

Figure S1. Heat map of the average amino acids identity (%) of proteins homologous to Re78 (REMIM1_PF00478) and Re79 (REMIM1_PF00479) of *Rhizobium etli* Mim1. The alignments were performed using ClustalW, version 1.2.4. of UniprotKB webserver.

(a)	R. etli Mim1 Re78 (AGS26143.1)	MIVSAATERL NDILVELPKY KSQLWEANVSS PYVRA YD LAI DNFQKTTKAQ	50
	Neorhizobium (CDN51818.1)	MIVASANQL GDILVKLPKY KSQLWELNVAS PYVRA YD AAV LSYNNEMKAQ	50
	Sinorhizobium (APG87637.1)	MIVSSATDRL TDILVELPKY KSQLWQSNVAN PYVRA YD LA VENFQRTLKAQ	50
	R. phaseoli (ANM08143.1)	--MSAATERL NDILVELPKY KSQLWEANVSS PYVRA YD LAI DNFQKTTKAQ	48
	Agrobacterium (WP_027675286.1)	--MSLATDRL NNILIELPKY KSQLWQLNVAS PYVRA YD LA DNYNKKMQEQ	48
	R. ruizarguesonis (NKQ86457.1)	--MSAATDRL NDILIELPKY KSQLWETNVAN PYVRA YD LA DNYQKTTKAQ	48
	R. oryziradicis (WP_075641707)	--MSSAKDRL TSIMDELDKK KSQLWEVSVVS PYVRSYDFAY NNYQNTLKAQ	48
	Rhizobium (WP_062451316)	--MSLAKERL NNILLELPKY ESQLWESNIVL PYSRA YD LAI YNLNKTKEQ	48
	Sphingomonas (WP_156477649)	----MADRL QTYRNQVSDA VDAWQANVCN PYIIAYNTAY KSYADVFKKQ	45
	Azospirillum (WP_089286101)	-----MSDII RNYLDELEAK AIRWRIFCR- DYASAYHIAH DQYKATWAAQ	44
	R. etli Mim1 Re78 (AGS26143.1)	EARD RM LAEL FVFSASILTG SIMMAAFATT SVRVLAGRAA LSVICNNNLN	100
	Neorhizobium (CDN51818.1)	GERDRATAEM FVLAASILTG SVMMAAFATT SVRVMAGRAI VRVICNNNLN	100
	Sinorhizobium (APG87637.1)	EARDKMTAEL FVFAASILTG SLVMAAFATA SLRVLAGRAA LRVICCNNLN	100
	R. phaseoli (ANM08143.1)	EARDRMLAEL FVFSASILTG SIMMAAFATT SVRVLAGRAA LSVICNNNLN	98
	Agrobacterium (WP_027675286.1)	AARDKMAEL FVMSASILTG SVMMAVFATT SLRVLAGRAA LRVICCNQLN	98
	R. ruizarguesonis (NKQ86457.1)	EARDRMLAEL FVFSASVLTG SIMMAAFATT SVRVLAGRTA LRVICNNNLN	98
	R. oryziradicis (WP_075641707)	VARDKMGAEI LVFTAGVLSG SVMMAFASS SLRVLAGRAL LRTICCNNLN	98
	Rhizobium (WP_062451316)	EARDKLKAEI FVMSASILTG SVMMAAFATT SLRVLAGRAA LHVICNNNLN	98
	Sphingomonas (WP_156477649)	DDSKDKAEL FVTAAILPVG SILMATAAT SVLRVIANRTA VRLLAGRSTS	95
	Azospirillum (WP_089286101)	EKS D KEKEM IYLALSVASS TVLMAAFATN SLRVLASRAA LNVICNNNLN	94
	R. etli Mim1 Re78 (AGS26143.1)	RTFNVMHAAA SNKVVM F ALG GVLDEAKKLA NKQVTSAAEK LTSSAPVASA	150
	Neorhizobium (CDN51818.1)	RTFNLVMAAN NNKAFM F ALG GVLDEAKKVA NKQVTKLVEG LTSTGTQISA	150
	Sinorhizobium (APG87637.1)	RTFDLMHAAA SNKAVM F ALG GILDEAKKFA GKQITSSAAEK LTSSAPIASS	150
	R. phaseoli (ANM08143.1)	RTFNVMHAAA SNKVVM F ALG GVLDEAKKLA NKQVTSAAEK LTSSAPVAAA	148
