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Supplementary Material

Three DUF1996 Proteins Localize in Vacuoles and Function in Fungal Responses to Multiple Stresses and Metal Ions

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Bba_EJP70292.1	DEIVSFGQIS.PHVHVVTGGTAFQQTQSN.EQARSARATIDCKLLIKSNYQFCQLYHQHRD.GRFLVTMQNAAYYIKRA.....C	79
Bba_EJP68966.1	DEIVNFKDVS.AFAHSLHGSSGLSASADG.SALKAGKCSORVEQCKKAYNTEFLYFQDSDTGKFLVTVQVGMGLAYLLN...GENIK	84
Bba_EJP64302.1	DEIINEGQLS.THVHAIVGGTGFHMSMSN.EDARNKNTIDCKALKIKSNYQFCQLYHQHHD.GSFLIKLLCIAAAYIDRT.....C	79
Cmi_EGX90679.1	DEIVNANSVS.AFAHSLHGSSGLSASADG.AALKGDCISORVTCCKKAYNTEFLYFQDIDTGGKFLVTVQVGMGLAYLLY...GDNIK	84
Cmi_EGX90023.1	DEIISFGQLS.SHVHTIVGGTGYMSMSN.EDARNRNITIDCKALKIKSNYQFCQLYHQYND.SSLDLIKMLCIAAAYIDRS.....C	79
Cmi_EGX93970.1	DEIMSFGQIS.AHVHVVTGGTAFQLAQSNAQARGARATIDCKILIKSNYQFCQLYHERHD.GRFLVTMQNAAYYIKRA.....C	79
Mro_XP_007822393.1	DEIINEAS.PHANTIHGNGFSESATT.ADLLDGDICISORVTCCKKAYNTEFLYFQDAKTEKFLVTVQVGMGLAYLLY...GDNIT	84
Mac_XP_007807914.1	DEILFEGVMS.PHVHAVVGGTRFALNLTN.EQARQAKATIDCKRLIKSNYQFCQLYHQRRD.GRFLVEMQG.....	69
Ani_XP_659411.1	DEIVSFGLYV.SHMSFFGSDAVNNTIST.SAELREGCSTARNPICFVYVIFLTLVLDKSAPEGTHQFITPMRFS.....	75
Ani_XP_681436.1	DELISFGEPS.YHVHAVVSGKGFGLTADM.ATLLDGDICISGVKCKKAYNTEFLYFVDNATGDSLVDVDEVGMLVYLLY...GDNVE	84
Afu_XP_753120.1	DEIVAFGTYT.SHMATFFGSDAITNVLPT.SAELQRGCSGDNPNELSVYVVTLYVVDGDTFTEVAIFRFSTYYTNSYSND.....S	80
Afu_XP_748048.1	DEIMDEGKPS.SHVHAIHSGGFSMSASG.ADLKASSCSICAVTCKKAYNTEFLYFIHEN.GDDEIVNQVGLLAYLLY...GENVT	83
Mor_ELQ61032.1	DELATFGQIPGRVHTVMGNAFANLTTYEQTKSTONSQSVTRKLSNYVFCLEFFRFDN.GSFAAVKNSGSLIYLLHR.....HD	82
Mor_ELQ58222.1	DELISFGVA.CHLHSIFGGGFGDNATY.DDLMASDCISCAVTCCKKAYNTEFLYFQDATTGQFLVTVQVGMGLAYLLFPNPEGLI	87
Bba_EJP70292.1	DYAFGRQNCNNATAPI.....APPRLRMITGDPFLRTYDQSSLAQCMISHVCELEGPNEGE.TPHMSRLPCGRMRAETFFPSCWDGQ	160
Bba_EJP68966.1	AFPDFFRMIAGSNARR..AYTAGNPEQADPKSLWHSMEGPKQHILEQRALGFICINYDRQAEGTLYRHKMPDKAYLDANCKDGRLEI	171
Bba_EJP64302.1	DYKFGRRDCRDAFGAI.....APPKGLRILSGDILRRTYDKSDKTNRAISHVCLDPGQD...YIELPQKLCNEMRTQVYFPCWDGK	158
