

Supplementary Information

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Table S1. Halogenase gene-positive strains detected by Hal3A/3B and information of PKS/NRPS genes and bioactivities.

Name	Accession Number	PKS I	PKS II	NRPS	Indicator Strains					Indicator Cancer Cells		
					<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>R. solani</i>	BL7402	A549	H160
MGR106	KF425740	+	+	+	—	—	—	—	—	—	—	+
MGR009	KF425718	+	+	+	+	—	—	—	—	—	—	—
MGR022	KF425724	+	+	+	+	—	—	—	—	—	—	—
MGR075	KF425733	+	+	+	—	—	—	—	—	—	—	—
MGR017	KF425722	+	+	+	+	+	—	—	—	—	—	—
MGR151	KF425751	+	+	+	+	—	—	—	—	—	—	—
MGR140	KF425748	+	+	+	+	+	—	—	—	—	—	—
MGR060	KF425730	+	+	+	—	—	—	—	—	—	—	—
MGR072	KF425732	+	+	+	+	+	—	+	—	—	—	—
MGR119	KF425743	+	+	+	—	—	—	—	—	—	+	—
MGR016	KF425721	+	+	+	—	—	—	—	—	—	—	—
MGR014	KF425720	+	+	+	—	—	—	—	—	—	—	—
MGR117	KF425742	—	+	+	+	—	—	—	—	+	—	+
MGR032	KF425726	+	+	+	+	—	—	—	—	+	+	+
MGR147	KF425749	+	+	+	—	—	—	—	—	—	—	—
MGR024	KF425725	+	—	+	—	—	—	—	—	—	—	—
MGR089	KF425737	+	—	+	+	+	—	—	+	—	—	+
MGR149	KF425750	—	+	+	—	—	—	—	—	—	—	—
MGR120	KF425744	+	+	+	—	—	—	—	—	—	—	—
MGR137	KF425747	+	+	+	+	+	—	—	—	+	+	+
MGR133	KF425745	—	+	+	+	+	+	+	+	+	+	+
MGR035	KF425727	+	+	+	+	+	—	—	+	+	+	+
MGR076	KF425734	+	+	+	+	+	—	+	+	+	+	+
MGR100	KF425738	—	—	+	—	—	—	—	—	—	—	+
MGR109	KF425741	—	—	+	—	—	—	—	—	+	+	+
MGR086	KF425736	—	+	+	—	—	—	+	+	+	+	+

Table S2. Deduced functions of ORFs in the *sha*-cluster from *S. albogriseolus* MGR072.

