

SUPPLEMENTARY MATERIAL

Penicillin acylase from *Streptomyces lavendulae* and aculeacin A acylase from *Actinoplanes utahensis*: two versatile enzymes as useful tools for quorum quenching processes

Rodrigo Velasco-Bucheli^{1, ‡}, Daniel Hormigo^{1, ‡‡}, Jesús Fernández-Lucas^{1, ‡‡}, Pedro Torres-Ayuso^{1, §}, Yohana Alfaro-Ureña¹, Ana I. Saborido¹, Lara Serrano-Aguirre¹, José L. García², Fernando Ramón^{1, ¥}, Carmen Acebal¹, Antonio Santos³, Miguel Arroyo^{1, a}, and Isabel de la Mata^{1, a*}

- ¹ Department of Biochemistry and Molecular Biology, Faculty of Biology, Universidad Complutense de Madrid (UCM), José Antonio Novás 12, E-28040 Madrid, Spain (rvelasc@unal.edu.co (R.V-B.); daniel.hormigo@universidadeuropea.es (D.H.); jesus.fernandez2@universidadeuropea.es (J.F-L.), pedro.torres-ayuso@nih.gov (P.T-A.), yohanaalfaro@gmail.com (Y.A-U.), asaborid@ucm.es (A.I.S.), larase01@ucm.es (L.S-A.), jlgarcia@cib.csic.es (J.L.G.), faramon@ucm.es (F. R.), ansantos@ucm.es (A.S.), acebal@ucm.es (C.A.), ansantos@ucm.es (A.S.), marroyos@ucm.es (M.A.), idlmata@ucm.es (I. D. L. M.))
 - ² Department of Environmental Biology, Biological Research Center (CSIC) Madrid, Spain
 - ³ Department of Genetics, Physiology and Microbiology, Faculty of Biology, UCM, Madrid, Spain
- * Correspondence: idlmata@ucm.es; Tel.: +34-913944150
- ^a These two researchers share the position of last author
- [‡] Present address: Roche Diagnostics GmbH, Penzberg, Germany
- ^{‡‡} Present address: Applied Biotechnology Group, European University of Madrid, Tajo s/n, E-28670 Villaviciosa de Odón, Spain.
- [§] Present address: Laboratory of Cell and Developmental Signaling, NCI, Frederick, USA
- [¥] Present address: Screening and Compound Profiling Institut de Recherches Servier, 125 Chemin de Ronde, 78290 Croissy-sur-Sine, France.

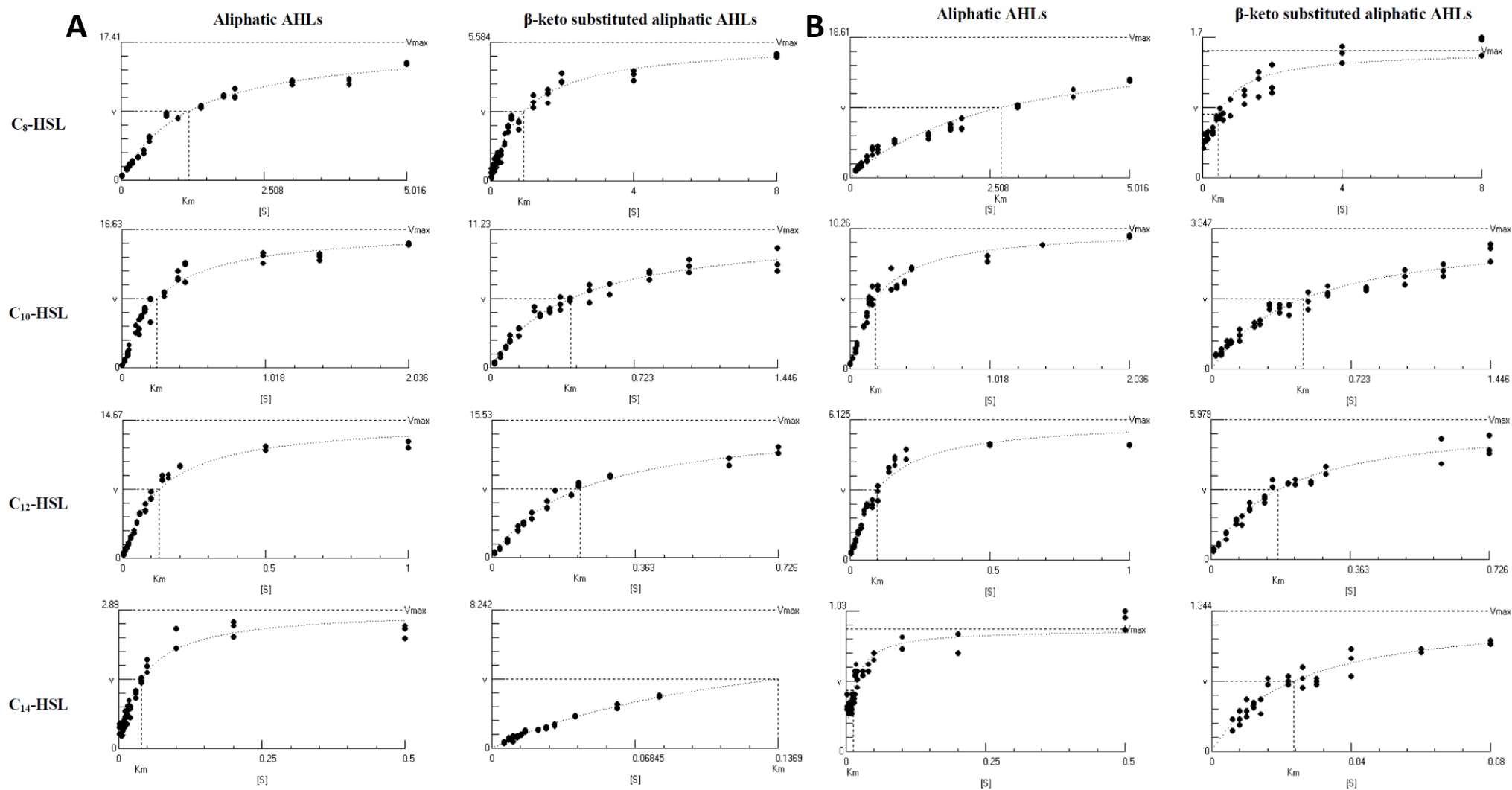


Figure S1. Hyperbolic regression of the activity at different concentrations of several AHLs: (A) *SIPA*, (B) *AuAAC* using Hyper32 program (available on <http://homepage.ntlworld.com/john.easterby/hyper32.html>). The x-axis represents substrate concentration (mM) and the y-axis represents reaction velocity (IU/mg). Reaction conditions: 45 °C, pH 8.0 and DMSO 20% (v/v).

