

Supplementary material

## **Novel Bifunctional Acylase from *Actinoplanes utahensis*: a Versatile Enzyme to Synthesize Antimicrobial Compounds and Use in Quorum Quenching Processes**

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KcPGA*	582	---LFLPAIKDATAN---LAENDPRRLVDKLASVGENLVNDGDKTYQQPGSAILNAWLTSMLKRTVVAAPVAPFGKW---YSASGYETTIDGPTSGLSINISVGAK-----ILYEALQDGKSPVQAVDLFGGKPEQE-VILAAIDDAWQITLSKRYGN-DVTGWKTPAMA	738
MacQ*	541	FGMGHTMALERLAGTDGYAGNKATSAVREMVLGSRVSAERFKDEVL-DLICTP--AQWTVNGAAVDAQAACAVILAA---WNNGRKKDS--GSHLWDEFWSRVF---TASLETVE---SAADLNTFR---INAAAAADAL---RC---ATAIAKVGQ---YA-LD---PRGEVLYA	696
PaPVA*	201	-----GVMVPGTNRAADRFRRA---SFYVNVNPNKILPGVAEKGTIEK---DRADLATA---SIIRNASV---EYSLPDM---PNIASTWRITVVD-HKSLQYFFESA	293
Pfma*	559	EVKELKPLAQIQIQONE-HQRQALELLKWSGNSMDSQSPISFIVMQREIRKLLKDELAGWVWQGGQDLTLDQIAST---LPLTTVVELLQHPQCVDSATSSSSNCCQLINAFKRALQVTSNCL---EDDIAGWNG---ELQHTVEVHTFFHIR-VLDGHTWRAP	720
PrPGA	513	NHLLTPTPTQATQ---LPSDMSVKLMSLQWQGIQMLSSDGKVIHPGSAILDILWKEMLATLQGVTAPEFWK---ELASGVET---ECPQSIAMSTGAK---LHESILIDKSPFSTLTPSGQFQND-VIRKTTVQNHLEKVD-NHAGTTPATA	735
PvdQ	513	RAVYALERIQQKQ---PLEAKTIEEMVTANHVPSADQVLPOLLRLCH---DNQGEKS---LARAACAIAQ---WDRGAMLDSSGSPFYFQFMRQFA---ELDGMKEI---DAQRPIDQ---FALDQPVATQVRC---ADADAARKEK---IP-DG---AWGDQLQVS	658
QqaR	526	PTMCKLELGRRLSGTGLPQRTFDIPTIQAATLRESNLTEGMYAADAAKLC-----QSAGG---AELQPCANLAA---WRRSSQSS---GAALWRFWRAR---AIPNVAVI---DPADFNTH---PNTADPAQATALLQ---REAAAALTA---IP-FD---GEVQGV	678
QuiP	580	GMALEPQAIADALPEAGRSRAREAYDRLMAPDGLKTASSSDAALYGFHESARQIFLDELGPEGDPAWKAFTETANLS---YSAGADHLLGRDSDPFWDITRTPOKEDKPAILARSLAAAVFCEQPLSERKAWQWGLKLTVEYQSDSSHAPYVLAGERPLGALIKGY	747
Slac1	235	-----PSVATMPFWSIGGLERNRL---LAMSTHMD-----LEGLSY---TETVARQKGTFDAAALNPFVQDPKT-----GDTYSPFFSMQYN-LDNGDITFRSL	319
Slac2	228	-----SALGTKLPADYSPLSRVYRG---LHHAESQD-----FKNMTY---QQTMAAQLLAFHSAVAI---QSVQAPFVDGP---KIAEGGVGPTFFFTVOYN-LKNGDVTYVSD	320
SlPVA*	542	RTEGATEDVAAAE-----RGRRLVTDIERQQLANRAPTGDVVAADVAKWCAALPGGTAVSGSSGTPVDVSAACPVLR---WDRSVDSDS---GALLDFDRFRKAA-AVFAAEILMKVLSDAADPVRTSG---INTAAPGVG---RHAADVTTELKASTIA-LMPLGEHQFV	696
SmPVA	545	RTEGGVEDVAAAMAK-----RGRRLTVADIERQQFAGRAPRTGDLAAADAAAACAKLPGGRTATPGDGRPVDVSGACGVLAR---WDRRLTGTGS---GALLDFDRFVALRVFVPAOLRVVSPAKPVTTENTINTAAPGVA---RAHAAAHVDRSRGASTIA-LDAPLGEHQFV	704
