACTTTCAGCAAC) and MicvicGluTransllrev1 (TTACTATCGACTCTCGAGAAATGGTTTCTGGTTTGTAGGAGTGAG) for *mvdD* per the manufacturer's supplied protocol. The italicized bases indicate the *Nde*I and *Xho*I 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 *Nde*I and *Xho*I. 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* DH5 $\alpha$  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 supernatant was decanted off and the cell pellet was stored at –20 °C until use.

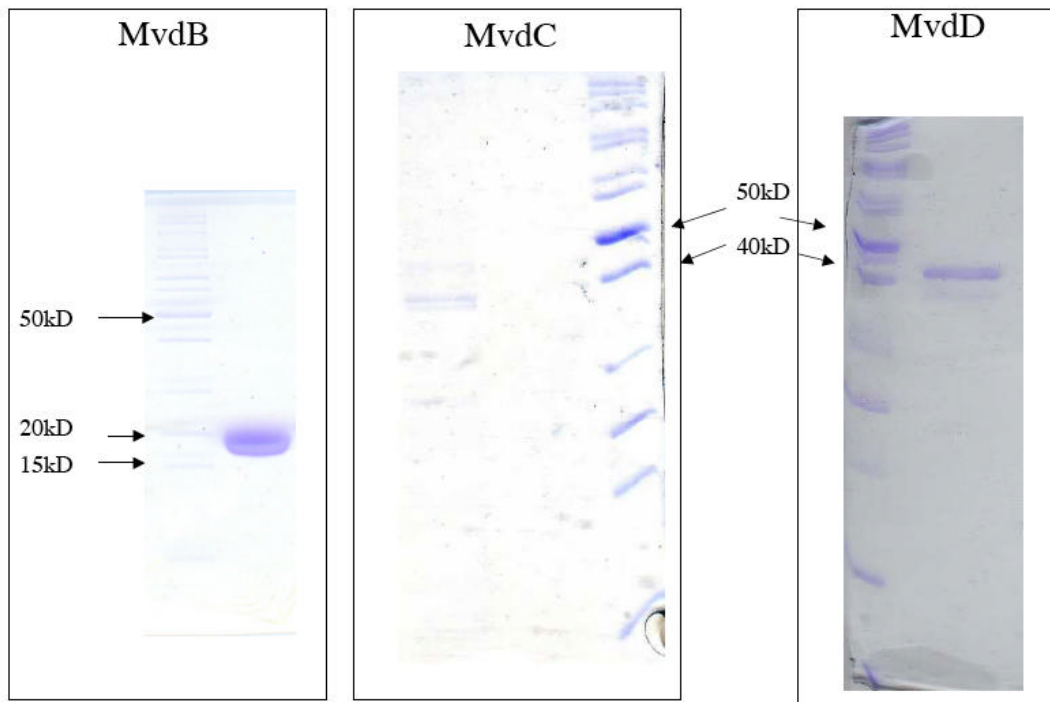
Both MvdC and MvdD were prepared as follows: The frozen cells were defrosted on ice and resuspended in 30 ml Lysis buffer [50 mM Tris, 300 mM NaCl, 5 mM imidazole, pH 8.0]. The cells were lysed by 30 sec intervals of sonication on ice. The cell debris was pelleted by centrifugation (10,000 g, 30 min, 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  $\beta$ -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.