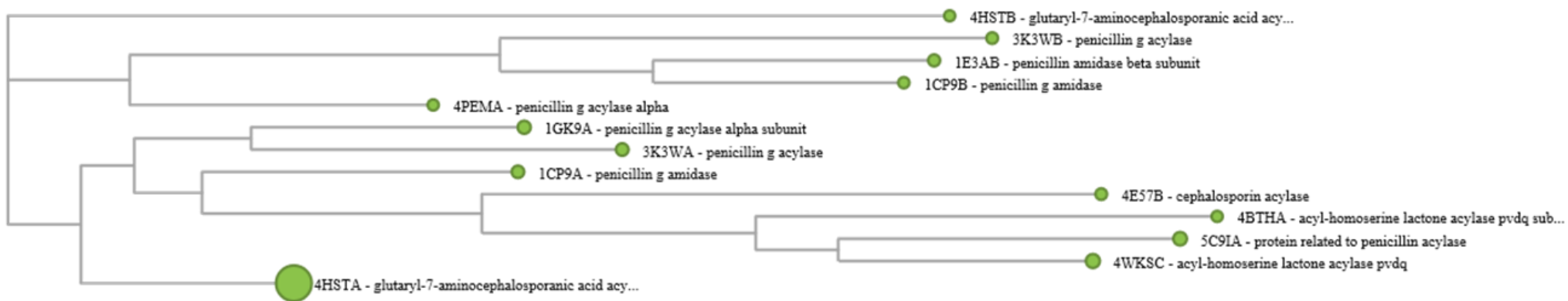


Figure S2. Structure-guided phylogenetic analysis of the Ntn-hydrolases superfamily as analyzed from 3DM database.

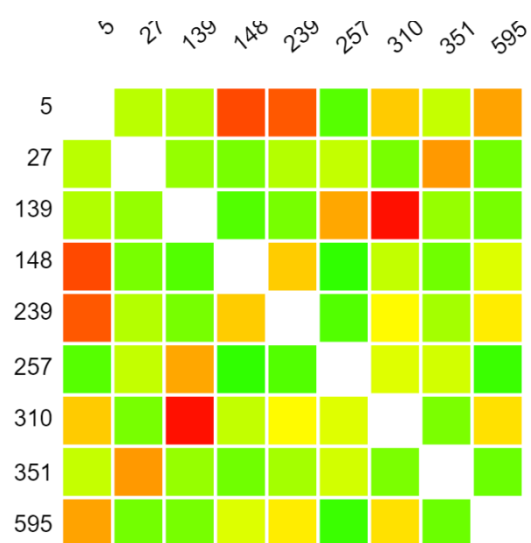


Figure S3. Correlated mutations matrix for the 5C9IA subfamily. Positions exhibiting the strongest mutational correlations (score above 0.9, orange-red) are shown. Such correlations are typically observed for residues involved in substrate specificity. The numbering scheme was generated by 3DM.

Table S1. Amino acids residues at different positions selected in *SIPVA* according to the structure-based alignment from the 3DM database.

3DM residue number	Residue number in <i>SIPVA</i>	Number of mutations in literature	Residue in <i>SIPVA</i> (Q539C0)	Residue in <i>AuAAC</i> (P29958)	Number of literature mutation reports containing keyword "Specificity"	Strong (score >0.9) mutational correlation within subfamily 5C9IA	100% conserved in subfamily 5C9IA	Comments
5	α 10		Y	R		148, 239 and 595		Putatively involved in substrate specificity
27	α 32		W	Y		351		Putatively involved in substrate specificity
35	α 40		C	C			YES	
40	α 45		G	S	2			
60	α 77		D	D			YES	
79	α 115		G	G			YES	
115	α 202	8	G	G	3			
116	β 1	28	S	S	4		YES	Catalytic
117	β 2		N	N			YES	
125	β 10	8	T	A	3			
137	β 22		P	P			YES	
138	β 23		H	H			YES	Catalytic
139	β 24	21	Y	F	3	257 and 310		Substrate-binding pocket
146	β 31	6	R	R				Substrate-binding pocket
147	β 32		F	F				Active site residue predicted by COBALT
148	β 33		W	Y		5		Substrate-binding pocket
162	β 47		G	G			YES	
165	β 50		L	L				Substrate-binding pocket
168	β 53		S	D				Substrate-binding pocket
172	β 57	7	S	E				Substrate-binding pocket
173	β 58		I	I				Substrate-binding pocket
176	β 61		N	N				Substrate-binding pocket
181	β 66		W	W			YES	
183	β 68	12	H	H				Active site residue predicted by I-Tasser & COBALT
184	β 69		T	T			YES	
185	β 70	7	V	V				Catalytic
187	β 72	11	T	T	5			
239	β 120		W	H		5		Putatively involved in substrate specificity

244	β125		G	G			YES	
257	β150		P	V		139		Putatively involved in substrate specificity
293	β186	19	V	V	6			Substrate-binding pocket
310	β203		Q	S		139		Putatively involved in substrate specificity
351	β266		P	D		27		Putatively involved in substrate specificity
355	β270		N	N			YES	
357	β272	9	N	N			YES	Catalytic
385	β301	8	R	R	4		YES	
476	β417		P	P			YES	
595	β544		R	K		5		Putatively involved in substrate specificity