Cmi_EGX90679.1	AFPFDFRMIAGSNTRR..AYTAGNPEQADPKSLWQSMGQTKQNLLEQRALGFICINYDRQPEGLYRHYMPDKAYLDANCKDGRLEI	171
Cmi_EGX90023.1	DYQPGRKNCRCGARGAI.....APPKGLRMLSGDTSRRTYDKNDKSRRAISHVICIDFGKD...YVELPQKPCNEMRSQVFFPSCWDGK	158
Cmi_EGX93970.1	DYAFGRNRCDGAFAPI.....APPRLRMISGDEPELRTYNPDSFAQCMISHVCELEGPNEGE.TPHMSRLPCGRMRAETFFPSCWDGH	160
Mro_XP_007822393.1	AFPPGFQMISSGDNDRR..TYTLGDARQFDPDRSQWAALGQTQEAALQRAIGFICINYAKNPEQTLYRHYLPKKAELDQNCTEGVRLEI	171
Mac_XP_007807914.1FQMVVGNASATGPEGLDPDQVQSWCEGDEAE.....EGKELAAFPTRTCTSTHLQTL	69
Ani_XP_659411.1	AYYELLNEAIEIPLPEN.....FQMVVGNASATGPEGLDPDQVQSWCEGDEAE.....EGKELAAFPTRTCTSTHLQTL	144
Ani_XP_681436.1	AFPFDFRMIAGDPFQR....NFTWPIPPPKSEWIGD.QASQAALRQRAIGFICINYAKAEPISLGRHFLPEKAYLDEHCTDGVRFEI	167
Afu_XP_753120.1	AIPQDFAMISGN.....ASAHSQAEADHPYNLEWFCGESSER.....ESDIALPTSTCDQHLQNL	138
Afu_XP_748048.1	AFPDNFRMIAGDTYLR....DFFWPIPPPKSEWIGK.QGSQEAALRQRAIGFICINYALDPEPSLGRHFLPNKTYLDEHCTDGVRFEL	166
Mor_ELQ61032.1	KKEPEYAKGLQFFPEG.....FRMIAGEPLARQRNDTQGRKQKAVSFFCLRGEF...TPATPYLPKMR....CKNGMRAQI	154
Mor_ELQ58222.1	AFPFGRMIAGDSLRRNYSEALGDVSKPDPKSIWAKLGQTSQPDLQRAIGFICINYAKAPEGSLSRHFLPKAYLDANCADGVRFEQ	176
Bba_EJP70292.1	NLD...SS.NHKD...HMAFPAVGAYNFVCEHSHPTAILSVFVFFFYDTGSLE.....DPNRIVWAMGPTFCYGLHGTYLQEW	232
Bba_EJP68966.1	MFPSCWNG.KDIDAKDHKSHMAYPDLVMTGTCHEGYETRVFSLMPEIWSVSPYKD.....RNGRFVFSNGDTEGYAYHAFMMGW	251
Bba_EJP64302.1	NLDSPDHK.SHASTEFRMRSFPGYDNGVCEHSHFVATVSVFNEFFWNTRQVQGG.....AFRRVYANGDTEGYGLHGTYLQEW	239
Cmi_EGX90679.1	MFPSCWDG.KNTDSSDHKSHMAYPDLVMTGTCHEGYETRVFSLMPEIWSVVPFKN.....RNGRFVFSNGDTEGYAYHAFMMGW	251
Cmi_EGX90023.1	NLDSPDHK.SHV.....AFPALGDYNTVCEHSHFVAIVSVFNEFFYNTIRQVQSK.....DFRRVYANGDTEGYGLHGTYLQEW	232
Cmi_EGX93970.1	NLD...SG.NHKD...HLAFAIGAINTVCEHSHFKAILSVFVFFFYDTGAIK.....NPNRIVWAMGPTFCYGLHGTYLQEW	232
Mro_XP_007822393.1	MFPSCWKGNAIDSEDDHKSHMAFPDQVMTGTCHEGYFVRTFSLYDPTIWNNTYAFKN.....RDGRFVSNGDTEGYGLHGTYLQEW	252
Mac_XP_007807914.1IMAFPAVGDYNTVCEHSHFVAILSVFVFFFYNTRAVK.....DFNRVWAMGPTFCYGLHGTYLQEW	132