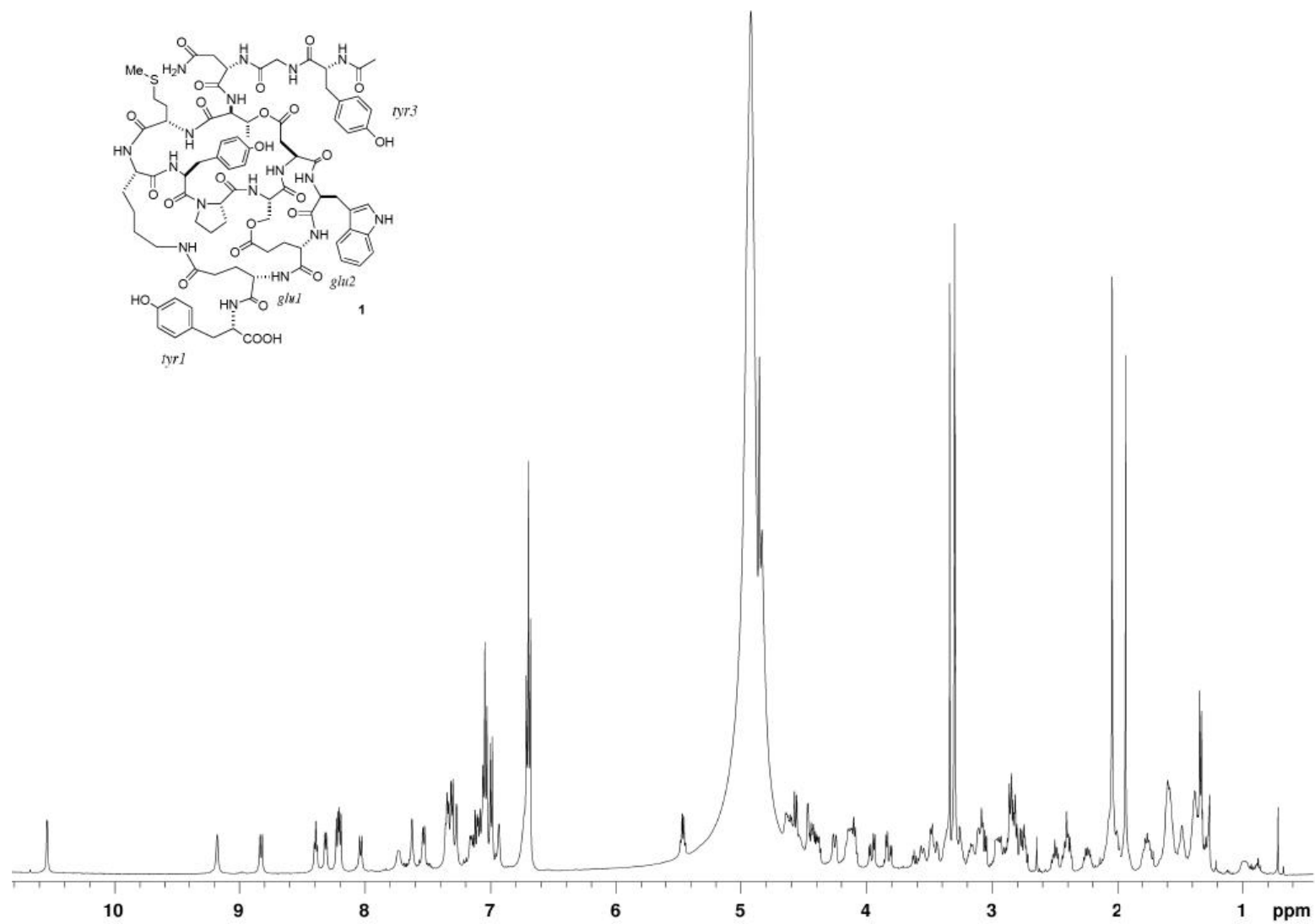
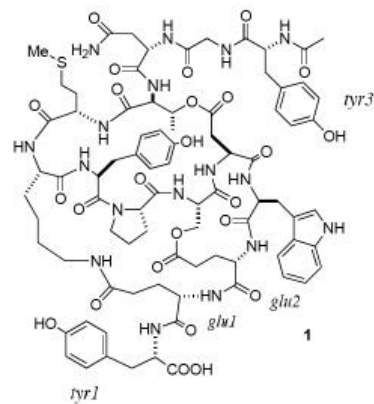
References:

<sup>1</sup> Sambrook, J.; Fritsch, E. F.; Maniatis, J. *Molecular Cloning: A Laboratory Handbook*

<sup>2</sup> 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..



<sup>1</sup>H-NMR spectrum of microviridin K in MeOH-d<sub>3</sub>.