BAF94155.1	--MKFNKLMIAMGMACGVML-----T-----GCNDESDSTLPTEPETQLQTF	40	Aac (<i>Shewanella</i> sp.)
BAV56778.1	-----MRESIHRGAWPRSVLTLAAVAALA-----AC-----GGSGG	31	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	-----MGMR--TVL---TGLAGM-----LLGS MMP-----VQAD-M	25	PvdQ (<i>P. aeruginosa</i>)
AA Y37014.1	-----MIISR P--LCS---FVFAGL-----SFAVILP-----AQALVE	28	HacA (<i>P. syringae</i>)
WP_010889514.1	MSRSPFSSVSLPARLLLGSLLLGLM-----L-----GGA	30	QqaR (<i>D. radiodurans</i>)
AAO41113.1	-----MMQGFALRGT LAMAALAALA-----GCASSTD-----GRWGS	32	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	-----MTHGFALRNTLAVAALAALA-----GCAGTAH-----GSRWDT	33	AaC (<i>R. solanacearum</i>)
WP_043523659.1	-----MRLKAAAIA---FGV-----I-----VATAAVP-----SPASGR	26	AuAAC (<i>A. utahensis</i>)
BAF51977.1	-----MGVILRLRLHSLRA---LAVGGLVLLTGAGALPAVAASD-----AARGER	41	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	-----MTFRNRLRL---FAVSG LALFTVSASLPPAAASG-----APEARH	37	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	-----MRLRNRLRL---LGVAGLALFTVSASLPPATASG-----AGQERH	37	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	-----MTLRNRLRL---LGVAGLALFTVSASLPPATASG-----TQETRH	37	SsCLA (<i>Streptomyces</i> sp.)

BAF94155.1	APNGLLKANIRRTTYGVPHIQADNLES LGFGSGY AQAQDNLCVLADGFIKANSQRSMYFG	100	Aac (<i>Shewanella</i> sp.)
BAV56778.1	GDGSTYSAEIRRTTGMVPHIKAGNWGSAGYGFYVQAQDNLC TMADSFLTYRGERSRHLG	91	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	PRPTGLAADIRWTAYGVPHIRAKDERGLGYGIGYAYARDNACLLAEIIVTARGERARYFG	85	PvdQ (<i>P. aeruginosa</i>)
AA Y37014.1	PGNQAAAEIRRTGFGVPHIVAANERGLGYGIGYAYA QDNLC LLANEVVTVNGQRSRYFG	88	HacA (<i>P. syringae</i>)
WP_010889514.1	ASAQTYQVQIQRTAHGIPHIQASDLGGIGYGVGYSYA QDNLC LLADQVMTVRGERSKFLG	90	QqaR (<i>D. radiodurans</i>)
AAO41113.1	LSDTGLSAEIRRTGFGIPHIRANDYASLG YGMAYAYA QDNLC LLADQVVTVNGERSKTFG	92	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	PSDTGLSAEIRRTGFGIPHIRANDYAGLGF GMAYAYA QDNLC LLADQVVTVNGERAKTFG	93	AaC (<i>R. solanacearum</i>)
WP_043523659.1	EHDGGYAALIRRASYGVPHITADDFGSLGFGVGVYQAEDNICVIAESVVTANGERSRWFG	86	AuAAC (<i>A. utahensis</i>)
BAF51977.1	PSADGLSAVIRYTEYGVPHILADDYADLGF GDGWAQAADAVCTLADGFVTVRGERARYFG	101	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	PSGGGLSATVRYTEYGIPHIVAKDYANLGF GTGWAQAADQVCTLADGFVTVRGERSKFFG	97	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	PSGGGLSAVIRYTEYGIPHIVAKDFAQLGF GTGWAQAADQVCTLADGFVTVRGERSRFFG	97	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	PSGSGLSAVIRYTEYGIPHIVAEDYAQLGF GTGWAQAADQVCTLADGFVTVRGERSRFFG	97	SsCLA (<i>Streptomyces</i> sp.)

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BAF94155.1	PHASIDFTTGQPTAEDNGNLISDFAYKALKIREQA-----EAKWPQFSERSRALIQGF	153	Aac (<i>Shewanella</i> sp.)
BAV56778.1	GSAQLVYNSTLGR---PRNIDSDFHHRHVISDEAVDRT-----MAAQPAKLLQMVEGF	141	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	SEGKSSAE-----LDNLPSDIFYAWLNQPEAL-----QAFWQAQTPAVRQLLEGY	130	PvdQ (<i>P. aeruginosa</i>)
AA Y37014.1	PDKATLEQ-----RNNMASDLLFQWLNTPOAL-----ADFWNAQPREIRQLMQGY	133	HacA (<i>P. syringae</i>)
WP_010889514.1	AEGKTVVGF-QPV---NNLSDSVFFKTVIEPGRL-----QAGYRD-QPQILALMRGY	137	QqaR (<i>D. radiodurans</i>)
AAO41113.1	PEGTVTVSF-KPI----PNLQSDAFFKGFIDEDGL-----RAGYAQMSPEARELLRGY	140	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	PDGTATVSF-KSI----PNTRSDAFFKGVDA AAL-----RDGYARMSPEARELLRGY	141	AaC (<i>R. solanacearum</i>)
WP_043523659.1	ATGPDD-----ADVRSDLFHRKAIDDRVAERLLEGPRDGV RAPSDDVRDQMRGF	135	AuAAC (<i>A. utahensis</i>)
BAF51977.1	PDAAPDRSF-SEA---STNLSSDLFFRGVRDARTVEKLLDRPA--PEGPSRQAKELMRGW	155	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	PDAAPDFSL-SSA---AKNLSSDLYFRGVRDSGTVEKLLKVPA--PAGPSRDAKESMRGF	151	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	PDAATDFSL-SSA---ATNLSSDLYFRGVRDSGTVEKLLKVPA--PAGPSRDVKETMRGF	151	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	PDAATDYSL-SSA---ATNLSSDLYFRGVRDSGTVEKLLKEPA--PAGPSRDVKETMRGF	151	SsCLA (<i>Streptomyces</i> sp.)