Protein	Amino Acids	Protein Homolog	positive/Identify	GenBank Accession No.	Proposed Function
ShaS	315	<i>RifS</i> , <i>A. mediterranei</i> U32	80/70	YP003762835	dehydrogenase
ShaT	338	<i>RifT</i> , <i>A. mediterranei</i> U32	50/39	YP003762836	dehydrogenase
Orf17	964	<i>Strvi_4056</i> , <i>S. violaceusniger</i> Tu 4113	54/42	YP004813994	transcriptional regulator
Orf18	391	<i>Strvi_8510</i> , <i>S. violaceusniger</i> Tu 4113	61/48	YP004818099	aromatic prenyltransferase
Orf19	475	<i>Strvi_8511</i> , <i>S. violaceusniger</i> Tu 4113	82/72	YP004818100	tryptophanase
Orf20	274	<i>Mar181_2538</i> , <i>M. posidonica</i>	51/32	YP004482488	type 11 methyltransferase
Orf21	239	<i>AMED_2499</i> , <i>A. mediterranei</i> U32	71/56	YP003764696	transferase
Orf22	418	<i>AMED_0649</i> , <i>A. mediterranei</i> U32	70/55	YP003762871	cytochrome P450
Orf23	152	<i>acpD</i> , <i>A. mediterranei</i> U32	60/39	YP003768008	NADH-azoreductase
Orf0	363	<i>AMED_0616</i> , <i>A. mediterranei</i> U32	84/76	YP003762838	cytochrome P450 hydroxylase
ShaA	5,464	<i>RifA</i> , <i>A. mediterranei</i> U32	78/71	YP003762839	PKS domains
loading					KR-ACP
Module1					KS-AT-DH-KR-ACP
Module2					KS-AT-ACP
Module3					KS-AT-KR-ACP
ShaB	3,626	<i>RifB</i> , <i>A. mediterranei</i> U32	79/72	YP003762840	PKS domains
Module4					KS-AT-DH-KR-ACP
Module5					KS-AT-DH-KR-ACP
ShaC	3,614	<i>RifC</i> , <i>A. mediterranei</i> U32	75/66	YP003762841	PKS domains
Module6					KS-AT-DH-KR-ACP
Module7					KS-AT-DH-KR-ACP-ACP
ShaD	1,854	<i>RifD</i> , <i>A. mediterranei</i> U32	77/68	YP003762842	PKS domains
Module8					KS-AT-DH-KR-ACP
ShaE	3,591	<i>RifE</i> , <i>A. mediterranei</i> U32	78/70	YP003762843	PKS domains
Module9					KS-AT-DH-KR-ACP
Module10					KS-AT-DH-KR-ACP
ShaF	259	<i>RifF</i> , <i>A. mediterranei</i> U32	75/64	YP003762844	N-acetyltransferase/amide synthase
Orf1	447	<i>Nat1</i> , <i>Streptomyces</i> sp. CS	88/78	ADM46362	halogenase
ShaG	356	<i>RifG</i> , <i>A. mediterranei</i> U32	84/78	YP003762846	aminodehydroquinase synthase
ShaH	445	<i>RifH</i> , <i>A. mediterranei</i> U32	72/64	YP003762847	aminoDAHP synthase
ShaI	270	<i>RifI</i> , <i>A. mediterranei</i> U32	77/70	YP003762848	aminoquinone dehydrogenase
ShaK	386	<i>RifK</i> , <i>A. mediterranei</i> U32	87/78	YP003762849	AHBA synthase
ShaL	362	<i>RifL</i> , <i>A. mediterranei</i> U32	76/69	YP003762850	Oxidoreductase
ShaM	209	<i>RifM</i> , <i>A. mediterranei</i> U32	87/80	YP003762851	phosphoglycolate phosphatase
ShaN	300	<i>RifN</i> , <i>A. mediterranei</i> U32	75/65	YP003762852	kanosamine kinase
Orf2	553	<i>mhpA</i> , <i>A. mediterranei</i> U32	75/68	YP003762868	FAD-dependent oxidoreductase
Orf3	64	<i>Fer</i> , <i>A. mediterranei</i> U32	72/61	YP003763095	Ferredoxin
Orf4	54	<i>ST1928_p132</i> , <i>S. rochei</i>	80/73	NP-851508	probable thioesterase
ShaR	244	<i>RifR</i> , <i>A. mediterranei</i> U32	81/66	YP003762870	Thioesterase
Orf5	445	<i>Nat4</i> , <i>Streptomyces</i> sp. CS	79/66	ADM46372	membrane ion antiporter
Orf6	280	<i>SACTE_0864</i> , <i>Streptomyces</i> sp. SirexAA-E	87/81	YP004801333	metallophosphoesterase
Orf7	438	<i>AMED_0655</i> , <i>A. mediterranei</i> U32	55/44	YP003762877	transcriptional regulator
ShaJ	144	<i>RifJ</i> , <i>A. mediterranei</i> U32	92/84	YP003762876	aminoDHQ dehydratase

Table S2. Cont.

Orf8	80	<i>SclaA2</i> , <i>S. clavuligerus</i> ATCC 27064	85/64	ZP08220974	DNA primase/helicase
Orf9	305	<i>Strvi_6475</i> , <i>S. violaceusniger</i> Tu 4113	93/91	YP004816175	resolvase domain-containing protein
Orf10	166	<i>Terracoccus</i> sp. 273MFTsu3.1	72/62	WP020141764	hypothetical protein
Orf11	417	<i>H340_20018</i> , <i>S. mobaraensis</i> NBRC 13819	85/79	ZP23080866	oxidoreductase
Orf12	198	<i>SPW_4890</i> , <i>Streptomyces</i> sp. W007	92/84	WP007454088	putative transcriptional regulator
Orf13	487	<i>Francci3_2757</i> , <i>Frankia</i> sp. CcI3	70/58	YP481846	EmrB/QacA family drug resistance transporter
Orf14	210	<i>SPW_4891</i> , <i>Streptomyces</i> sp. W007	84/71	WP007454090	putative methyltransferase
Orf15	221	<i>SteO22.29c</i> , <i>S. tenjimariensis</i>	90/85	CAI59998	putative transposase
Orf16	204	<i>SteO22.29c</i> , <i>S. tenjimariensis</i>	86/83	CAI59998	putative transposase

Figure S1. Overlapping fosmids that cover the whole enduracidin biosynthetic cluster in *S. atrovirens* MGR140. The positions of the five sets of primers (P1–P5) used to located the enduracidin biosynthetic cluster from *S. atrovirens* MGR140 are labeled.