Ani_XP_659411.1	WFPDCANP..DTFETAYSKNKFAFEGYGENWCEIGMYKIPRLRFSIRYDLRKLDPG.....WDGEPFLELPCGNSFCSHGFIINGW	224
Ani_XP_681436.1	MFPSCWNG.KDIDSDDHASHVAYPDLVMTGTCHEGFETRIVSLFFETIWNNTYAFKD.....RDGYFALSTGPTFCGYHAFMMGW	247
Afu_XP_753120.1	LFPQCENP.....DDHSEYDFADESNTCEGMLQMPQLRYRVQYDTRKSVAPDGWSG...PAPFQLSCSDTFGDCYCFHAFINGW	215
Afu_XP_748048.1	MFPSCWNG.KDVDSDDHSHVAYPDLVMTGTCHEGFETRIVSLFFETIWNNTYAFKD.....KEGYVYIANGPTFCGYHAFMMGW	246
Mor_ELQ61032.1	HFPACWNG.KDNDSPDHSHVAYPDLVMTGTCHEGFETRIVSLFFETIWNNTYAFKD.....KEGYVYIANGPTFCGYHAFMMGW	242
Mor_ELQ58222.1	MFPSCWNG.VDVDSPDHRSVAYPDLVMTGTCHEKSHPKRLISLFFETIWNNTAFAFG.....RNGRFVFSNGDTEGYGLHGTYLQEW	256

Figure S1 Sequence features of domain regions in the DUF1996 family proteins of several filamentous fungi. NCBI accession code of each protein follows the fungal name (Ani, *Aspergillus nidulans*; Afu, *Aspergillus fumigatus*; Bba, *Beauveria bassiana*; Cmi, *Cordyceps militaris*; Mac, *Metarhizium acridum*; Mro, *Metarhizium robertsii*; Mor, *Magnaporthe oryzae*). Red: 100% identity. Blue: 75% identity.

Bba_EJP70292.1	CFIVSFGQISPHVHVVTIGTAFQQTQSNEQARSARATIDKLLDRSNWQQLNHRHDGRREIVTMQENAAAIKRRACDYAPGRQNC	88
Bba_EJP68966.1	CFIVNEKDVSAHAHSLHSSGLSASADGSALKAGKCTSRVEQKRSAYWTFSLIFQDSDTGFREIVTQVWGMLAYLLNGDNIAKAFPDD	89
Bba_EJP64302.1	CFIINEGQLSTFVHAIVGGTFHMSMSNEDARNRKNITDKALDRSNWQQLNHRHDGRREIVTMQENAAAIKRRACDYAPGRRDC	88
Cmi_EGX90679.1	CFIVNENSVAHAHSLHSSGLSASADGAALGGDCSTRVITQKRSAYWTFSLIFQDSDTGFREIVTQVWGMLAYLLYGDNIKAFPDE	89
Cmi_EGX90023.1	CFIISFGQLSSHVHTIIVGGTYMMSNEDARNARNITDKALDRSNWQQLNHRHDGRREIVTMQENAAAIKRRACDYAPGRKNC	88
Cmi_EGX93970.1	CFIIMSFGQISAHVHVVTIGTAFQLAQSNQAARGARATIDKILLNSNHWQQLNHRHDGRREIVTMQENAAAIKRRACDYAPGRRNC	88
Mro_XP_007822393.1	CFIINENEASPHATIHASNGFSEFSEATTADLLDGDCTSRVITQKRSAYWTFSLIFQDAKTEKREIVTQVWGMLAYLLYGDNITAFPFG	89
Mac_XP_007807914.1	CEILFSGVMSPHVHVAVVGTIRFALNLNEQARQAKATIDKRLDRSNWQQLNHRHDGRREIVEMQG.....	69
Consensus	dpi p s h h g t c dks yw p ly q d g felv g y d	
Bba_EJP70292.1	NNATAPIAPPRLRMIT...GIPFLRTYDQSSLAQQAISHVCLLEGFNEGETPHMSRLPCGRMRA...ETFFPSQNDGQND...SSNEK	168