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Figure S4

BAF94155.1	TAGYNQYLADVEVGKQTAEPFCGGQPWVKP	I	VPEDVVTYLFSIALLPGAANFLDLIFYAN	213	Aac (<i>Shewanella</i> sp.)		
BAV56778.1	AAGYNRYVREAKAGG-SAHAACRSEAWVQP	I	TARDVWRRIYAANLAGGYSNFAEAIANAQ	200	MacQ (<i>Acidovorax</i> sp.)		
AAG05773.1	AAGFNRFLEADG----KTTSTCLGQPWLRA	I	TATDDLRLRRLRLVEGGVGFADALVAAA	186	PvdQ (<i>P. aeruginosa</i>)		
AAAY37014.1	VAGYNRSLAEQTTQ--GLPQPC-AAEWVRP	I	STDDLRLRRLRLVEGGVGFADALVAAA	190	HacA (<i>P. syringae</i>)		
WP_010889514.1	VAGVNRYLDRTPPE--QWPSACRNADWVRP	L	TELDMVRLGEEKAIQASAGAMVSAITSAR	195	QqaR (<i>D. radiodurans</i>)		
AAO41113.1	IAGFNRYLKDTPPA--NFPAACRNAAWVRP	L	TLGDMMRMGEEKAIQASAGAMLAGIVAAQ	198	AiiD (<i>Ralstonia</i> sp.)		
WP_011002462.1	IAGYNRYLKDTPPA--DFPAACRNAAWVRP	L	TLADMMRMGEEKAIQASAGAMLAGIVDAQ	199	AaC (<i>R. solanacearum</i>)		
WP_043523659.1	VAGYNHFLRRTGVHR-LTDPACRGKAWVRP	L	SEIDLWRTSWDSMVRAGSGALLDGIVAAT	194	AuAAC (<i>A. utahensis</i>)		
BAF51977.1	AAGYNAWLRQH---K-VTDPACRGKGWVRP	V	TATDVARRGYALTVLGGQGalADAITEAR	211	SmPVA (<i>S. mobaraensis</i>)		
AAU09670.1	AAGYNAWLRQN-RDR-ITDPACRGASWVRP	V	TALDVAVRGFALAVLGGQGRGIDGITAAQ	209	SlPVA (<i>S. lavendulae</i>)		
AAT68473.1	AAGYNAWIAQN---R-ITDPACRGASWVRP	V	TALDVAARGYALAVLGGQGRGIDGITAAQ	207	AlhM (<i>Streptomyces</i> sp.)		
BAD07025.1	AAGYNAWIAQN---R-ITDPACRGASWVRP	V	TALDVAARGYALAVLGGQGRGIDGITAAQ	207	SsCLA (<i>Streptomyces</i> sp.)		
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BAF94155.1	PGDGQEYMPRIVGPAQSQEQTAFVSDMQSKLLARAARITTPETNPRDL	G	SNWGLGKDKT	273	Aac (<i>Shewanella</i> sp.)		
BAV56778.1	PPQAKAGAEPAAF-----E-PGRTRAPSLQVGGELGV	G	SNNMYGFGTAAAT	244	MacQ (<i>Acidovorax</i> sp.)		
AAG05773.1	PPGAEKVA---LSG-----EQAQVVAEQ-RRQRFRLER	G	SNAIAVGSERS	227	PvdQ (<i>P. aeruginosa</i>)		
AAAY37014.1	PPAQKPLQVDAQQ-----AQALQLAAA-RNQRFALER	G	SNAVAIGRELS	234	HacA (<i>P. syringae</i>)		
WP_010889514.1	PPQAGASTA----A-----PRPDIAAFNRQYRFDLPI	G	SNGWAFGSEAT	236	QqaR (<i>D. radiodurans</i>)		
AAO41113.1	PPGRTVPAEREIPP-----QAVDTVALDRELQLRDMPI	G	SNGWAFGADAT	243	AiiD (<i>Ralstonia</i> sp.)		
WP_011002462.1	PPGRAPVTHTVVPP-----HAVDVAALDRELRLRDLPI	G	SNGWAFGSAAT	244	AaC (<i>R. solanacearum</i>)		
WP_043523659.1	PPTAAGPASAP-----EAPDAAAIAAALDGTSAIG	G	SNAAYGLGAQAT	236	AuAAC (<i>A. utahensis</i>)		
BAF51977.1	PPAARTVGGGKA-V-----ADPAAAAARAYFAARHDTGM	G	SNAVAFSGAAT	255	SmPVA (<i>S. mobaraensis</i>)		
AAU09670.1	PPTAAPPAAGVT-P-----KEAAAAAQ-RLLSTQNADM	G	SNAVAFRGSTT	252	SlPVA (<i>S. lavendulae</i>)		
AAT68473.1	PPTAAPPAAGVT-P-----EEAASAAK-RLLSAQNADM	G	SNAVAFDGSTT	250	AlhM (<i>Streptomyces</i> sp.)		
BAD07025.1	PPTAAPPAAGVT-P-----EEAATAAE-RLLSTQNADM	G	SNAVAFDGSTT	250	SsCLA (<i>Streptomyces</i> sp.)		
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BAF94155.1	ENGRGMVLGNP	H	FPHTGNLRFWQSHITIPGQLDMMGGSLVGMPPINIGF	N	KDLAWHTF	333	Aac (<i>Shewanella</i> sp.)
BAV56778.1	GEGSGVLFGNP	H	WYWKGPDRFYQAQLTIDGEANVSGVSLGLP-VIQIGF	N	DSVAWSHTV	303	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	ADGKGMLLANP	H	FPWNGAMRFYQMHLTIPGRLDVMGASLPGLP-VVNIGF	S	RHLAWHTV	286	PvdQ (<i>P. aeruginosa</i>)
AAAY37014.1	ANRGMLLANP	H	FPWGGMRFYQMHLTIPGKLDVMGAALPGLP-LINIGF	N	QHLAWSHTV	293	HacA (<i>P. syringae</i>)
WP_010889514.1	TNGRGLLLGNP	H	FPWETSNRFYQLHLTLPGQFDVMGASLGGMP-VVNIGF	N	QDVAWHTV	295	QqaR (<i>D. radiodurans</i>)
AAO41113.1	ANRRGVLLGNP	H	FPWT'TNRFYQVHLTVPGKLDVMGASIAAFP-VVSI	G	FNKDVAWHTV	302	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	ENRRGVLLGNP	H	FPWT'TNRFYEVHLTVPGKLDVMGASIAAFP-VVSI	G	FNQDVAWHTV	303	AaC (<i>R. solanacearum</i>)
WP_043523659.1	VNGSGMVLANP	H	FPWQGAERFYRMHLKVPGRYDVEGAALIGDP-IEIGHN	R	TVAWSHTV	295	AuAAC (<i>A. utahensis</i>)
BAF51977.1	ANRRGLLLGNP	H	YPWHGNRRFWQSQQTIPGELNVSGASLLGSP-TVQIGH	N	ASMAWSHTV	314	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	ANRGMLLLGNP	H	YPWDGRRFWQSQQTIPGELNVAGSLLGST-TVSIGH	N	ADVAWSHTV	311	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	VNGRGLLLGNP	H	YPWQGGRRFWQSQQTIPGELNVSGASLLGAT-TMSIGH	N	PDVAWSHTV	309	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	VNGRGLLLGNP	H	YPWQGGRRFWQAQQTIPGELNVSGASLLGAT-TISIGH	N	ADVAWSHTV	309	SsCLA (<i>Streptomyces</i> sp.)
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Figure S4