SsCIA	540	RTEGATEDVASMAD-----RGRRLRVGDLIRQQQFANRAPAGDLAAASEAAKWCAALPGGTAVSGDGTDPVDVSAACPVLR---WDRTVSDSDS---GALLDFDRFRKAS-SAPAAELNRTFSDPADPVRTSG---INTAAPVLG---RAHADAVALRAGASTIA-LDAPLGEHQFV	694
AuAHLA*	698	TRAG-----ETIPVHGAPHALGVNLVITPTWR-AGAGNVDMVHGS-----SFTQVVEFGATCAPR-ARTLLTYSQSADPTSEHADCTRLFSRST-VVTSFSTERE---HASPVLQSQIRLTERAPR--	808
Aac (WP_011002462)	691	RLPD-----GDIALHCGEEYEGVLNKLQSLPIGPQGLQ---WDEGT-----SYVQVGFDAQCEVA---QALLVYGESTDPASSEADQMRAFSKR-WIRLPSSEAAADPALKVTKLSQ-----	795
Aac (BAF94155)	713	EKSLPYGTASGARLPWEGSHNAEGGNFVSTLSGDDTLIPQHKYAPLMDVVTGKAMASGMTAKGYQVRYGSSWMMMAVSTDECEVA---RGILITYSESSNLSPSFIDGSLNLYSSKSERPLLIKEDAPAVISTTETLTLOKAQ---	855
AcPGA	762	MVFA-----PKNFIQVQADAKAVLCYRATNRGTENNMTVFDGKS-----VRAMVVAFGQSG-----FVAPDGTFSSTFDGFDLYNTFG-SKRVVSTADEVRNATSEETLRYPR---	863
AfPGA	713	HRFS-----DKNFTICPQTMPGNITFAFTGYQNRGTENNMTVFDAGK-----VEFCDAMPEQSG-----FTDRNGVRSFYEYDQLKLVENFE-CKTMDVTHADVRNAAQSSTMLLIQPPF---	816
AhaP	711	LDAGKN---NQHIPHSGYSGEGLFNVARGAGVNEAGNYIINNGP-----TYMQSVTFDSHCPVV---EALLASQAGDTTTPPHDCTRRYSDDK-WIRLESKSESKQAVGEVILIRE---	819
AhlM*	695	VRNG-----KRLPIGSGTESLGIWNKTEPFVWNAAGGGYTESSGS-----SYIQVQWDDSRCPV-ARTLLTYSQSENPSFYSQDTRLYAGER-MVTSFSCERARSFDRVVRVHERR---	804
AibP	469	-----NHTVTNANWRNANQSFVETDPSLRTVDLAN-----LDDSG---HHAH---QSG-----HAFICQVYVIESWKLE---HEPMLQOQVTTANTSAKILOHPR---	465
AiiC	786	TTSG-----NHTVTNANWRNANQSFVETDPSLRTVDLAN-----LDDSG---HHAH---QSG-----HAFICQVYVIESWKLE---HEPMLQOQVTTANTSAKILOHPR---	847
AiiD	690	HAPD-----GSIALHCGEEYEGVLNKLQTLPIGPKGLP---WYFGT-----SYVQVTFDDQCEVA---DAILTYGESTDHA---HAFICQVYVIESWKLE---HEPMLQOQVTTANTSAKILOHPR---	794
AmiE	453	LPVN-----QIIHQQPYADKVT-----IKFAELLQIT---GQPKPLDE-----	490
AtPVA*	273	LTFN-----VWFDMTKLDLSK-----ETGAVKKLDLGM---HITYSGMA-----NESIKDTKPKFKGLG---	326
AuAAC*	674	SRGE-----RRIPIHSGRGEAGTFNVITNPLVPGVG-YPOVHGT-----SFMAKCLG-PHGFS---GRQILTYAOSTNPNFVYADTVLYSRKG-WDTIKVTEAC---AADPNLRVVRVPAQRGR---	782
AvPGA	703	MTFG-----EKSLIEPHGYG---SMTPIIEMNRGSENHYIEMTPKG-----PSGFNITPFGQIG-----LVKDKGTIS---YVDGLVMEFAEWK-EKPYIE---KHKHYGAATNVSAINMSK---	802
BlBSH	292	DDPA-----RYSYAMADYMD-----SSELISVAR-----	316
BmPGA	703	MTFG-----EKSLIEPHGYG---SMTPIIEMNRGSENHYIEMTPGT-----PSGFNITPFGQIG-----LVKDKGTIS---YVDGLVMEFAEWK-EKPYIE---KHKHYGAATNVSAINMSK---	802