Bba_EJP68966.1	FRMIAGSNARRAYTAGNPEQDPDKSLWHSMGEPKQHILEQRALGFNCLNYDKQAEGTLYRHKMPDKAYLDANCRDGIHIEIMFPSCWN	178
Bba_EJP64302.1	RDAPGAIAPPKGLRILS...GDTLRRTYDKSDKTNRAISHVCLDPGQD..YIELPQKLCNEMRT...QVFFPSQNDGKNIDSPDHKSHA	169
Cmi_EGX90679.1	FRMIAGSNTRRAYTAGNPEQDPDKSLWQSMGQTKNLLQRALGFNCLNYDKQPEGTLYRHYMPDKAYLDANCRDGIHIEIMFPSCWD	178
Cmi_EGX90023.1	RGARGAIAPPKGLRMLS...GDTSRRTYDKNDKSRQRAISHVCLDPGKD..YVELPQKPCNEMRS...QVFFPSQNDGKNIDSPDHKSHV	169
Cmi_EGX93970.1	DGAPAPIAPPRLRMISS...GPELRTYNSDFQAQAISHVCLLEGFNEGETPHMSRLPCGRMRA...QTFPPSQNDGHNID...SGNHK	168
Mro_XP_007822393.1	FQMISGDNDRRTYTLGDARQHPDRSQWAALGQTTQEAQAQRAIGFNCNLVAKNPEQTLYRHYLPKRAELDQNCDEGVRIEIMFPSCWK	178
Mac_XP_007807914.1	69
Consensus	d c dg l	
Bba_EJP70292.1	D.....HMAFFAVGAYNFVCFESHETAILSVFYFFFYDGSLE..DPNLLWAMGEPVGYCLFGYILQEN	232
Bba_EJP68966.1	G.XDIDAKDHKSHMAYPDLVMTGTCHEGYETRVPSLMFHTIWSVSPYKD.RNGHFVFSNGETESYFYAFQFMGN	251
Bba_EJP64302.1	S.....TEFRRMSFPSIGDYNGVCFESHVVAIVSVFNFFFWNTRQVQGGAFRWVYANGETVGYCLFGYILQEN	239
Cmi_EGX90679.1	G.XNIDSSDHKSHMAYPDLVMTGTCHEGYETRVPSLMFHTIWSVVPFKN.RNGHFVFSNGETESYFYAFQFMGN	251
Cmi_EGX90023.1AFPALGDYNTGVCETHVVAIVSVFNFFFYNTRQVQSKDFRWVYANGETVGYCLFGYILQEN	232
Cmi_EGX93970.1	D.....HLAFAIGAAYNTGVCQSHKAILSVFYFFFYDGAIR..NPNLLWAMGEPVGYCLFGYILQEN	232
Mro_XP_007822393.1	GGNAIDSEDHKSHMAFPDQVMTGTCHEGYFVRTPSILYTIWNTYAFKN.RDGHFVFSNGETESYFYAFQFMTEW	252
Mac_XP_007807914.1IMAFFAVGDYNTGVCQSHVAILSVFYFFFYNTRAVK..DFNLLWAMGEPVGYCLFGYILGN	132
Consensus	tg cp p s e t r v gd tgyg hgd gw	

Figure S2 Sequence features of domain regions in the DUF1996 proteins of four filamentous fungal insect pathogens. NCBI accession code of each protein follows the fungal name (Bba, *Beauveria bassiana*; Cmi, *Cordyceps militaris*; Mac, *Metarhizium acridum*; Mro, *Metarhizium robertsii*). Red: 100% identity. Blue: 75% identity.

Table S1. Paired primers designed for manipulation of three VLP-coding genes (*vlp1–3*) in *B. bassiana* and identification of their deletion and complementary mutants.