	Agrobacterium (WP_027675286.1)	RTTFNFAHVS ESKVLM F ALG GVLDEVKLA GKHTAAVEG LTSSDAIASA	148
	R. ruizarguesonis (NKQ86457.1)	RTFDLMHAAA TSKPVM F ALG GVLDEAKKLA NKQVTSAAEK LTSSAPIASA	148
	R. oryziradicis (WP_075641707)	RTFDAVHAVT NNAKAM F ALG SVLDKAKGIA GHITA A VEN FTVRSGIQS	148
	Rhizobium (WP_062451316)	RTFEAMRAAS ANKALM F ALG SVLDEAKKLA GKQVTSAVEG LVSSASIASA	148
	Sphingomonas (WP_156477649)	RALGVYNAVA SNATAT F AI G KALDIVKDET GKA I KDSVVK TMSNTRDLIA	145
	Azospirillum (WP_089286101)	RTFNLALHLSV NNKAAM F ALG AVMDAADAKA KKYIEEKAGT LTRSA P PAV	144
	R. etli Mim1 Re78 (AGS26143.1)	PTALNFKTRI DDFIAINHVC VHEFIRGVQQ DNTISEAGKS RLADLVGQTP	200
	Neorhizobium (CDN51818.1)	PTA N FM H VSRL MDFIRVSHC THSFIEGVKE DNSISETHKA QVADMAARIP	200
	Sinorhizobium (APG87637.1)	PTAINFLTRI EDFINVNHIC VHEFIAGVR DRAISETDKL KVADLVGKTP	200
	R. phaseoli (ANM08143.1)	PTALNFKTRI DDFIAINHVC VHD F VRGVQH DNTISEAGKS RLADLVGQTP	198
	Agrobacterium (WP_027675286.1)	PTALNFKTRM EDFINVNHIC VHNFVQGVRD DASISDANKT HIAELVGKTP	198
	R. ruizarguesonis (NKQ86457.1)	PTALNFMTRI ADFIDTNHIC VHFDFVRGVQQ DNSISEANKS RLADLVGKTP	198
	R. oryziradicis (WP_075641707)	QT S FNFLTRL QDFVNVSFIA VHEFTGVGRN DNSIKESDKL KLADMVEATP	198
	Rhizobium (WP_062451316)	PTGLNFESRM KTFVNDNHIC VH S FVQGVRD DGSLKDDDKM HIAELATKMP	198
	Sphingomonas (WP_156477649)	TDPLNRDQKL NSWLINHKLL AYADA A IER SRSMSERDKQ RAYDLLRAAP	195
	Azospirillum (WP_089286101)	TNP A VLQNDL INYVESNQLY AMEVAFAIRD DKSLSEADRA ILVGS L KRAP	194
	R. etli Mim1 Re78 (AGS26143.1)	FCNP P ESRRV DENRLSQKME LLFYMSAVALD SD K L V TYAPS TGGTVGGVGR	250
	Neorhizobium (CDN51818.1)	FCNPPSGSSI DEPKLANKM E LLFYMSSVLD SD R L V TYAPA TGGSF G MGQ	250
	Sinorhizobium (APG87637.1)	FCNP P AGRRV DENRLSQKME LLFYMSAVALD SD R L V T F V P TGGSVPGTAR	250
	R. phaseoli (ANM08143.1)	FCNP P ESRRV DENRLSQKME LLFYMSAVALD SD K L V TYAPS TGGTVGGIGR	248
	Agrobacterium (WP_027675286.1)	FCNP P EARRV DENRLSQKME LLFYMDA V LE SD K L V KFASV TSSTMILL-Q	247
	R. ruizarguesonis (NKQ86457.1)	FCNP P ESRRV DENRLSRKME LLFYMSAVALD SD K L V TYVPA TGGTVGGMGR	248
	R. oryziradicis (WP_075641707)	FWL L PRANRV DENKLAQ O ME LLFYMTSVDL SD T L V RHAPS IGN G IGGGIG	248
	Rhizobium (WP_062451316)	FCN P QSRRI DENRLSQKME LLFYMDA V LE SD K L V TYAPA SGERAMG--R	246
	Sphingomonas (WP_156477649)	IANKPN G -KI DP A R L SPK I E LGFYMLW L D S DELVTQ T AP AGPYGGGG--	242
	Azospirillum (WP_089286101)	LFKPPVN-EI KRERLTDHIE LSLHMSAMLD SD F L V DIPMH EMSTAGGLGS	243