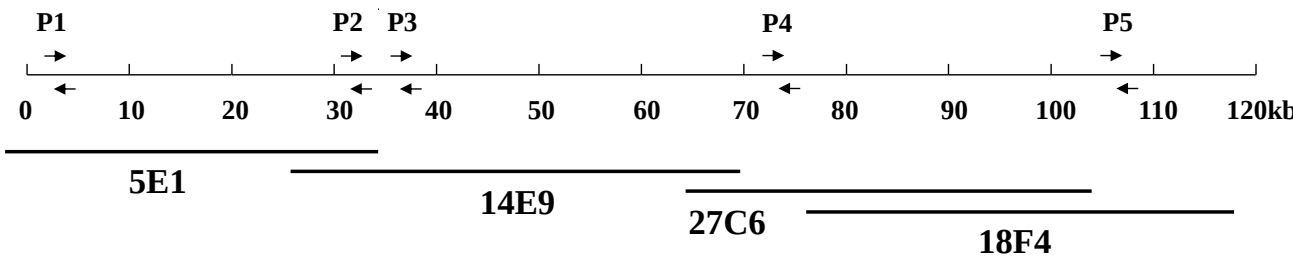


Figure S2. Overlapping fosmids that cover the whole putative halogenated ansamycin biosynthetic cluster in *S. albogriseolus* MGR072.

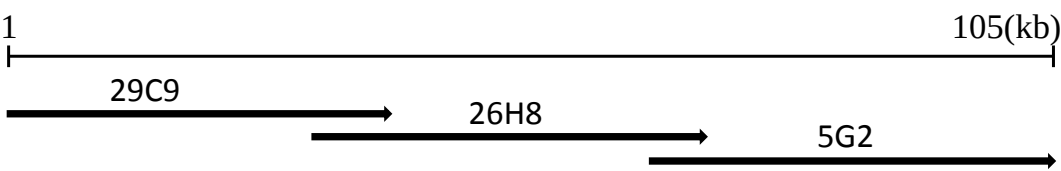


Figure S3. Phylogenetic tree constructed using 16S rRNA of halogenase positive antagonistic strains. Tree topography and evolutionary distance are given by a neighbor-joining method with 1,000 replicates of bootstrapping. Bootstrap values providing $\geq 50\%$ support are indicated. The scale bar indicates 0.01 substitutions per nucleotide position. The dashed rectangle indicated the clade of putative enduracidin producer.