Primers	Sequences (5'–3')*	Purpose
Vlp1-F/R	AGCTTTGTTTAAACATGCCCTCAAATTGGTCACTC / AAAAAGACTAGTTACCGGGTCCCCGGTAACC	Cloning <i>vlp1</i> cDNA
Vlp2-F/R	AGCTTTGTTTAAACATGTTCAAGCTTACTACCGCCGTG / AAAAAGACTAGTGCTCCGGCGGACGTGAGC	Cloning <i>vlp2</i> cDNA
Vlp3-F/R	AGCTTTGTTTAAACATGCTTTCTAAATACCTTGTTC / AAAAAGACTAGTGAGAGCAACACAGCGTTGG	Cloning <i>vlp3</i> cDNA
Vlp1up-F/R	AAACCGGAATTCTCTACCAATGAACCTTGTCTG / AAACCCAAGCTTCAAGAAGAAACGAAGAACGGAG	Cloning <i>vlp1</i> 5'(1501 bp)
Vlp1dn-F/R	AACTAGTCTAGAATGCTAAGCGACGGCAGAGAAT / AAAAAAGTTAACTTGC GGCTCTGAAAGGTGAAAT	Cloning <i>vlp1</i> 3'(1525 bp)
Vlp2up-F/R	AAACGCGGATCCACCTACCTACCTAATCGTTGG / AAACCCAAGCTTCTTTGATGGTAAGGAGTGGAC	Cloning <i>vlp2</i> 5'(1584bp)
Vlp2dn-F/R	AA CTAGTCTAGAACTCCTACCGCTTCTACCAACC / AAAGGAAGATCTCCAGCCAGCCCTTCTCTTT	Cloning <i>vlp2</i> 3'(1588 bp)
Vlp3up-F/R	AAACCGGAATTCGCGAGTCTGGTCTTGAGCATT / AAACGCGGATCCAAGGTAGCAATGGGGGAGAG	Cloning <i>vlp3</i> 5'(1424 bp)
Vlp3dn-F/R	AACTAGACTAGTGGCATTGAGGGAGAAGATTG / AAAAAAGTTAACGT CAGGCTCTTCTTCTGTTTC	Cloning <i>vlp3</i> 3'(1637 bp)
Vlp1fl-F/R	<u>GGGGACAAGTTTGTACAAAAAGCAGGCT</u> GAGGCTCCAACCCATAGACATT/ <u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> CTTGACGGCGACCATCATTTAC	Cloning full-length <i>vlp1</i>
Vlp2fl-F/R	<u>GGGGACAAGTTTGTACAAAAAGCAGGCT</u> TCCGTTGACCAAGTGCCTGAGT/ <u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> GAATGTGTTGCCTGGTGAGACG	Cloning full-length <i>vlp2</i>
Vlp3fl-F/R	<u>GGGGACAAGTTTGTACAAAAAGCAGGCT</u> TAGAAAGGCAATCCCTCCAT/ <u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> TTATCCGTCACAAGTAGTCCGT	Cloning full-length <i>vlp3</i>
pVlp1- F/R	AAATCCTTGATACCGTCTCCGT / AAATCGCAGTATTCAGTGTCCG	PCR detection of <i>vlp1</i>
pVlp2- F/R	ACACCATCTCAGACTGCTCATC / CTGCTGCGTCCAGAGTATTTTG	PCR detection of <i>vlp2</i>
pVlp3- F/R	TACCTTGTTGCTCTCTCCCCCA / GCGTATCGGTTTTCCGGCTTTTG	PCR detection of <i>vlp3</i>
sbVlp1-F/R	TTGTGCGACTGAATACTGC / CATGGGATCTTCCGCTTCT	For Southern probe of <i>vlp1</i>
sbVlp2-F/R	TTCTTTCATTGGCGAGGAT / TGAGTGGCTGGTGGTGG	For Southern probe of <i>vlp2</i>
sbVlp3-F/R	ACAGCCAATGCCACCTAC / TTGCCAAACCCTTCGGATA	For Southern probe of <i>vlp3</i>
qVlp1-F/R	GGCAATCCTGTCCGTGTT / CAGGTAGTCGCCGTGGAG	qRT-PCR detecting <i>vlp1</i>
qVlp2-F/R	CCACTCCTACCGCTTCTAC / GTGTTGCCGTTGGTGATG	qRT-PCR detecting <i>vlp2</i>
qVlp3-F/R	GGAGGTTGGCTTGAATGG / ATGGCGTAGAATCTCTGTTG	qRT-PCR detecting <i>vlp3</i>
q18S-F/R	TGGTTTCTAGACCGCGTAA / CCTTGGCAAATGCTTTCGC	qRT-PCR of 18S rRNA

* Underlined regions: introduced cleavage sites of restriction enzymes for the deletion of *vlp1* (*EcoRI/HindIII* and *XbaI/HpaI*), *vlp2* (*BamHI/HindIII* and *XbaI/BglII*) and *vlp3* (*EcoRI/BamHI* and *SpeI/HpaI*). Italicized and underlined regions: the gateway exchange fragments for each *vlp* complementation.