	R. etli Mim1 Re78 (AGS26143.1)	DIEYSKKAIP QLPSAT-D Y KSIS P RLTGR PFQPYDPGQR IEYDNIGSVV	299
	Neorhizobium (CDN51818.1)	DVEYSRK S IS QMPSNTGH Y KAASP K FVGR PFV P YDPGQR IEYENIG S GI	300
	Sinorhizobium (APG87637.1)	ELVLARK K IP QMPSAA-D Y RETAPRLTGK LLQPFP E PGTR IEYSNIGSAV	299
	R. phaseoli (ANM08143.1)	DIEYGKKAIP QLPSAT-D Y KSIS P RLTGR PFQPYDPGQR IEYDNIGSVV	297
	Agrobacterium (WP_027675286.1)	DVEFSRTPIA QLPSAT-D Y REIGPQSGGI PITSKNIGQR VKYENLGSQI	296
	R. ruizarguesonis (NKQ86457.1)	DIEYSRK A IP QLPSAT-D Y KATS P RFIGR PLQFFEPGQR VEYN N GS A V	297
	R. oryziradicis (WP_075641707)	EGIRS K ERIN QMPTAK-D Y KESEP K IIRG --ATYEPGOR IEYDDLG S VV	295
	Rhizobium (WP_062451316)	DVEYGS K PIS QMPSAA-N Y KAIAPKF T GR PFSPYDPGQR VEYN N GS A V	295
	Sphingomonas (WP_156477649)	--RYSS K PI D ALPSS K -D Y R-----GN TDPKRGP I QW VGVT R GDV	282
	Azospirillum (WP_089286101)	VFGAKER P IP QL S AS-D Y RGTPF R --T SLGVVHAQQS VRVERPG P IV	289
	R. etli Mim1 Re78 (AGS26143.1)	RERINTLSRL TGNSSFYPEQ NS-VEKLFID PTSGAQMVKA EQIINRISAD	348
	Neorhizobium (CDN51818.1)	RDRINKL S IS TGNGP F PYNQ SF-VERKLFD PTGHAQMVK A EQIINKLYND	349
	Sinorhizobium (APG87637.1)	RERIDTLSRQ YGGSP F PYQQ NF-AEK K IID PTG A QMIRA EQIINR L SAD	348
	R. phaseoli (ANM08143.1)	RERINTLSRL TGNSSFYPEQ NS-LEKLFID PTSGAQMAKA EQIINR L SAD	346
	Agrobacterium (WP_027675286.1)	RQRIDTLSKL TGNSSFYPEQ SA-VER-FFD PTGNAQLLKA EQIINR L SIE	344
	R. ruizarguesonis (NKQ86457.1)	RDRINTLSRL SGNSSFYPEQ NF-AEK K IID PTGSTQLAKA EQIINR L STE	346
	R. oryziradicis (WP_075641707)	RARI D TL S RA VTGSP F PYQQ SIGMRIAGND PTGREQMVKA EHII S RLSAQ	345
	Rhizobium (WP_062451316)	RERIDMLSRM TGNSSFYPEQ NF-AERLLID PTGNAQMVK A EQIINR L SAS	344
	Sphingomonas (WP_156477649)	EDQIDKVHQ VRGSAFY A PG GW-FGKSDVN QARLKEV A ERVLA W SDK	331
	Azospirillum (WP_089286101)	QTKIDE N GR IFNEKFFSAS SWQGGQN G PM P---ALVRA ETTLNRLAEEA	335
	R. etli Mim1 Re78 (AGS26143.1)	ARP K LTD V R MI 360	
	Neorhizobium (CDN51818.1)	ARP R QLTD V R MI 361	
	Sinorhizobium (APG87637.1)	ARP K QLV D VR MI 360	
	R. phaseoli (ANM08143.1)	ARP K QLTD V R MI 358	
	Agrobacterium (WP_027675286.1)	ARP K ELTD V K VI 356	
	R. ruizarguesonis (NKQ86457.1)	ARP K QLTD V R MI 358	
	R. oryziradicis (WP_075641707)	TRPKQLSDVF MM 357	
	Rhizobium (WP_062451316)	ARP K DITD V R MM 356	
	Sphingomonas (WP_156477649)	TOP A PLGL R T- 342	
	Azospirillum (WP_089286101)	SRPLQLDAAR V- 346	