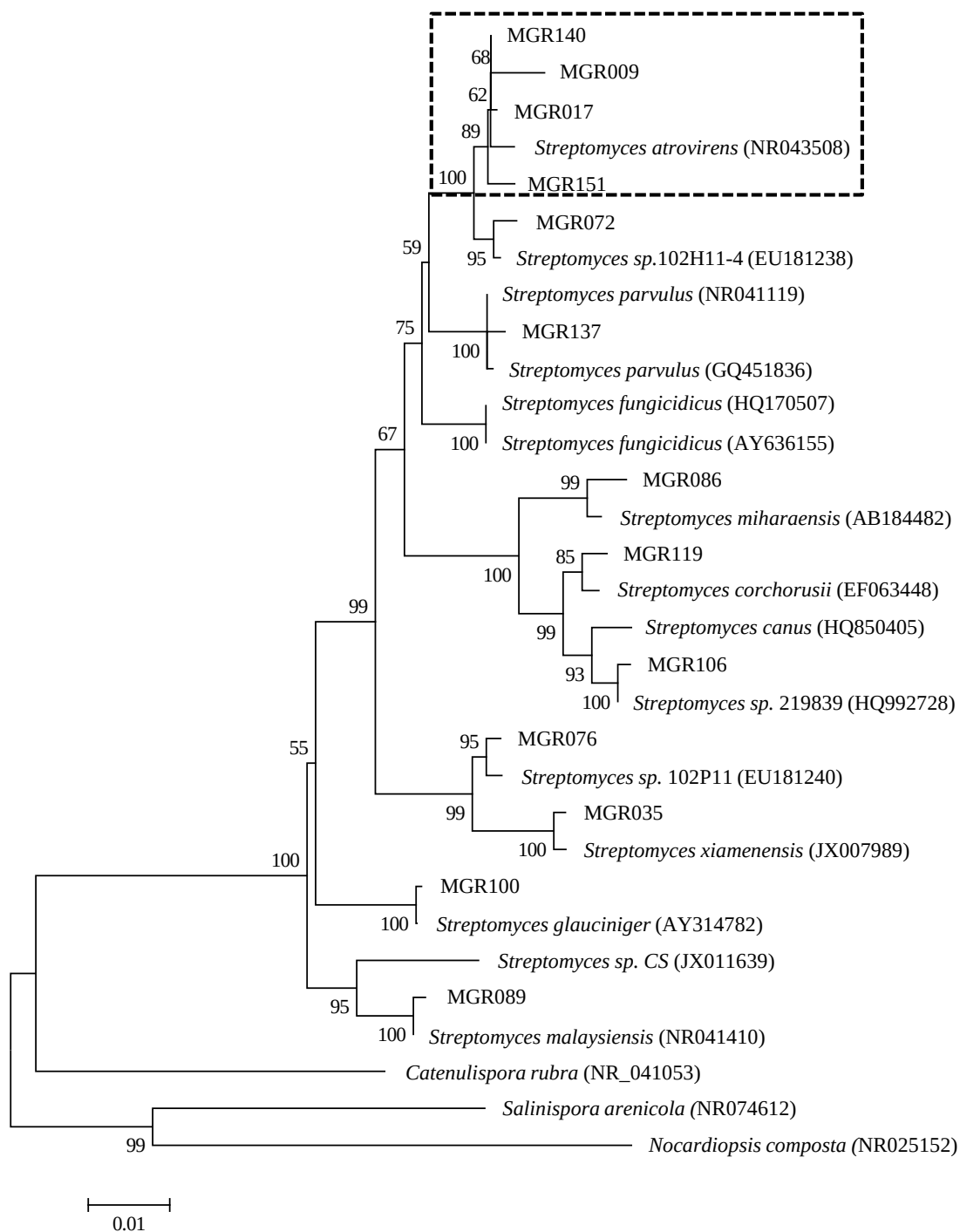


Figure S4. Alignment of *orf1*, *nat1* and *asm12*. *Orf1*, halogenase of shamycin biosynthesis from *S. albogriseolus* MGR072; *nat1*, halogenase of naphthomycin biosynthesis from *Streptomyces* sp. CS; *asm12*, halogenase of ansamitocin biosynthesis from *Actinosynnema pretiosum* ATCC31565; motif I, GxGxxG; motif II, WxWxIP.