BAF94155.1	STAEHFVMYNLELVSGDRQLQYLFDGQPMPIKNETVSVILVNAGPAGMLVAEKDIYTTAKGP	393	Aac (<i>Shewanella</i> sp.)
BAV56778.1	STARRFQFFQLSLVQGEPTSILRQDGVVPMKPKPATITVPSRNADGSVSDVTRTLYHSEFGP	363	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	DTSSHFTLYRLALDPKDPRLYLVDGRSLPLEEKSVVIEVRGADGKLSRVEHKVYQSIYGP	346	PvdQ (<i>P. aeruginosa</i>)
AAAY37014.1	DTSKHFTLHRLQLDLPKDPSTRYLLDQSVAMGKQVSVVDVKQADGSLKSVPRIVYSSIFGP	353	HacA (<i>P. syringae</i>)
WP_010889514.1	STDKRFTLAALTLVPGDPLSYVKDQQRRLQRRTAVIEVKTANGPRL-HTRTVYFTPEGP	354	QqaR (<i>D. radiodurans</i>)
AAO41113.1	STGRRFTLFELKLAEGDPTTYLVDGTPHKMTTRTVAFDVKLPDGRLEERRTHTFYDTIYGP	362	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	STGRRFTLFELRLAEGDPTTYLVDGTPHKMTARTVAFDVKLPDGRLEERRTHTFYDTIYGP	363	AaC (<i>R. solanacearum</i>)
WP_043523659.1	STARRFVWHRLSLVPGDPTSYVVDGRPERMRARTVTVQTGS-----GVSRTFHDTTRYGP	350	AuAAC (<i>A. utahensis</i>)
BAF51977.1	ATGVPMNLYELKPAFGDPRSILVDGRPERMTERKVTVAVRGADGRLANVTRSQWWTRYGP	374	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	ATGVTLNLHQLTLDPADPTVYLVLDGKPKQRTQRTVAVPVKGA----APVTRTQWWTRYGP	367	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	ATGVTLNLHQLTLDPADPTVYLVLDGKPERMTKRTVSVPVKGA----ADVTRTQWWTRYGP	365	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	ATGVTLNLHQLSLDPADPTVYLVLDGKRRMTQRTVSVPVKGG----ADVTRTQWWTRYGP	365	SsCLA (<i>Streptomyces</i> sp.)
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BAF94155.1	MVEAPPSLAPFGWDDGSFMIQDANMANMDPVDHWMANLATNKDEFQQAQFKDYDGVITEN	453	Aac (<i>Shewanella</i> sp.)
BAV56778.1	LVNLAGLNPALAWSQGTAFAIRDINGENFRTLRTWMRWNQAKSLDEFIAIQKEEASIPWV	423	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	LVVWPG---KLDWNRSEAYALRDANLENTRVLQQWYSINQASDVADLRRRVEALQGIWV	403	PvdQ (<i>P. aeruginosa</i>)
AAAY37014.1	VVQWPG---KLDWDSKFAFSLRDANLQNDRVLQQWYAMDKADSLKAFQDSVRKIQGIWV	410	HacA (<i>P. syringae</i>)
WP_010889514.1	LVNLPAA--GLTWTQYAFALRDANRNNTRMLATWLGFAKASVRDIRASLN-VQGIWV	411	QqaR (<i>D. radiodurans</i>)
AAO41113.1	VLSMPG--GMPWTTQKAYALRDANRNNTRSVDSWLHIGQARDVAGIRQAIG-NLGIWV	419	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	VVSMPAA--GMPWTTQKAYALRDANRGNTRMIDAWLHIAQAGDVAGIHRAG-NLGIWV	420	AaC (<i>R. solanacearum</i>)
WP_043523659.1	VAVVPG---TFDWTPATAYAITDVNAGNNAFADGWLRMGQAKDVRALKAVLDRHQFLWV	407	AuAAC (<i>A. utahensis</i>)
BAF51977.1	VVTGLGDDLPLPWTGSAALADPNAAQFRFFDSSGLGSKARDVRGVREALRRTQGLPWV	434	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	VVTSLGAALPLPWTASTAYALNDPNVAVNLRADTSLGFSKARSTAGIERALHRSQGLPWV	427	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	VVTSMGAAALPLPWTATTAYALNDPNATNLRMADTGLGFSKARSTKDVERSLRNQGMWV	425	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	VATSMGAGLPLPWTASTAYALNDPNATNLRMADTGLGFGKARSTGDVERALHRSQGMWV	425	SsCLA (<i>Streptomyces</i> sp.)
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BAF94155.1	NTMFADKE-GNAFYIDDSVTPGLSESAVVVLKTSPPDIKAAKQQRAGFTILPGNTSLFSF--	510	Aac (<i>Shewanella</i> sp.)
BAV56778.1	NTVAVGRGSAKAWYADIGAVPNVSPAQTAACCTPPFGMAVGQALPNVPFFDGSRSECDWLT	483	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	NTLAADEQ-GNALYMNQSVVYPYLKPELIPACAIQQL-----VAEGLPALQGGDSRCAWSR	457	PvdQ (<i>P. aeruginosa</i>)
AAAY37014.1	NTLAVDAQ-GQALYMNLSVVPNVDAARLARCSDPRI-----GT-ELIVLDGSRSECNWEV	463	HacA (<i>P. syringae</i>)
WP_010889514.1	NTIAADRA-GSALYADISSPNVSAQQQACTPPPLA-PLFPAAGLAVLDGSHSACDWKT	469	QqaR (<i>D. radiodurans</i>)
AAO41113.1	NTIATDRN-GRALFADVSTTPDVPAAELQRCAPSPLAGKLFKDGAGLVLLDGSRGTCNWQV	478	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	NTIAADRN-GRALFVDVSATPDVSAETLKRCAAPSPLAERLFGKAGLVLLDGGARSTCNWQV	479	AaC (<i>R. solanacearum</i>)
WP_043523659.1	NVIAADAR-GEALYGDHSVVPVPTGALAAACIPAPF-QPLYASSGQAVLDGSRSDCALGA	465	AuAAC (<i>A. utahensis</i>)
BAF51977.1	NTVAADSA-GHSLYTQSQVLPVPTDEVQRSCATALG-RRTYPESGLAVLDGSRGACALGR	492	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	NTIAADRS-GNSFFSQSQVLPRIITDELAARCSTPLG-QATYPSAGLAVLDGSTSACALGS	485	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	NTIAADRA-GHSFFAQSQVLPRIITDDLAERCSTPLG-RATYPASGLAVLDGSRKDCALGS	483	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	NTIAADRA-GRSFFAQSQVLPRIITDALAERCSTPLG-RATYPASGLAVLDGSRKDCALGS	483	SsCLA (<i>Streptomyces</i> sp.)
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Figure S4