(b)	<i>R. etli</i> Mim1 Re79 (AGS26144.1)	--MLLTGLIK QGFLGMRWP LAAVTIVGVM AASAAAMVGA SGSTDQSLLQ IEAVSSRLSA 58
	<i>Neorhizobium</i> (CDN51817.1)	-MKLISFFSP SVRTGPILRQ SRNVAVAFAM AWAPAFAAA SDEIDETLLQ IERVGPRLSA 59
	<i>Sinorhizobium</i> (APG87638.1)	-----MIAGT SNPIDQSLVQ IEQVNRLSG 25
	<i>R. phaseoli</i> (ANM08142.1)	--MLLTDFLK RGLMGMRWRV LVAAGFTGAM VASAAMVGA SGSTDQSLLIE IEAVSSRLSA 58
	<i>Agrobacterium</i> (WP_027675287.1)	MLFNDFTSV KKICLLKLC A FACLVCVILL TASHVSADQA NNTIDSVVIR AERLDQRLLAD 60
	<i>R. ruizarguesonis</i> (NKQ86458.1)	-----MGMRWP LIAALVVCAM AAAAGMVGA SDTDQSLLQ IEQVASRLSG 47
	<i>R. oryziradicis</i> (WP_175577311.1)	-----MIRGLKHS IRGGLCLGM GMLSPVAAAC DPSIDASLVQ IEQVGERVAN 48
	<i>Rhizobium</i> (WP_062451254.1)	MMLLVDTVAV LNRVRKVTFI IAAPVLAILM TGCDAGAESS KSSVDASLLR AERVDERLAL 60
	<i>Azospirillum</i> (WP_143270660.1)	-----M RGQELSSLW SGLLVLVTL LLAGCSRSGE AEKSDETMQQ MEQVASLYQS 51
	<i>Aureimonas</i> (WP_183201854.1)	-----MRWT AAALALVGVL AAIPLGRAMA DPAGDERYLR LEDAERVYAG 44
	 <i>R. etli</i> Mim1 Re79 (AGS26144.1)	
	<i>Neorhizobium</i> (CDN51817.1)	TPAAVEVTAK AAADRSVPV -DAQPLLETA ARSSFDADGM LAEISKAVAE AGSDA-VDPR 116
	<i>Sinorhizobium</i> (APG87638.1)	MPSEVENVVK AATDRGNDEA SKAGPILEPV VRASFPKGM VAEISHSLAE VEGGK-IDPA 118
	<i>R. phaseoli</i> (ANM08142.1)	APSAVTKSVK ALAENGNAEV PAAEALLETA VHSSFDSESN GAAILKNIGE VDGGS-IDPE 84
	<i>Agrobacterium</i> (WP_027675287.1)	TPGAVGSVK AAADRSVPAS -DAQPLLETA ARSSFDADGM FAEISKAVAE AGSDA-VDPR 116
	<i>R. ruizarguesonis</i> (NKQ86458.1)	INAMVENVSK IEAAGTKLKV AKTPKPLMLKA ISQKF DLLQK KRTIVAAIEQ PDNDY-QNFK 119
	<i>R. oryziradicis</i> (WP_175577311.1)	TPAAVENVK AAADRGISAT SDAQPLLETA ARSSFKVDSM FAEISKAVGE AGDDA-VDPR 106
	<i>Rhizobium</i> (WP_062451254.1)	AGLLADNVLK YISDQEGLHD QKADSMIESA TRDAFNAQAF VTEIQNSMQN VGNSS-IDAQ 107
	<i>Azospirillum</i> (WP_143270660.1)	PQALIRNAAS ARRDDKNADI AKTEPLLISS SEKAFDAGEM RKKLVSIEA TPGQY-ADVA 119
	<i>Aureimonas</i> (WP_183201854.1)	MPGLVDIVGG DVKEFGAGV DADLPLLRQS VVKAFDPADL LARVRRELKA VDAGGGAEVP 111
	 <i>R. etli</i> Mim1 Re79 (AGS26144.1)	MAEFARMQGP LIEVSDRREL PALAERTGL AAQTFAVAGA RDAMLERLRR TPGEV---PV 101
	 <i>R. etli</i> Mim1 Re79 (AGS26144.1)	AFAKAAAAWH EGQAKVARMY QAQDQVAKE IETRLLADPK GPGIKALADL MAGPDLAVES 176
	<i>Neorhizobium</i> (CDN51817.1)	TLAKAAAEEF DGRKKIAEY RSQDQIAKQ IEARIAGADD GPRIKQLADL MASPELAET 178
	<i>Sinorhizobium</i> (APG87638.1)	AFARAAAAFE EGRGKIAIRY EAQDQTAKE EARLADPET GARLRQLTDL MAAPELAVES 144
	<i>R. phaseoli</i> (ANM08142.1)	AFAKAAAAWH EGQAKVARMY QAQDQVAKE IETRLLADPK GPGIKALADL MAGPDLAVES 176
	<i>Agrobacterium</i> (WP_027675287.1)	ALDKAVSALI VSYAQIDKTY TDKEVAGKS IQARLGNPKT RETVNDVANL MAGPKLAADA 179
	<i>R. ruizarguesonis</i> (NKQ86458.1)	AFAKVAAALE AGQAKIARMY EAQDQTAKEAD IEMRLANPDN GPRISQLADL MAGPDLAVDT 166
	<i>R. oryziradicis</i> (WP_175577311.1)	RALKIADDLN ASRKIETLIA QSKGDAALQT MDAEIVKKAD SPREQLAQQL MASPDLAET 167
	<i>Rhizobium</i> (WP_062451254.1)	AFDTATAALA DGYRAVDKMY ADKDEDGFKK IETRLLASPEA KSTIEEVASL MASPDLAET 179