Motif I																		
orf1	MAKETADVVI	GGGPAGSVCA	YVLAKQGH	SVVLL	LEKELSS	RFHIGESL	LPYMMGL	FERIG	60									
nat1	MSDNSTDVVI	GGGPAGALSAYQ	LAKQGH	SVVLL	LEREEFP	RFHIGESL	LPYMMGL	FDRIG	60									
asm12	...MLDAIVM	GGGPAGSVCA	AVLARQGR	SVLV	LERQEFP	RFHIGESM	LPYMVGL	LERHG	56									
Consensus	d v	gggpag	a la	qg sv	le	rfhiges	lpym gl	r g										
orf1	LRETVA	QGYVPK	EGGEFIDP	TEKKFF	EG.	VFRADFTK	QGVGRH	NAFQVERAK	FDRMLA	119								
nat1	IREAVETK	QGYVPK	EGGEFIDP	NEEKFF	SD.	VFRADFSK	QGDGRY	DRAFQVERAK	FDGMLV	119								
asm12	LLDAVRE	QGYVVK	RGGEFIDP	TGTKFF	RAGV	FRADFAKT	GDGRHH	ETQOVERSH	FDRVNL	116								
Consensus	v	gyv k	ggefidi	p kff	vfradf	k g gr	fqver	fd										
orf1	EQAEAAGAR	VLF	G	ANVN	ELMD	GDRM	VG	VRYERD	G	ESHEVSS	AYVVDAS	GRSGRI	ANRFG	179				
nat1	EEAGKAGAE	VHL	G	ATV	GELLME	GDRM	VG	VRYTKD	G	QEHEVRS	RYVVDAS	GRAGR	VAHKFG	179				
asm12	DQARAAGAT	VREGA	Q	VVGL	LEE	EGGR	V	GVRYREGG	V	EREERAR	YVVDAT	GRAGV	VANRFG	176				
Consensus	a	aga v	ga v	ll	g r	vgvry	g	e	yvvd	gr g	a	fg						
Motif II																		
orf1	LRKTLEK	LRMVAV	FRHYS	GLDERH	NPG	VEGDIQ	VGAH	DDG	WVAI	PLTK	DTIS	VGTV	MPR	239				
nat1	LRERLEK	LRMVAV	FRHYE	GLDEK	HN	LGVEG	DIQV	GAH	ADG	WVAI	PLSK	DVIS	VGTV	MPR	239			
asm12	LRMIED	LRMVAV	FHRD	GLDEA	HN	PGHEG	DIQV	GSH	SDG	WVAI	PLSADR	IS	VGTV	MHR	236			
Consensus	lr	e lrmvav	f h	glde	hn	g egdiq	vg h	dg	w	wai	pl	d is	vgtv	r				
orf1	DVLRGATT	QERF	DEHLAR	VPRIVAR	LTGTR	PSMDL	KVETD	YCYHT	DTVT	TGPGW	LMVGD	AG	299					
nat1	DVFRAST	PEEV	FEEH	KARIPR	IVARLT	DTRPT	MDLK	VETD	YCYHS	DTVT	TGPGW	VMVGD	AG	299				
asm12	DRLRGRT	PAEAF	AEHVER	VPRINQ	RLTGT	SATS	DFW	VETD	YSYHS	DQVT	TGPGW	VMVGD	AG	296				
Consensus	d	r t	e f eh	r pri	rlt t	d	vetdy	yh	d	vtgpgw	mv	gdag						
orf1	CFGDPMF	SGGVLV	ATATA	VRAAET	TLGEV	LADP	SAEDR	LLERY	S	SNYF	KTG	YD	TYIR	LIHSY	359			
nat1	CFGDPMF	SGGVLV	ASATA	VRAAET	TLSDA	LNNP	EDADR	LIDEY	ANF	KTG	YD	TYIR	LIHAF	359				
asm12	CFGDPMF	SGGVLV	GMATG	AEAAE	ALGRAL	DS	PADEE	QALTG	YSN	F	KTG	YD	TYVRL	LIFS	356			
Consensus	c	fgdpmf	sggvlv	at	aae l	l p	y n	fktgy	dy	rli								
orf1	YDGELV	AMAADA	ARST...	DRE	TL	E	KYLM	R	LI	GGDF	WSEH	NSVA	QEMRR	RKEW	DTFEP	FQ	416	
nat1	YDGELV	AVAADA	AGR	TT...	DRE	TL	E	RYL	R	LI	GGDF	WSEH	NAVA	QEMRR	TEW	DTFSP	FQ	416
asm12	YEGELL	PALAE	AHSE	AGNL	SEAD	MEMY	VVRL	LI	GGDF	W	SAR	NP	ANAL	RANPA	WSTFS	PFE	416	
Consensus	y	gel	a a	e y	rl	ggdf	w	s	n	va	r	w	t	f	p	f		
orf1	R	VYGCPS	SYPHL	DELDR	KERSE	ARV	RRVTAG	446										
nat1	R	VFGCPS	SYPHL	DEQDR	KERA	ESR	VRRVTQG	446										
asm12	P	VHRC	PVYPE	LDVAEL	GGLAG	TVGR		441										
Consensus	v	cp	yp	ld	r													

Figure S5. Alignment of *orf2*, *mhpA* and *nat2*. *orf2*, oxidoreductase of shamycin biosynthesis from *S. albogriseolus* MGR072; *mhpA*, oxidoreductase of rifamycin biosynthesis from *A. mediterranei* U32; *nat2*, oxidoreductase of naphthomycin biosynthesis from *Streptomyces* sp. CS; motif I, GXGXXG; motif II, DGXXSXXR; motif III, GDXXH.