BAF94155.1	-----SGPTPYERAPKLEERSDFVQNS	NDSFWSTNLNEPLTYFSPMYGPEAG-QLSLRTR	563	Aac (<i>Shewanella</i> sp.)
BAV56778.1	DADSVQKGA VGVS RMP SLQRDDYVGNM	NDSYWLAVNHAPLTGYPAIFGPAGTSAQTLRTR	543	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	DPAAAQAGITPAAQLPVLLRRDFVQNS	NDSAWLTNPASPLQGFSP LVSQE-K-PIGPRAR	515	PvdQ (<i>P. aeruginosa</i>)
AA Y37014.1	SAEAAQAGIYPSSRQPQLLRTDFVQHS	NDSAWMVNPAAPLQGFSP LISQDGQ-PLGQRRAR	522	HacA (<i>P. syringae</i>)
WP_010889514.1	DPASRV PGLRAPDKMPVLIRQDFVANS	NNSAWLANPAAPQTGLDPLVGEVNA-PQSPRTR	528	QqaR (<i>D. radiodurans</i>)
AAO41113.1	DPASPVPGLVAPARMPVLERDDYVANS	NDS SWLTNPAQKLTGFSPVMGSVDV-PQRLRTR	537	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	DPASPVPGLIAPARMPALERDDFVANS	NDS SWLTNPAQKLTGFPPIMGETDV-PQRLRTR	538	AaC (<i>R. solanacearum</i>)
WP_043523659.1	DPDAAVPGILGPASLPVFRDDYVTNS	NDSHWLASPAAPLEGFPRI LIGNERT-PRSLRTR	524	AuAAC (<i>A. utahensis</i>)
BAF51977.1	DRDALQPGVFGPSAMPTLVDAPYAENS	NDSAWLANADRPLTGYPRIFGDIGT-ERSFRTR	551	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	DRDAVQPGIFGPGRMP TLKNA PYVENS	NDSAWLTNADRPLTGYERVFGTTAT-QRSIRTR	544	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	DRDAVQPGIFGPGRMPVLKNQ PYVENS	NDSAWLTNADRPLTGYERVFGTIAT-PRSMRTR	542	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	DPDAVRPGIFGPGRMPVLKNQ PYVENS	NDSAWLTNAERPLTGYERVFGTIAT-PRSMRTR	542	SsCLA (<i>Streptomyces</i> sp.)
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BAF94155.1	MGLTLMQDAA-GS----DGKFNLEELEAAVLSNRSYLAELVLQDLIAQCEAQGSTPVVVS	618	Aac (<i>Shewanella</i> sp.)	
BAV56778.1	MGHTMALERLAGTDGYAGNKATS AVVRE MV LGS RV FSAERFKDEVLDLICTPA---QWTV	600	MacQ (<i>Acidovorax</i> sp.)	
AAG05773.1	YALSRLQ GKQ-----PLEAKTLEEMVTANHVFSADQVLPDLLR LCRDNQGEK----	562	PvdQ (<i>P. aeruginosa</i>)	
AA Y37014.1	FALDRLESLKT-----AGKISVENLQAMVMDNEVYQAGQVLPDLLTFCASELGDD----	572	HacA (<i>P. syringae</i>)	
WP_010889514.1	MGLLEIGRRLSGTDGLPGRTFDIPTLQATLLRESNLTGEM YAADA AKLCQSAG-----	581	QqaR (<i>D. radiodurans</i>)	
AAO41113.1	I GLIEIGRRLAGTDGLPGNRIDL PNLQAMIFSNANLAGQLVLGDL LAACKATPAP-----	592	AiiD (<i>Ralstonia</i> sp.)	
WP_011002462.1	I GLIEIGRRLAGADGLPGNRIDL PNLQAM LFRNTNLAGHLVLGDLLEACKAARDA-----	593	AaC (<i>R. solanacearum</i>)	
WP_043523659.1	LGLDQIQQLRAGTDGLPGKGF T T AR LWQVMFGNRMHGAELVRDDLVALCRRQPTA--TAS	582	AuAAC (<i>A. utahensis</i>)	
BAF51977.1	GGVEDVAAMAK-----RGRLTVADLERQQFAGRAP TGD LAAADAAAACAKLPGG RATGP	605	SmPVA (<i>S. mobaraensis</i>)	
AAU09670.1	GAIEDVAAMAE-----RGRLRVTDLERQQ LANRAP TGD LVAADVAKWCAALPGGTAVGS	598	SlPVA (<i>S. lavendulae</i>)	
AAT68473.1	GAIEDVASMAD-----KGRLRVADLQRQQFANRAPAGELAA SEVAKWCAALPGGTAVGT	596	AlhM (<i>Streptomyces</i> sp.)	
BAD07025.1	GAIEDVASMAD-----RGRLRVGDLQRQQFANRAPAGDLAA SEAAKWCAALPGGTAVGS	596	SsCLA (<i>Streptomyces</i> sp.)	
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BAF94155.1	ASLSKDLTSACAALKAWNGKQDNDSKGGALLREFAHQF----	SQKTMLTKGFD PANAATT	674	Aac (<i>Shewanella</i> sp.)
BAV56778.1	NGAAVDAAQACAVLAAW DNRGRKDSRGS H L W D E F W S R V ----	PTASLFTVPFSAADPLNT	656	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	-----SLARACAALAQWDRGANLDSGSGFVYFQRFMQR--FAELDGAWKEPFDAQRPLDT	615	PvdQ (<i>P. aeruginosa</i>)	
AA Y37014.1	---AARLAPLCAALKDWDGRADLNSGIGFVYFQKIMTS--MQAVASRWRVAFDPQDPVHT	627	HacA (<i>P. syringae</i>)	
WP_010889514.1	---GAELQPACNALAAWDRRSSQESRGAALWREFWRRR-R--AIPNVYAVPFD PADPVNT	635	QqaR (<i>D. radiodurans</i>)	
AAO41113.1	---DADV RDGCAALGQWNRTSNADARAHLRFRE FWMRA-K--DIAQVHAVEFDPADPVHT	646	AiiD (<i>Ralstonia</i> sp.)	
WP_011002462.1	---DADV R N G C V A L G R W N R T A A A N A R A A H L F R E F W M R A - K --	DIPQVYATDFNPDDPIYT	647	AaC (<i>R. solanacearum</i>)
WP_043523659.1	NGAIVDLTA ACTALS R F D E R A D L D S R G A H L F T E F A L A G -----	GIRFADTFEVTDPVRT	636	AuAAC (<i>A. utahensis</i>)
BAF51977.1	DGRPVDVSGACGVLARWDRRLTTGSRGALLFDRFVRALVREVPADRLWRVPFDPKPVTT	665	SmPVA (<i>S. mobaraensis</i>)	
AAU09670.1	SGTPVDVSAACPVLRWDRSVSDSRGALLFDRFWRKA-AAVPAAELWKVPFDAADPVRT	657	SlPVA (<i>S. lavendulae</i>)	
AAT68473.1	GGTPVDVSDACAVLRWDRSVSDSRGALLFDRFWRKT-SAVPAAELWKTPFDPADPVRT	655	AlhM (<i>Streptomyces</i> sp.)	
BAD07025.1	DGTPVDVSAACRVLRWDRVTVDSDSRGALLFDRFWRKA-SSAPAAELWRTPFDPADPVRT	655	SsCLA (<i>Streptomyces</i> sp.)	
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Figure S4