	<i>Azospirillum</i> (WP_143270660.1)	AFLAANDFI RTQSAVEAES RVDPGKVIQS ATDAYEVRAD KG RIDALAGA MAAADIQVEG 171
	<i>Aureimonas</i> (WP_183201854.1)	AFDAIVARMR AVEAEPASRS LEALQAGEAD AVAHYEARPD RASIDAVGDA MAAPDLARET 161
	 <i>R. etli</i> Mim1 Re79 (AGS26144.1)	AVTWQTMYLL LEALSSADAE KLASVSDKL QSEMNGVIAS LRAR---SEN EKPLPKDMAR 233
	<i>Neorhizobium</i> (CDN51817.1)	ALTAQVMVFS LEAFSDPNSA ELASASSEKR KSELGGVIAS LRDR---TEN EKPVPRDIAR 235
	<i>Sinorhizobium</i> (APG87638.1)	AFSQLMYAA MEAFSDPSAA ELASSS---- EETPHVIAS LRSR---NEN EKPIAKDAAR 195
	<i>R. phaseoli</i> (ANM08142.1)	AVTWQTMYLS LEALSSADAE KLASVSDKL QSEMNGVIAS LRAR---SEN EKPLPKDMAR 233
	<i>Agrobacterium</i> (WP_027675287.1)	ASFQOAVGVA LKIISDPPGY ELNEEDQKKE LADLPNFLAN LRER---EVN QSAYSRVITE 236
	<i>R. ruizarguesonis</i> (NKQ86458.1)	AFTAQLMYLC LEALNSNDL TLASASKDKL QSEMVGVISS LRSK---SEN EKPVPKDIAR 223
	<i>R. oryziradicis</i> (WP_175577311.1)	AVTAQAMVVA VHAFNNSDPS QFAALSQEQR QMDMTOILSY LQQK---TEN EKPYSKDANR 224
	<i>Rhizobium</i> (WP_062451254.1)	AVTQQAISAV LTMETGSPAL DPSSEEHKQL KATLPSLLPE FRNR---PDQ GGAYSKESAK 236
	<i>Azospirillum</i> (WP_143270660.1)	TLLGLRLQGA AALRHLKEPV LWQGLDDQGR DRYADQFTRR TRAEG---DAN HSPPRYEIAR 229
	<i>Aureimonas</i> (WP_183201854.1)	AVTGLRVWKI YETLLTGNMID EMRAMTPALV ETGIREVLDL TRAPDDPEYG APPLRKEVLR 221
	 <i>R. etli</i> Mim1 Re79 (AGS26144.1)	ADEKARLTFV LATMPPEDLS VLTTEFYESSG GKA[KRDALVE SYRRVSDQAN KQMLGQYFQA 293
	<i>Neorhizobium</i> (CDN51817.1)	MEEKARLTFV LAALPKDDLS VLSDFYQSAB GKA[KRQALVE TYRQVSDQAN TRMLEKYFSA 295
	<i>Sinorhizobium</i> (APG87638.1)	LEEQQQLSFI LATLTSYEDLS VLLDFYRSSG GKT[KRQALVD SYVKVSDQAN TKMLQAYFSA 255
	<i>R. phaseoli</i> (ANM08142.1)	ADEKARLTFV LATMPPEDLS VLTTEFYESSG GKA[KRDALVE SYRRVSDQAN KQMLGQYFQA 293
	<i>Agrobacterium</i> (WP_027675287.1)	EYARNELSYA LATLSSDQLD TIKMFYSSPT GIAKTEALKK TYKDILSENT SIALDQYFRL 296
	<i>R. ruizarguesonis</i> (NKQ86458.1)	ANEKTRLTFV LATLSPEDPS VLTDFYESPQ GKA[KRDALIA SYRQASDQAN TEMLGQYFQA 283
	<i>R. oryziradicis</i> (WP_175577311.1)	ANEKLRLGLA LAGLSDGDL YLTNLQSPPE GKA[KRAALLD RYKRLSTDAN QKMLQDYFTQ 284
	<i>Rhizobium</i> (WP_062451254.1)	AFETAGLTFV LATLTDKDL SALKSFYAGSY GKSKRQELLG AQQQVWDAAA TTMLTTYIEE 296
	<i>Azospirillum</i> (WP_143270660.1)	FLAIGRLTII LLRLPEPSLK ALEDFYNSDL GKA[KRkalva AAVAQSDADT KQMMVEYLQ 289
	<i>Aureimonas</i> (WP_183201854.1)	EQARMVKRMV LSHLPEADVA ALAAFYASDV GKA[KRRALVE AFGARNDEDG ARFIRQLLER 281
	 <i>R. etli</i> Mim1 Re79 (AGS26144.1)	LAEYFKTHPR PQQQQ 308
	<i>Neorhizobium</i> (CDN51817.1)	LAGYFKTHPR PQQQ- 309
	<i>Sinorhizobium</i> (APG87638.1)	LANYLKTHPR PQQQ- 269
	<i>R. phaseoli</i> (ANM08142.1)	LAEYFKTHPR PQQQQ 308
	<i>Agrobacterium</i> (WP_027675287.1)	IFAEGVKN-- ----- 304
	<i>R. ruizarguesonis</i> (NKQ86458.1)	LADYLKTHPR PQQQQ 298
	<i>R. oryziradicis</i> (WP_175577311.1)	LADNLTKRKS NN--- 296
	<i>Rhizobium</i> (WP_062451254.1)	LAKQHPAAVK AQ--- 308
	<i>Azospirillum</i> (WP_143270660.1)	LR----- 291
	<i>Aureimonas</i> (WP_183201854.1)	ERSRTP---- ----- 287