BspPVA	295	DNSR-----TSASIMAEINL-----SQDLITFEWDR-----KOLKQLNQVNVMS---	335
BsPVA	293	DNRQ-----TQKNILFHEDLD-----CLEPKVESAKA-----EESHELN---	327
EcPGA	741	LTFR-----ANNFEPVQAAAEEETHQAEYQNRGTENDMTVFSPTTSD---RPVLAWDVAFQSG-----FIAPDGTVDIEYDGLVMEYFNG-RKSLWLTQDVEAHKESQEVLVQR---	846
EFBSH	289	DNSQ-----TTATDMNKENLE---KSLIIVPMVE-----TOCNRYANLEHHHHHH---	331
HacA	701	SISG-----KQIPIHSGPAGLGVYNAMQTAVG---KDGKRETVSGT-----SYIQVVFDEQCEPKA---QGLLASESSNPEBASSDCTEAFSKKO-WALFSTEQCADPAYEVQVISEEDPR---	779
HacB	672	AAPG-----THEVPNNLSSSIRPAPVPGVGYGSTRRIIDFADP---AHSIGINPVQSG---VFFDRYSDCAKAFVNGE-XLPQBSSEKDVAEHTGVLRIAPGA---	795
KcPGA*	739	LTFR-----ANNFEPVQAAAEEETHQAEYQNRGTENDMTVFSPTSGN---RPVLAWDVAFQSG-----FIAPDGDADRYDGLVMEYFNG-RKSLWLTQDVEAHKESQEVLVQR---	844
MacQ*	697	TRGG-----TRLPLVSCGAMGYFTITCSENDITGGYSDMGQPNAS---NSYMQVVSFPASSVQA---HTFLTYSLSDDPARFHCQVTKAYSAGO-WLRVHTEAEFGNADRYRTATVKE---	806
PaPVA*	294	VSPN-----IFWDLKKNIFAP---RGSSAAKLDLGEHSTIISGQA-----SGHFKPAQFFEFAGLLEHHHHH---	355
Pfma*	721	AGGS-----IHSVNVAAASYDKSQGFQNFQAGMQRQIVMGDP-----EQHFYNNSTQSG---NPT---YIVWVKESRVF---ERFDIAEPSQLAVVELIAGD---	809
PrPGA	736	LTFR-----ENNFIHSGPAGLGVYNAMQTAVG---KDGKRETVSGT-----SYIQVVFDEQCEPKA---QGLLASESSNPEBASSDCTEAFSKKO-WALFSTEQCADPAYEVQVISEEDPR---	844
PrPGA	659	TRCQ-----ERAIIDGQHGPGVNAIQSVR---KGDHLEVGGT---SYIQVTFPFGQIG---RGLIAYSQSSDPRFHTDGLVMEYFNG-RKSLWLTQDVEAHKESQEVLVQR---	837
QqaR	679	VRGG-----DPTSLERAEFEGVLDKIDFNPLAPGGVGVGNAS---SYIQVTFPFGQIG---RGLIAYSQSSDPRFHTDGLVMEYFNG-RKSLWLTQDVEAHKESQEVLVQR---	761
QuiP	748	LDRGP-----YPAGGDHTLTDVSAYCWQQDFDTWILPAMRLVDFGQS---EPKGVNNSQSG---EAVLITYSQSSNPEBASSDCTEAFSKKO-WALFSTEQCADPAYEVQVISEEDPR---	785
Slac1	320	MSGK-----EIKFNLIEDTKQF---EIKFNLIEDTKQF---KTPMHADIMAQ-----VDKGAQTITWSKM---	359
Slac2	321	YEGH-----QIKFNFAEVSVG---KEPVCANLSVD-----AHEGRAAPFTFGKCS---	361
SlPVA*	697	VRNG-----KRIPVSGTESLGIWNKIEPVWNPAAAGGYTEVSAGS---SYIQVQWDDSRCPV-ARTLLTYSQSSNPNPSFYSQDTRLYAGER-MVTSFSCERARSFDRVVRVHERR---	806
SmPVA	705	VRKG-----KRIPVSGTESLGIWNKIEPVWNPAAAGGYTEVSAGT---SYIQVQWDDSRCPV-ARTLLTYSQSSDPRFHTDGLVMEYFNG-RKSLWLTQDVEAHKESQEVLVQR---	814
SsCIA	695	VRNG-----KRLPIGSGTESLGIWNKTEPQWNAAGGGYTESSGS-----SYIQVQWDDSRCPV-ARTLLTYSQSSNPNPSFYSQDTRLYAGER-MVTSFSCERARSFDRVVRVHERR---	804