BAF94155.1	PNTLTTDGSAL-----VALAHAALNLEAAGFALDAPLGDVQFVEKSLPYGTASGARLPWP	729	Aac (<i>Shewanella</i> sp.)
BAV56778.1	PRGINAAAA---DALRQAMATAIARVGGQSGYALDAPRGEVLYAT-----RGGTRLPLY	706	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	PQGIALDRPQVATQVRQALADAAAEEVEKSGIPDGARWGDLQVST-----RGQERIAIP	668	PvdQ (<i>P. aeruginosa</i>)
AAAY37014.1	PSGLAIENPSVATALRAAMLAAVDDVAKAGLPAGSKWGDIVQSS-----ISGKQIPIH	680	HacA (<i>P. syringae</i>)
WP_010889514.1	PRGLNTADPAAQTALLGALREAAAALTAAGIPFDAPLGEVQGVV-----RGGDFISLP	688	QqaR (<i>D. radiodurans</i>)
AAO41113.1	PRGLRMNDATVVRTAVFKALKEAVGAVRKAGFALDAPLGTVQAAH-----APDGSIALH	699	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	PRGLRMHDATVRATVFKALKDAVGAVRAAGFALDAPLGTVQAVR-----LPDGDIALH	700	AaC (<i>R. solanacearum</i>)
WP_043523659.1	PRRLNTTDPVVRT----ALADAVQR--LAGIPLDAKLGDIHTDS-----RGERRIPIH	683	AuAAC (<i>A. utahensis</i>)
BAF51977.1	PNTLNTAEPGVAR----ALAAAVHDLRSAGIALDAPLGEHQFVV-----RKGKRIPIV	714	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	PRGLNTAAPGVGK----ALADTVTELKAAGIALNAPLGEHQFVV-----RNGKRIPIV	706	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	PRGLNTAAPGVGR----ALADAVAELRAAGIALDAPLKGKHQFVV-----RNGKRLPIG	704	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	PRGLNTAAPVLGR----ALADAVAELRAAGIALDAPLGEHQFVV-----RNGKRLPIG	704	SsCLA (<i>Streptomyces</i> sp.)
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BAF94155.1	GSHNAEGGFNVFSTSLSGDDTLIPQHKYAPLMDVVTGKAMASGMTAKGYQVRYGSSWMMMA	789	Aac (<i>Shewanella</i> sp.)
BAV56778.1	GGCGAMGYFTITCSENDITQ-----GGYSMDGQPNASNSYMQV	744	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	GGDGHFGVYNAIQSVR--K-----GDHLE---VVGGSYIQL	700	PvdQ (<i>P. aeruginosa</i>)
AAAY37014.1	GGPAGLGVYNAMQTV--GK-----DGKRE---VVSGETSYLQV	713	HacA (<i>P. syringae</i>)
WP_010889514.1	GGAEFEGVLDKIDFNPLAPG-----GYRGV---VGNASSYIQT	723	QqaR (<i>D. radiodurans</i>)
AAO41113.1	GGEEYEGVNLKQLTLPVIGPK-----GLPVY----FGTSYIQT	732	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	GGEEYEGVNLKQLSLPIGPQ-----GLQVD----EGTSYVQT	733	AaC (<i>R. solanacearum</i>)
WP_043523659.1	GGRGEAGTFNVITNPLV--PG-----VGYPO---VVHGTSFVMA	717	AuAAC (<i>A. utahensis</i>)
BAF51977.1	GGTEALGAWNKVESRWDGSR-----GGYPE---VSMGTSYVQA	749	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	GGTESLGIWNKIEPVWNPAA-----GGYTE---VSAGSSYIQA	741	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	GGTESLGIWNKTEPVWNAAG-----GGYTE---VSSGSSYIQA	739	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	GGTESLGIWNKTEPQWNAAG-----GGYTE---VSSGSSYIQA	739	SsCLA (<i>Streptomyces</i> sp.)
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BAF94155.1	VSFTD--EGPVARGILTYSESSNILSPSFTDQSNLYSSSKSFRPLLFKEADIAPAVISTT	847	Aac (<i>Shewanella</i> sp.)
BAV56778.1	VSFPA--SGVQAHTFLTFSLSDDPASPHHGDYTKAYSAGQ-WLRVFPFTEAEITGNADYRT	801	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	VTFPE--EGPKARGLLAFSQSSDPRSPHYRDQTELF SRQQ-WQTLFPFSDRQIDADPQLQR	757	PvdQ (<i>P. aeruginosa</i>)
AAAY37014.1	VTFDE--QGPKAQGLLAFSESSNPQSAHSSDQTEAFSFKKQ-WQALPFTEQQIKADPAYEV	770	HacA (<i>P. syringae</i>)
WP_010889514.1	VGFTD--SGVQAEAVLTYSSNPESPYFSDQTRLFSRSE-WVKLPFTQPEIEADPTRTV	780	QqaR (<i>D. radiodurans</i>)
AAO41113.1	VTFDD--QGPVADAILTYGESTDHASPHAFDQMRAYS GKH-WNRLPFSEAAIAADPALKV	789	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	VGFDA--QGPVAQALLVYGESTDPASPHAFDQMRAFSAKR-WIRLPFSEAAIAADPALKV	790	AaC (<i>R. solanacearum</i>)
WP_043523659.1	VELG--PHGPSGRQILTYAQSTNPNSPWYADQTVLYSRKG-WDTIKYTEAQIAADPNLRV	774	AuAAC (<i>A. utahensis</i>)
BAF51977.1	VGFDGGPCPVTARTLLTYQSDDPASAHSSDQTELF SKGR-MTRGRFCERDILASPLLRV	808	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	VGWDNSRCPV-ARTLLTYQSSENPNPSPHYSDQTRLFSGER-WVTSRFCEKDIARSPQLKV	799	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	VGWDDSRCPV-ARTLLTYQSENPRSRHFSQDQTRLYAGER-WVTSRFCEKDIARSPDLRV	797	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	VGWDDSRCPV-ARTLLTYQSENPKSPHYSDQTRLYAGER-WVTSRFCEKDIARSPDLRV	797	SsCLA (<i>Streptomyces</i> sp.)
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Figure S4