Figure S2. Complete alignment of Re78 and Re79 with homologous proteins. (a) Re78 (ReMim1 AGS26143.1) alignment showed 39 identical amino acids (red). (b) Re79 (ReMim1 AGS26144.1) alignment presented 11 identical amino acid (red). The amino acid position of residues is indicated on the right side of the sequences. The NCBI ID is shown on the left of each sequence.

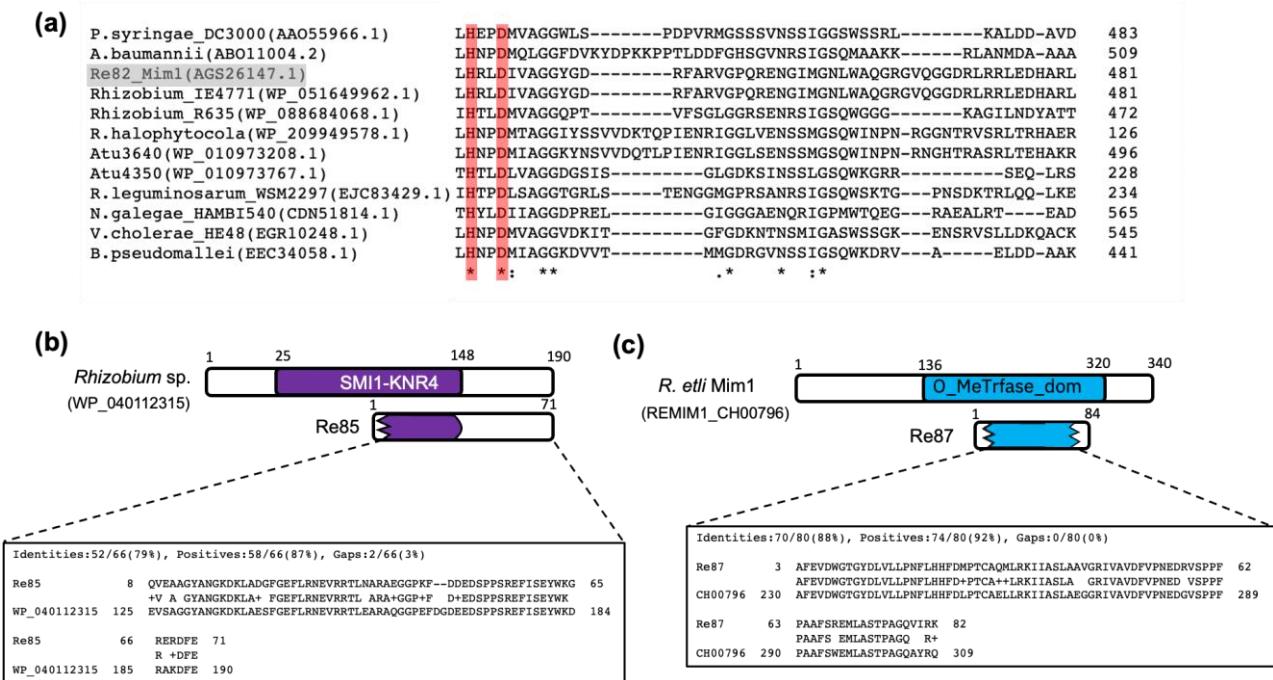


Figure S3. Relevant characteristics of the Re82, Re85 and Re87 proteins encoded in the T6SS cluster of ReMim1. (a) Partial sequence alignment of Re82 and members of a superfamily of T6SS-dependent DNase effectors. The HxxD catalytic motif is highlighted in red. Asterisks and dots indicate conserved amino acid and conservative changes, respectively. The amino acid position of residues shown is indicated on the right of the sequences. The NCBI ID are shown on the left of each sequence (b) Alignment of protein Re85 with a representative of a BLAST search, *Rhizobium* sp. (WP_040112315). SMI1-KNR4 domain is highlighted in purple. (c) Alignment of protein Re87 with a representative of a BLAST search, *Rhizobium etli* Mim1 (REMIM1_CH00796). O-methyltransferase domain is highlighted in blue. Organism names and protein accession numbers are on the left and the amino acid position in the alignments is indicated on each side of the sequences.

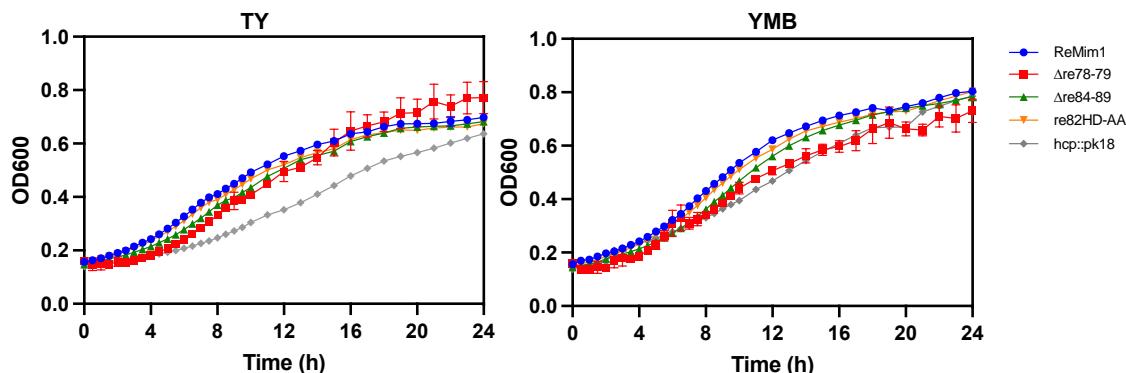


Figure S4. Growth curves of *Rhizobium etli* Mim1 strain and T6SS-derivative mutants in TY and YMB media at 28 °C.