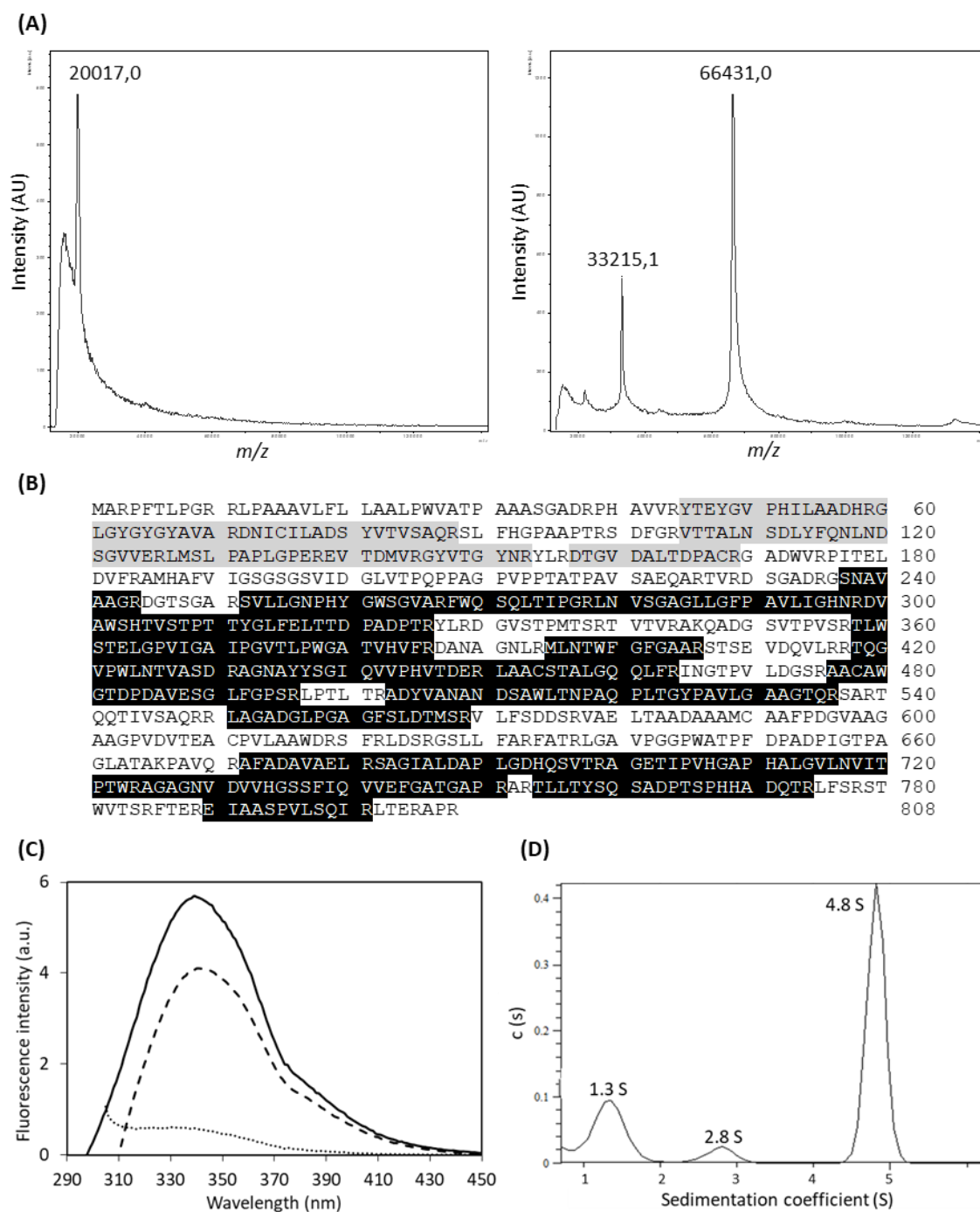
**Supplementary Figure S1.** Multiple amino acid sequence alignment of *AuAHLA* and other acylases from the Ntn-hydrolase superfamily by ClustalW. Ntn-hydrolases with reported AHL acylase activity: AiiD, from *Ralstonia* sp. XJ12B (NCBI accession no. AAO41113); PvdQ, from *Pseudomonas aeruginosa* PAO1 (AAG05773); AhlM, from *Streptomyces* sp. M664 (AAT68473); QuiP, from *P. aeruginosa* PAO1 (AAG04421); HacA, from *Pseudomonas syringae* B728a (AAY37014); HacB, from *P. syringae* B728a (AAY39885); AiiC, from *Anabaena* sp. strain PCC7120 (BAB75623); Aac, from *Shewanella* sp. Strain MIB015 (BAF94155); QqaR, from *Deinococcus radiodurans* R1 (WP\_010889514); MacQ, from *Acidovorax* sp. MR-S7 (BAV56778), PfmA, *Pseudoalteromonas flavipulchra* JG1 (ASS36259); AmiE, *Acinetobacter* sp. Ooi24 (BAP18758); AibP, from *Brucella melitensis* (AAL53453); Aac, from *Ralstonia solanacearum* GMI1000 (WP\_011002462); SlPVA, from *Streptomyces lavendulae* ATCC 13664 (AAU09670), *AuAAC*, from *Actinoplanes utahensis* NRRL 12052 (WP\_043523659); AhaP from *Psychrobacter* sp. M9-54-1 (WP\_200663734.1); Slac1, from *Shewanella loihica* PV-4 (ABO22553.1); Slac2, from *Shewanella loihica* PV-4 (ABO24671.1); and *AuAHLA*, from *A. utahensis* NRRL 12052 (WP\_052163432). Ntn-hydrolases with reported penicillin G acylase activity: EcPGA, from *Escherichia coli* ATCC 11105 (P06875); KcPGA, from *Kluyvera citrophila* DSM 2660 (P07941); AcPGA, from *Achromobacter* sp. strain CCM 4824 (AAY25991); BmPGA, from *Bacillus megaterium* ATCC 14945 (Q60136); AfPGA, from *Alcaligenes faecalis* ATCC 19018 (AAB71221); AvPGA, from *Arthrobacter viscosus* ATCC 15294 (P31956); and PrPGA, from *Providencia rettgeri* (AAP86197). Ntn-hydrolases with reported penicillin V acylase activity: SmPVA, from *Streptomyces mobaraensis* (BAF51977); AtPVA, from *Agrobacterium tumefaciens* (5J9R); PaPVA, from *Pectobacterium atrosepticum* (4WL2);