BAF94155.1	ELTLQKAQ-	855	Aac (<i>Shewanella</i> sp.)
BAV56778.1	ATVKE----	806	MacQ (<i>Acidovorax</i> sp.)
AAG05773.1	LSIR-----	761	PvdQ (<i>P. aeruginosa</i>)
AAV37014.1	QVISEEPDR	779	HacA (<i>P. syringae</i>)
WP_010889514.1	VQLSE----	785	QqaR (<i>D. radiodurans</i>)
AAO41113.1	MRLSQ----	794	AiiD (<i>Ralstonia</i> sp.)
WP_011002462.1	TKLSQ----	795	AaC (<i>R. solanacearum</i>)
WP_043523659.1	YRVAQRGR-	782	AuAAC (<i>A. utahensis</i>)
BAF51977.1	VRVAQR---	814	SmPVA (<i>S. mobaraensis</i>)
AAU09670.1	VRVHERR--	806	SlPVA (<i>S. lavendulae</i>)
AAT68473.1	VRVHERR--	804	AlhM (<i>Streptomyces</i> sp.)
BAD07025.1	VRVHERR--	804	SsCLA (<i>Streptomyces</i> sp.)

Figure S4. Sequence alignment of SlPVA and AuAAC with other homologues from the AHL acylase group A. Alignment was performed with Clustal Omega (<https://www.ebi.ac.uk/Tools/services/rest/clustalo>). Acylase homologues used in the alignment include Aac, from *Shewanella* sp. strain MIB015; MacQ, from *Acidovorax* sp. MR-S7; PvdQ, from *Pseudomonas aeruginosa* PAO1; HacA, from *Pseudomonas syringae* B728a; QqaR, from *Deinococcus radiodurans* R1; AiiD, from *Ralstonia* sp. XJ12B); Aac, from *Ralstonia solanacearum*; SmPVA, from *Streptomyces mobaraensis*; AhlM, from *Streptomyces* sp. M664; SsCLA, from *Streptomyces* sp. FERM BP-5809. ‘*’, identical residue; ‘.’, conserved substitution; ‘dot’, semi-conserved substitution. Confirmed signal peptides are indicated in blue, whereas predicted signal peptides according to SignalP server (<http://www.cbs.dtu.dk/services/SignalP/>) are indicated in purple. Conserved residues of relevance to autoproteolysis and catalysis in known acylases belonging to Ntn-hydrolase family are shaded in yellow. Residues related to long acyl-chain recognition are shaded in cyan. Variant residue positions predicted (by 3DM) to be involved in substrate specificity are shaded in grey.