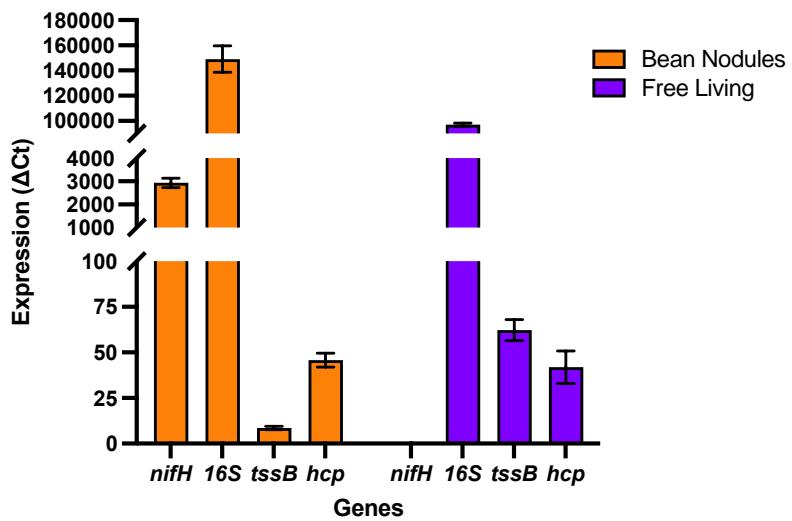


Figure S5. RT-qPCR analysis of T6SS genes *tssB*, *hcp* and *16S* rRNA gene (constitutive) and *nifH* gene (expressed in symbiotic condition). RNA from *tssB*, *hcp* and *16S* rRNA were analysed in free-living cells (TY medium) and RNA from *tssB*, *hcp* and *nifH* were analysed from bean nodules induced by *Rhizobium etli* Mim1, 28 dpi. Data were normalized using expression of *rpoD* gene. Bars values are average of two biological assays with three replicates.

Table S1. Bacterial strains and plasmids, related to Experimental Procedures.

Strain or Plasmid	Relevance Characteristics	Reference or Source
<i>Escherichia coli</i>		
DH5α	Host for DNA cloning	Gibco-BRL
BL21(DE3)	Host for protein expression	New England Biolabs
S17.1	Host for conjugation	[72]
HB101	Host for conjugation, triparental mating	[73]
<i>Rhizobium</i>		
<i>Rhizobium etil</i> Mim1 (ReMim1)	Wild-type strain able to induce nodules in <i>Phaseolus vulgaris</i> plants	[74]
hcp::pk18	ReMim1 <i>hcp</i> mutant, Km ^r	[21]
Δre78-79	ReMim1 <i>re78</i> and <i>re79</i> deletion mutant	This work
re82HD-AA	ReMim1 derivative by site-directed mutagenesis in amino acids H431 and D434 has been substituted for Alanines	This work
Δre84-89	ReMim1 derivative with <i>re84</i> to <i>re89</i> genes deleted	This work
Plasmids		
pCR2.1-TOPO	Vector for optimize cloning PCR products, Amp ^r , Km ^r	Invitrogen
TOPO.Re79StrepTag	pCR2.1-TOPO derivative containing <i>re79</i> gene	This work
pCR2.1-P6	pCR2.1-TOPO derivative containing P6 region (1242-bp) of T6SS from ReMim1	This work
pSCA	Vector for optimize cloning PCR products, Amp ^r , Km ^r	Agilent Technologies
pSCA-re82	pSCA derivative containing <i>re82</i> gene	This work
pSCA-HD→A	pSCA derivative containing <i>re82</i> H ₄₃₁ A	This work
pSCA-AD→A	pSCA derivative containing <i>re82</i> H ₄₃₁ A-D ₄₃₄ A	This work
pK18mobsacB (pK18)	Suicide vector; <i>sacB</i> gene for double crossover event selection, Km ^r	[75]
pK18mobsacB.Δre78-79	pK18 derivative to generate a <i>re78-79</i> deletion	This work
pK18mobsacB.Δre84-89	pK18 derivative to generate a <i>re84-89</i> deletion	This work
pK18mobsacB.re82HDAA	pK18 derivative to generate <i>re82</i> H ₄₃₁ A-D ₄₃₄ A	This work
pET22b(+)	T7 promoter vector with N-terminal signal peptide (PelB), Amp ^r	Novagen
pET22b.Re78SP	pET22b derivative expressing Re78 with a N-terminal PelB sequence	This work
pET22b.Re78	pET22b derivative expressing Re78	This work
pBAD33	Plasmid for cloning, L-arabinose inducible, araBAD promoter, Cm ^r	[76]
pBAD33.Re79	pBAD33 expressing immunity protein Re79, Cm ^r	This work
pLMB509	Broad-host range expression vector with a taurine inducible promoter, Gm ^r	[77]
pLMB509-Re79StrepTag	pLMB509 derivative expressing Re79 with a C-terminal Strep-tag	This work
pMP220	Cloning vector with a promoter-less <i>lacZ</i> gene, Tc ^r	[78]
pMP220-PTssA	pMP220 cloned P6 of ReMim1 and <i>tssA</i> region, Tc ^r	This work
pMP220-PHcp	pMP220 cloned P6 of Remim1 and <i>tssH</i> region, Tc ^r	This work
pHC60	Green Fluorescent Protein (GFP) gene under a constitutive promoter, Tc ^r	[79]
pCMB13	Contains the <i>Discosoma</i> Red fluorescent protein (DsRed) gene under a constitutive promoter, Sp ^r	[80]

Antibiotic Abbreviations: Amp (ampicillin), Km (kanamycin), Sp (spectinomycin), Tc (tetracycline), Gm (gentamicin), Cm (chloramphenicol).

Table S2. Primers used in this study, related to experimental procedures.