*BspPVA*, from *Bacillus sphaericus* (3PVA); and *BsPVA*, from *Bacillus subtilis* (2OQC). Ntn-hydrolases with reported bile salt hydrolase activity: *BfBSH*, from *Bifidobacterium longum* (2HF0), and *EfBSH*, from *Enterococcus faecalis* (4WL3). Ntn-hydrolases with reported cyclic lipopeptide acylase activity were as follows: *SsCLA*, from *Streptomyces* sp. FERM-BP5809 (BAD07025). Bifunctional acylases (with both penicillin and AHL acylase activities) are indicated by asterisks and, among them, *SlPVA* and *AuAAC* present aculeacin A acylase activity as well.

**Supplementary Table S1.** Amino acids involved in the catalysis predicted by COBALT sequence alignment using *AuAHLA*, *SIPVA*, *AuAAC* and other 174 acylases, and their characteristics\*.

Subunit	Residue			Secondary structure	Conserved		Most common	
	<i>SIPVA</i>	<i>AuAAC</i>	Residue		Residue	Side chain	Residue	Side chain
$\beta$ (catalytic residues)	$\beta$ Ser1	$\beta$ Ser1	$\beta$ Ser1	Coil	S (100 %)	Polar (100 %)	S (100 %)	Polar (100 %)
	$\beta$ His23	$\beta$ His23	$\beta$ His23	Coil	H (84 %)	Basic (87 %)	H (84 %)	Basic (87 %)
	$\beta$ Val70	$\beta$ Val70	$\beta$ Val70	Coil	V (33 %)	Nonpolar (63 %)	V (33 %)	Nonpolar (63 %)
	$\beta$ Asn272	$\beta$ Asn268	$\beta$ Asn274	Coil	N (99 %)	Polar (100 %)	N (99 %)	Polar (100 %)
$\alpha$ (substrate binding pocket)	$\alpha$ Ala154	$\alpha$ Met149	$\alpha$ Val157	Helix	A (22 %)	Nonpolar (66 %)	A (22 %)	Nonpolar (66 %)
	$\alpha$ Gly158	$\alpha$ Gly153	$\alpha$ Gly161	Coil	M (6 %)	Nonpolar (66 %)	G (34 %)	Polar (55 %)
	$\alpha$ Thr167	$\alpha$ Val162	$\alpha$ Val170	Coil <sup><i>SIPVA</i></sup> Helix <sup><i>AuAAC</i>, <i>AuAHLA</i></sup>	V (12 %)	Nonpolar (66 %)	F (14 %)	Nonpolar (60 %)
					T (9 %)	Polar (25 %)		
$\beta$ (substrate binding pocket)	$\beta$ Gly8	$\beta$ Ala8	$\beta$ Arg8	Coil	G (28 %)	Polar (36 %)	P (41 %)	Nonpolar (47 %)
	$\beta$ Thr10	$\beta$ Ala10	$\beta$ Gly10	Coil	A (5 %)	Nonpolar (47 %)	K (41 %)	Basic (80 %)
	$\beta$ Tyr24	$\beta$ Phe24	$\beta$ Tyr24	Coil	R (4 %)	Basic (17 %)	L (43 %)	Nonpolar (77 %)
	$\beta$ Arg31	$\beta$ Arg31	$\beta$ Arg31	Helix <sup><i>SIPVA</i></sup> Strand <sup><i>AuAAC</i>, <i>AuAHLA</i></sup>	Y (4 %)	Polar (12 %)	R (24 %)	Nonpolar (53 %)
	$\beta$ Phe32	$\beta$ Phe32	$\beta$ Phe32	Helix <sup><i>SIPVA</i></sup> Strand <sup><i>AuAAC</i>, <i>AuAHLA</i></sup>	F (24 %)	Nonpolar (77 %)	W (42 %)	Nonpolar (72 %)
	$\beta$ Trp33	$\beta$ Tyr33	$\beta$ Trp33	Helix <sup><i>SIPVA</i></sup> Strand <sup><i>AuAAC</i>, <i>AuAHLA</i></sup>	F (24 %)	Nonpolar (72 %)	Y (62 %)	Polar (69 %)
	$\beta$ Leu50	$\beta$ Leu50	$\beta$ Leu50	Coil	W (14 %)	Nonpolar (31 %)	L (40 %)	Nonpolar (86 %)
	$\beta$ Ser53	$\beta$ Asp53	$\beta$ Phe53	Coil	Y (62 %)	Polar (69 %)	L (27 %)	Nonpolar (75 %)
	$\beta$ Ser57	$\beta$ Glu57	$\beta$ Leu57	Strand	S (6 %)	Polar (25 %)	V (21 %)	Nonpolar (59 %)
	$\beta$ Ile58	$\beta$ Ile58	$\beta$ Ile58	Strand	D (1 %)	Acidic (1 %)	I (40 %)	Nonpolar (93 %)
	$\beta$ Ser67	$\beta$ Ser67	$\beta$ Ser67	Strand	F (2 %)	Nonpolar (75 %)	G (38 %)	Polar (98 %)
	$\beta$ His68	$\beta$ His68	$\beta$ His68	Strand	S (9 %)	Polar (26 %)	H (26 %)	Nonpolar (49 %)
	$\beta$ Thr69	$\beta$ Thr69	$\beta$ Thr69	Coil	E (1 %)	Acidic (1 %)	T (94 %)	Polar (99 %)
	$\beta$ Thr72	$\beta$ Thr72	$\beta$ Thr72	Helix	L (18 %)	Nonpolar (59 %)	T (23 %)	Polar (54 %)
	$\beta$ Val186	$\beta$ Val182	$\beta$ Leu188	Strand	I (40 %)	Nonpolar (93 %)	Q (20 %)	Nonpolar (54 %)
					S (20 %)	Polar (98 %)		
					H (26 %)	Basic (26 %)		
					T (94 %)	Polar (99 %)		
					T (23 %)	Polar (54 %)		
					V (16 %)	Nonpolar (54 %)		
					L (15 %)	Nonpolar (54 %)		