			Motif I	
orf2	MSTTRPHDP.....YESPTDTG	DVAVVGNGPVGAALSVL	37	
mphA	MTDT.....DVVIVGNGPIGATLSVL		21	
nat2	MSPGRTLTRSHPTRAREPGRTEGVSMVSVSPDDAMTSTSSPEADVLVVGYPISALAVL		60	
Consensus		t t dv vg gp g l vl		
orf2	LAQRGLRVTVLERRRPYLLPRATSFDFGETARLLAATGVGDRFGEITEPATGYQWHTAAG		97	
mphA	LAQRGWRVTVLERRRPYKLPATSFDFGETARLLAATGIGPDLGRITAPANGYQWQTADG		81	
nat2	LARRGWRVTVLERRRPYTLPRATSFDFGETARLLAGTGVGGELGRITEPGTGYQWRTADG		120	
Consensus	la rg rvtvlerr rpy lpratsfdgetarlla tg g	g it p gyqw ta g		
orf2	ETLLDIAFSPTGRYGWPDANTMHQPALELLADRAAALPGVTVLRGRRVVDLAERDDRV		157	
mphA	QTLDDIAFTTDGPYGWPDANTMHQPALEELLAARAADLPGITVLRGHEVVAITDGDSFVQ		141	
nat2	KTLDDIEETTEGPYGWPDANTMHQPALEELLAARAGELRDITVVRDRRVVDIADGPAGVT		180	
Consensus	utllldi f g ygwpdantmhqpale lla ra l	tv r vv v		
			Motif II	
orf2	LTVETDDGAARTVVAHVVGCDGANSFVREHLGVPMTDLGFSYEWLLCDVELREPREFTP		217	
mphA	VTATGEDDVPRTISGRVVGCDGANSFVREHLDPVPTDLGFSYEWLLCDVRLNEPREFVP		201	
nat2	VTAEDEGATRTFSARVVGCDGANSFVDRMGVSVTDLGFSYEWLLCDVELREPRAFVP		240	
Consensus	ut rt wvvgcdgansfvr	v tdlgfsyewllcdv l epr f p		
orf2	TNVQICDPARPTTLVGSGPGHRRWEFMRPLPGESEAEINRPEETAWRLKPFVGPETATLL		277	
mphA	TNVQICDPARPTTLVGSGPGRRRWEFMRPLPGENAAELNKDETAWRLMAPFGVGPETATLL		261	
nat2	TNVQLCDEVRPTTLVGSGPGHRRWEFMRPLPGERAADLNREETAWRLAPFGVGPETATLL		300	
Consensus	tnvq cdp rpttlvgsgpg rrwefmrplpge a ln	etawrl pfgvtp ta ll		
			Motif III	
orf2	RSTTYIFRAAWADEWRSGRVLLAGDAAHLMPPFAGQGMCSGIRDAANLAWKLDLTLRGLA		337	
mphA	RSTTYIFQARWADRWVGHVLLAGDAAHLMPPFAGQGMCSGIRDVTNLAWKLDLTLRGLA		321	
nat2	RSTTYIFQARWADRWLGHMLLAGDAAHLMPPFAGQGMCSGVRDVANLAWRLDLVLRGTA		360	
Consensus	rsttyif a wad wr g llagdaahlmppfagqgmcs g rd	nlaw ld l lrg a		
orf2	DASLLDITYAEERRQHVKESILSSVQLGRVICVTDPAAAAERDATVLAGRRGRTGPGAFDA		397	
mphA	PESLLDSYGDERREQAREAILASVQLGRVICVTDPAAAAERDSTVLANRRGKP.AGRPEP		380	
nat2	PESLLDITYTEERKAQVRESILASVQLGRVICVTDPAAAAERDATVLANRRGTG...REEP		417	
Consensus	sllld y er e il svqlgr icvtdpaaaaerd tvla rrg	p		
orf2	AKPLSAGLLRAPACAPRA....PAGEVVPQWRVSTAGGTGLFDAVVGGFVLLTTDDPRP		453	
mphA	AKPLTGGLRHESPG.....AGVVPVQGRVQVGDTGLFDDVIGRGFVLLTTEETHS		431	
nat2	ARPITCGILLHRPEGAAADTRVPPAGEVLPHPARLLRPSGGEPLEDPVGRGFVLLTASEAEP		477	
Consensus	asp gl g ag v p r	g gfvll t		
orf2	ALGEDRWSFLAALDTRVVRLLSPPGTVLPPEDTEP.....GLTDVIDTDGIFYRAYLS		504	
mphA	DFLTELGAHVVRLLDEG.....VDVDDVYRPELA		459	
nat2	DLTPERLSFLREVGARVVRLWPDGTSHGATDGTGDTGDTGDTGDLVDAVDAGVLRGFLG		537	
Consensus	fl vvrl	d d r l		
orf2	EHDASALMVRPDHVFVGAARGAEDTAALVDCRLDRRLATAVEPAGGPHRR		552	
mphA	RFGAASVLRPDYHVFGTG.GPGGLEALVGALRDLRLAAVPTG.....		501	
nat2	RYGTALLVRPDYHVFGAADPAGVAALVDDLRLRLTAPVAPAGRAD		585	
Consensus	a vrpdp hvfg a alv lr rl vp			