Primer	Sequence (5'-3')	Use
P78.F	CCGGCCATATGATCGTGTGGCTGCAACTGA	Cloning of <i>re78</i> in pET22b without PelB
P78.R	CCGGGCTCGAGTTAATGGTGTGGTGAATCATCCTGACATC	
P78sp.F	CCGGGGATCCGTCGGCTGCAACTGAGCGGCA	Cloning of <i>re78</i> with PelB (N-terminal)
P78sp.R	CCGGGCTCGAGTTAATGGTGTGGTGAATCATCCTGACATC	
P79.F	CCGGCCATATGCTGTTGACCGGTCTTATCAAG	Cloning of <i>re79</i> in pBAD33
P79.R	CCGGCACCGCTTAATGGTGTGGTGTGGCTGCTGTTGCCGGGG	
P79.NdeI	CCGGGCATATGCTGTTGACCGGTCTTATC	Cloning of <i>re79</i> in
P79.NdeIStrep	CCCGGCATATGTTACTTTGAACTGCGGGTGGCTCCAGCTAGCCTGCTGCTGTTGCG G	PCR2.1 TOPO and pLMB509
P78.1	CCTCACCAAGGTAGCGATT	
P78.2	CACCAAACGTGGCTTGCGTACTTGGGGAGTTCTACCAGA	Deletion of <i>re78</i> to <i>re79</i>
P79.3	GGCAAGCCACGTTGGTGGCGCTGGCGGAGTATTCAA	
P79.4	CGACCGAGGTCTTGTCCATC	
P84.1	GCGAAAACGGGATTATGGC	
P84.2	ACCCGCTTCATAGCGATCATCGGTCCAACG	Deletion of <i>re84</i> to <i>re89</i>
P89.1	TGATCGCTATGAAGCGGGTCGATTAGGAGG	
P89.1	GCTGGCGCGCAATACAATTA	
P82.F	GATTGGCAGGGTAGGCAGAT	Site-directed mutagenesis in
P82.R	TGAAGACGGGATTGTAGGCG	which amino acids H ₄₃₁ and D ₄₃₄ have been substituted for Alanines
PH.A.F	CTGGCCGCACTGGCACGCCCTGATATT	
PH.A.R	AATATCAAGGCGTGCCAGTGCAGGCCAG	
PD.A.F	TGGCACGCCCTGCTATTGTGGCGGG	
PD.A.R	CCCGCCACAATAGCAAGGCGTGCCA	
pMP220 F	AGCTCCTGAAAATCTCGTCG	Confirmation of P6 orientation in pMP220
pMP220 R	TAAAAAACCCACTCCGGCAA	
P6.1	GTCGAGCATTGCCGGT	Amplification of ReMim1 P6 promoter region
P6.2	CCGACATCCGTGCGACA	
78.RT.F	GAGCGTTGAACGATATTCT	
78.RT.R	CTGACATAGGGACTGGAAAC	
79.RT.F	TGACGGAATTCTATGAGAGC	
79.RT.R	GGGATGGGTCTTGAAATACT	
81.RT.F	TTCTGCCAGAGAACCCAAAC	
81.RT.R	CCATAGGCCTTACTCCAGCA	
82.RT.F	GACGTAGATGATGTGGAGGA	
82.RT.R	ACTGGTTCTCTCTCGGTCT	
83.RT.F	AGTGTCTCGGACCTCTGAA	
83.RT.R	CTCGACCCCTATAACGGACAA	
84.RT.F	CTATTCCGAGACAGGAATG	qPCR of T6SS genes
84.RT.R	GACTTCAACTGCGAATGAAT	
85.RT.F	GTTCCCTGCGAACGAAAGT	
85.RT.R	GTTCCCGACCCCTCCAGTAT	
86.RT.F	GGCAGTGGGTCTGGACATAG	
86.RT.R	GATACGACTGCGGAGCACAC	
87.RT.F	CTGCCCTCGAGGTAGATTGG	
87.RT.R	CGATGATCTTCGGAGCATT	
88.RT.F	ACCGCACGCTATCAGCTC	
88.RT.R	GTCGAGAACCGGTTTCAG	
89.RT.F	GCTGCCGGATCATAGTCAA	
89.RT.R	GGCAGCAAGGTTGAAAAGT	

hcp.RT.F	TTTCGGAAGTGATCCTCAC
hcp.RT.R	ATATAGGTGTCGCCGGGATT
nifH.RT.F	GGTCTTCATGATGCCGAAGT
nifH.RT.R	CTAGCCGAGAAGATCCATGC
tssB.RT.F	AAGCTCCGTTCCGTCTTCT
tssB.RT.R	ATCCGACACAGCCTCTCAAG
16S.RT.F	ACGTGCTACAATGGTGGTGA
16S.RT.R	CAGAGTGCAATCCGAACTGA
rpoD.RT.F	GATGAAGTCGATCGGCAATCTG
rpoD.RT.R	GCTTCGACCATTCCCTCTGG

Table S3. Proteomic analysis of Re78/79 from secretomes of *Rhizobium etli* Mim1 and hcp::pk18 mutant.

Protein	Accession	MW (kDa)	SignalP ^a	Score ^b		Peptides ^c		PSM ^d	
				ReMim1	hcp::pk18	ReMim1	hcp::pk18	ReMim1	hcp::pk18
Re78	S5S7Y0	39.5	No	160.97	11.68	18	2	54	4
Re79	S5SXD8	32.8	Yes	42.25	35.21	6	6	14	13

^a Signalpeptide identified by SignalP 5.0; ^b Measure of the goodness of fit of experimental peptide fragments to theoretical spectra; ^c Total number of distinct peptide sequences identified; ^d Relative Peptide-Spectrum-Match.