\*Secondary structure of each residue, percentage of conserved amino acids and most common residue on each position and side chain properties.



**Supplementary Figure S2.** Purified *AuAHLA* analysis. **(A)** MALDI-TOF analysis of  $\alpha$ -subunit (left) and  $\beta$ -subunit (right) of *AuAHLA*. **(B)** Mass fingerprint of  $\alpha$ -subunit (highlighted in grey) and  $\beta$ -subunit (highlighted in black) of *AuAHLA*. **(C)** Fluorescence emission spectra of *AuAHLA*:  $\lambda_{\text{ex}} = 275$  nm (solid line),  $\lambda_{\text{ex}} = 295$  nm (dashed line) and contribution of tyrosine residues to the emission spectra (dotted line). **(D)** Analytical ultracentrifugation analysis of *AuAHLA*:  $c(s)$  distribution as a function of sedimentation coefficient (S).

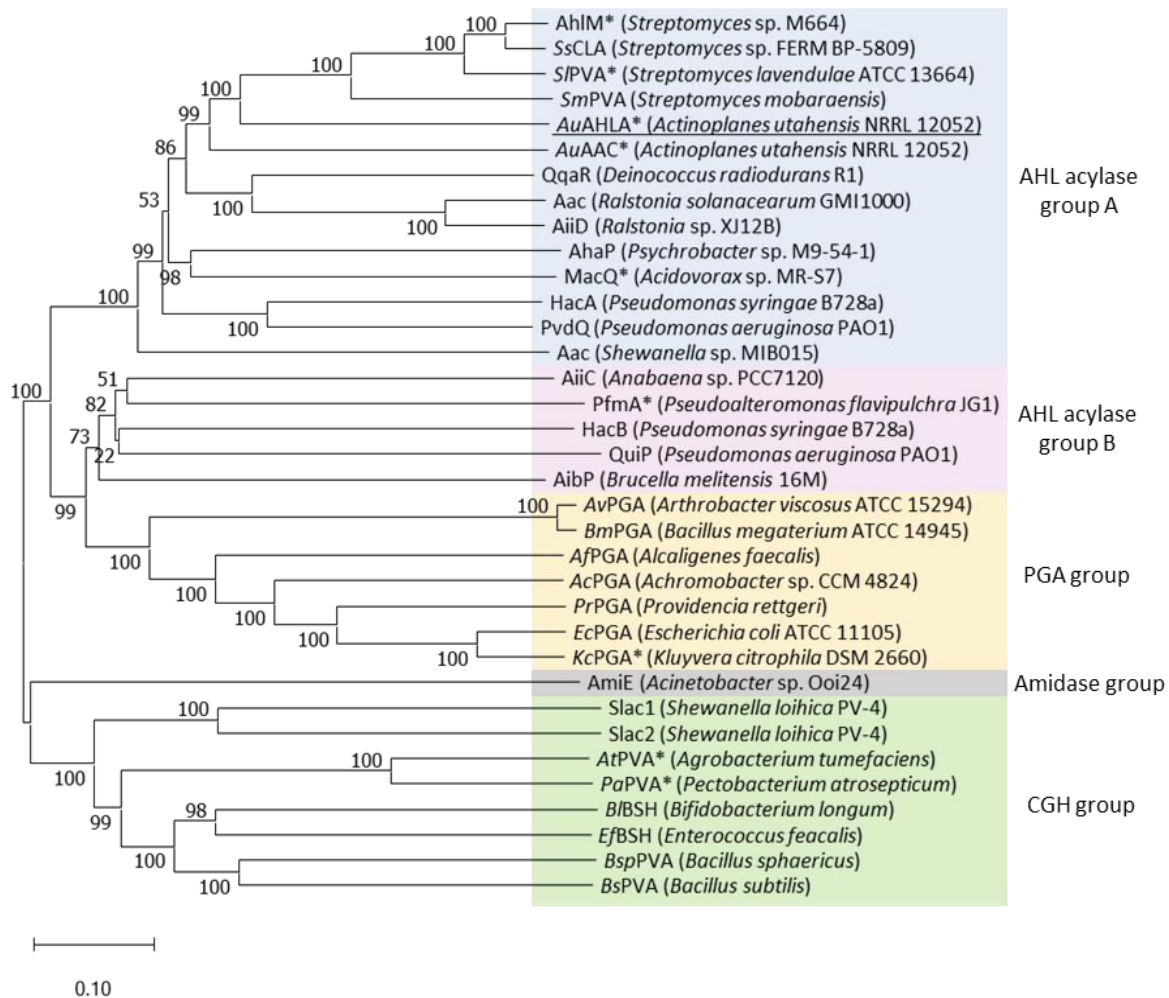
**Supplementary Table S2.** Purification table of *Au*AHLA by ionic exchange chromatography.

Step	Activity (IU/mL)	Protein concentration (mg/mL)	Specific activity (IU/mg)	Yield (%)	Purification (fold)
Cell-free broth	0.19	0.042	4.5	100	1
SP Sepharose	0.60	0.011	54.5	37	12
Ultrafiltration	8.40	0.096	87.5	22	19

**Supplementary Table S3.** MRM parameters of AHLs analysis.

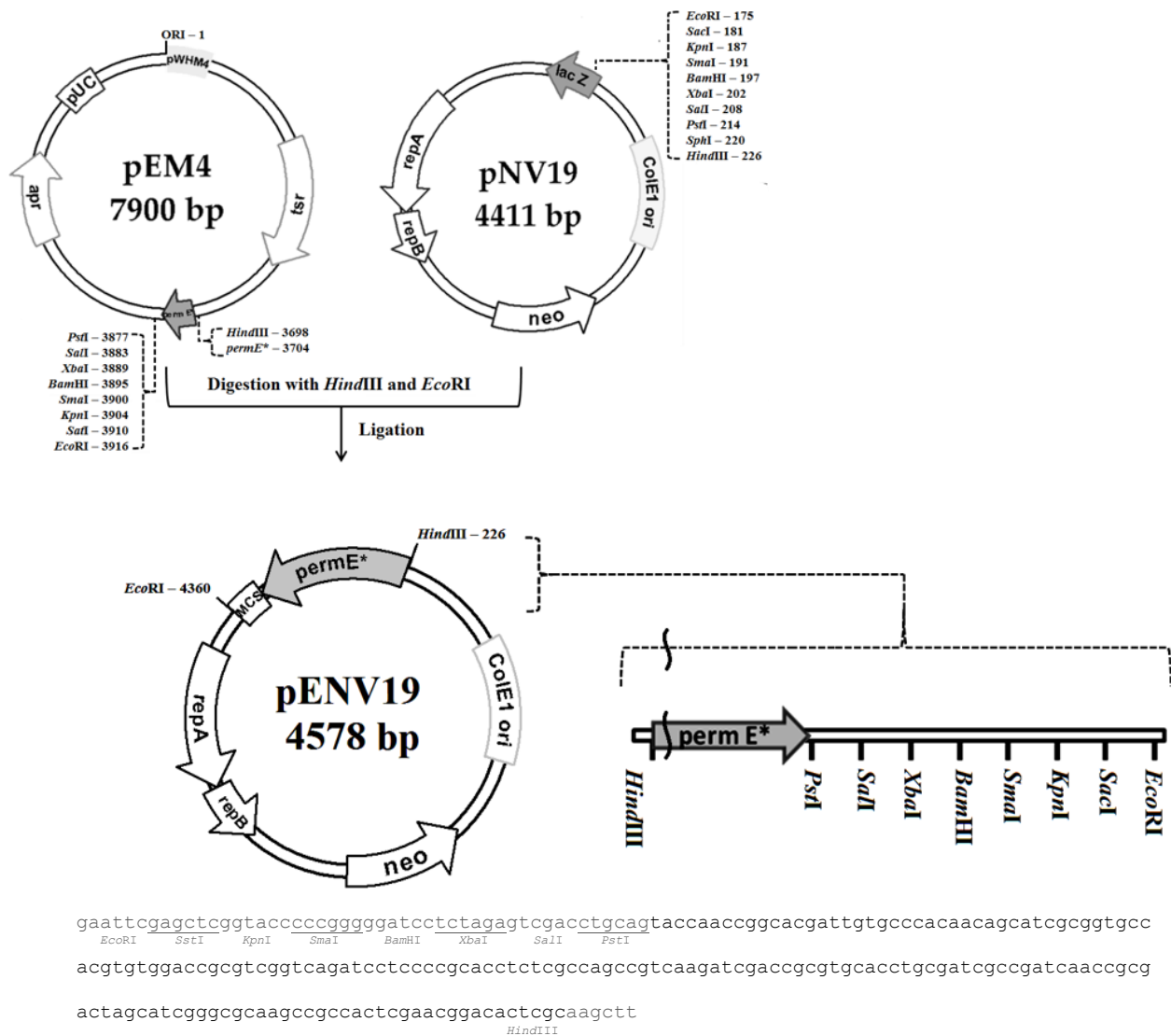
AHLs	Retention time (min)	Parent ion ( <i>m/z</i> )	Product ions ( <i>m/z</i> )	Collision Energy (V)
C <sub>4</sub> -HSL	2.9	172.10	43.05	-27
			71.00	-12
			102.05	-14
C <sub>6</sub> -HSL	4.7	200.10	42.95	-30
			98.95	-12
			102.05	-12
C <sub>8</sub> -HSL	5.7	228.10	210.20	-15
			109.10	-15
			102.10	-15
C <sub>10</sub> -HSL	6.2	256.10	238.30	-17
			102.10	-17
			109.10	-17
C <sub>12</sub> -HSL	6.6	284.10	266.20	-17
			109.10	-17
			102.10	-17
3-oxo-C <sub>6</sub> -HSL	3.4	212.10	85.10	18
			87.05	10
			57.05	30
3-oxo-C <sub>10</sub> -HSL	5.8	268.10	143.15	13
			141.20	20
			57.05	35
3-oxo-C <sub>12</sub> -HSL	6.3	296.30	171.20	14
			169.20	17
			57.10	11





**Supplementary Figure S3.** Phylogenetic analysis of *AuAHLA* and other acylases from the Ntn-hydrolase superfamily. Evolutionary analysis was conducted in MEGA X and the phylogenetic tree was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the p-distance method and are in the units of the number of amino acid differences per site. Ntn-hydrolases with reported AHL acylase activity: AiiD, from *Ralstonia* sp. XJ12B (NCBI accession no. AAO41113); PvdQ, from *Pseudomonas aeruginosa* PAO1 (AAG05773); AhIM, from *Streptomyces* sp. M664 (AAT68473); QuiP, from *P. aeruginosa* PAO1 (AAG04421); HacA, from *Pseudomonas syringae* B728a (AAY37014); HacB, from *P. syringae* B728a (AAY39885); AiiC, from *Anabaena* sp. strain PCC7120 (BAB75623); Aac, from *Shewanella* sp. Strain MIB015 (BAF94155); QqaR, from *Deinococcus radiodurans* R1 (WP\_010889514); MacQ, from *Acidovorax* sp. MR-S7 (BAV56778), PfmA, from *Pseudoalteromonas flavipulchra* JG1 (ASS36259); AmiE, *Acinetobacter* sp. Ooi24 (BAP18758); AibP, from *Brucella melitensis* (AAL53453); Aac, from *Ralstonia solanacearum* GMI1000 (WP\_011002462); SIPVA, from *Streptomyces lavendulae* ATCC 13664 (AAU09670), *AuAAC*, from *Actinoplanes utahensis* NRRL 12052 (WP\_043523659); AhaP, from *Psychrobacter* sp. M9-54-1 (WP\_200663734.1); Slac1, from *Shewanella loihica* PV-4 (ABO22553.1); Slac2, from *Shewanella loihica* PV-4 (ABO24671.1); and *AuAHLA*, from *A. utahensis* NRRL 12052 (WP\_052163432). Ntn-hydrolases with reported penicillin G acylase activity: *EcPGA*, from

*Escherichia coli* ATCC 11105 (P06875); KcPGA, from *Kluyvera citrophila* DSM 2660 (P07941); AcPGA, from *Achromobacter* sp. strain CCM 4824 (AAY25991); BmPGA, from *Bacillus megaterium* ATCC 14945 (Q60136); AfPGA, from *Alcaligenes faecalis* ATCC 19018 (AAB71221); AvPGA, from *Arthrobacter viscosus* ATCC 15294 (P31956); and PrPGA, from *Providencia rettgeri* (AAP86197). Ntn-hydrolases with reported penicillin V acylase activity: SmpPVA, from *Streptomyces mobaraensis* (BAF51977); AtPVA, from *Agrobacterium tumefaciens* (5J9R); PaPVA, from *Pectobacterium atrosepticum* (4WL2); BspPVA, from *Bacillus sphaericus* (3PVA); and BspPVA, from *Bacillus subtilis* (2OQC). Ntn-hydrolases with reported bile salt hydrolase activity: BBSH, from *Bifidobacterium longum* (2HF0), and EfBSH, from *Enterococcus faecalis* (4WL3). Ntn-hydrolases with reported cyclic lipopeptide acylase activity were as follows: SsCLA, from *Streptomyces* sp. FERM-BP5809 (BAD07025). Bifunctional acylases (with both penicillin and AHL acylase activities) are indicated by asterisks and, among them, S1PVA and AuAAC present aculeacin A acylase activity as well.



**Supplementary Figure S4.** Shuttle vector pENV19 construction.