

SUPPLEMENTARY INFORMATION

Table S1. UHPLC-HRMS data of AHL Standards. Rt: Retention time. Theoretical and Observed mass correspond to the pseudo-molecular ion $[M + H]^+$.

Standard	Molecular formula	Theoretical mass	Observed mass	Rt (min)	Limit of detection (nM)
C4-HSL	C8H13NO3	172.0968	172.0968	5.27	>500
C6-HSL	C10H17NO3	200.1281	200.1281	8.43	3.64
OXO-C6-HSL	C10H15NO3	214.1074	214.1072	7.56	10.90
C7-HSL	C11H19NO3	214.1438	214.1440	8.83	5.33
C8-HSL	C12H21NO3	228.1594	228.1594	9.27	6.50
OXO-C8-HSL	C12H19NO4	242.1387	242.1381	8.69	6.15
OH-C8-HSL	C12H21NO4	244.1543	244.1540	8.55	-
C9-HSL	C13H23NO3	242.1751	242.1748	9.57	7.37
C10-HSL	C14H25NO3	256.1907	256.1907	9.90	4.56
OXO-C10-HSL	C14H23NO4	270.1700	270.1699	9.43	2.91
OH-C10-HSL	C14H25NO4	272.1856	272.1856	9.25	3.23
C11-HSL	C15H27NO3	270.2064	270.2063	10.13	9.11
C12-HSL	C16H29NO3	284.2220	284.2220	10.46	5.07
OXO-C12-HSL	C16H27NO4	298.2013	298.2013	10.04	21.28
OH-C12-HSL	C16H29NO4	300.2169	300.2169	9.87	2.39
C13-HSL	C17H31NO3	298.2377	298.2377	10.63	14.78
C14-HSL	C18H33NO3	312.2533	312.2533	10.93	11.82
C14:1-HSL	C18H31NO3	310.2377	310.2370	10.51	9.41
OXO-C14:1-HSL	C18H29NO4	324.2169	324.2170	10.23	4.71
OXO-C14-HSL	C18H31NO4	326.2326	326.2322	10.56	6.79
OH-C14-HSL	C18H33NO4	328.2482	328.2482	10.42	36.58
C15-HSL	C19H35NO3	326.2690	326.2689	11.15	15.11
C16-HSL	C20H37NO3	340.2846	340.2846	11.34	16.30
C16:1-HSL	C20H35NO3	338.2690	338.2704	10.93	14.68
OXO-C16:1-HSL	C20H33NO4	352.2482	352.2497	10.61	6.75
C18-HSL	C22H42NO3	368.3159	368.3155	11.66	10.48
C18:1-HSL	C22H39NO3	366.3003	366.3003	11.40	28.56

Table S2. UHPLC-HRMS data and identification of 3-OH-C9-HSL in *A. fischeri* strains.

Rt: Retention time. Theoretical and observed mass correspond to the pseudo-molecular ion $[M + H]^+$. Bold characters in the fragmentation column are highlighting the daughter ions characteristic of the lactone ring.

Strain	Rt (min)	Observed mass	Molecular Formula	Delta ppm	Fragmentation	Identification			
						Name	Molecular Formula	Molecular Weight	Theoretical Mass
ES213	8,9	258,1701	C13H24NO4	0,115	70.041 (16.93), 74.061 (9.53) , 75.027 (16.64), 91.055 (14.94), 93.037 (31.41), 102.055 (12.41) , 105.070 (10.50), 109.076 (66.51), 122.084 (18.27), 135.092 (53.91), 137.060 (14.71), 151.075 (81.97), 229.097 (23.28), 244.120 (21.08), 257.128 (13.45), 257.175 (16.47), 258.110 (21.03), 259.144 (100.00)	OH-C9-HSL	C13H23NO4	257,1627	258,1700
MB13B1		258,1702		0,718	70.041 (10.26), 74.061 (9.68) , 75.027 (12.38), 91.055 (7.87), 93.037 (16.40), 102.055 (35.76) , 105.070 (6.38), 109.076 (31.04), 122.084 (10.24), 135.092 (52.27), 137.060 (8.18), 151.075 (33.32), 229.097 (23.46), 244.120 (23.04), 257.128 (6.53), 257.175 (6.67), 258.110 (9.27), 259.144 (100.00)				
KB2B1		258,1698		-0,185	70.041 (16.69), 74.061 (10.36) , 75.027 (12.97), 91.055 (9.90), 93.037 (15.18), 102.055 (18.65) , 105.070 (9.32), 109.076 (54.58), 122.084 (12.89), 135.092 (53.85), 137.060 (10.73), 151.075 (69.36), 229.097 (24.46), 244.120 (18.15), 257.128 (21.52), 257.176 (18.03), 258.110 (17.91), 259.144 (100.00)				
KB4B5		258,1696		-0,395	70.041 (14.37), 74.061 (13.51) , 75.027 (18.06), 91.055 (12.21), 93.037 (29.99), 102.055 (20.07) , 105.070 (12.15), 109.076 (56.22), 122.084 (12.35), 135.092 (45.94), 137.060 (10.86), 151.075 (72.83), 229.097 (22.02), 244.120 (18.48), 257.128 (23.68), 257.177 (14.71), 258.109 (18.25), 259.144 (100.00)				
ES114		258,1700		0,214	70.041 (9.43), 74.061 (10.47) , 75.027 (36.97), 91.055 (8.93), 93.037 (100.00), 102.055 (14.35) , 105.070 (9.00), 109.076 (45.02), 122.084 (9.23), 135.092 (76.51), 137.060 (9.31), 151.075 (51.00), 229.097 (29.82), 244.120 (11.06), 257.128 (15.74), 257.176 (13.72), 258.110 (22.61), 259.144 (100.00)				

Table S3. UHPLC-HRMS data and identification of oxo-C16:2-HSL in *A. fischeri* strains. Rt: Retention time. Theoretical and observed mass correspond to the pseudo-molecular ion $[M + H]^+$. Bold characters in the fragmentation column are highlighting the daughter ions characteristic of the lactone ring.

Strain	Rt (min)	Observed mass	Molecular Formula	Delta ppm	Fragmentation	Identification			
						Name	Molecular Formula	Molecular Weight	Theoretical Mass
ES114	9,97	350,2326	C20H32NO4	-0,043	56.050 (2.68) , 57.071 (19.57), 69.071 (3.46), 71.086 (24.65), 73.066 (3.34), 74.061 (13.01) , 89.060 (75.83), 95.086 (0.69), 102.055 (17.33) , 105.070 (100.00), 106.074 (7.71), 109.102 (2.31), 123.085 (1.89), 127.112 (9.03), 133.086 (17.83), 146.081 (6.71), 224.128 (17.40)	OXO-C16:2-HSL	C20H31NO4	349,2253	350,2326
					56.050 (1.26) , 57.071 (22.15), 69.071 (4.62), 71.086 (32.15), 73.066 (2.63), 74.061 (10.98) , 89.060 (55.17), 95.086 (2.30), 102.055 (17.11) , 105.070 (100.00), 106.074 (7.70), 109.102 (3.48), 123.081 (2.07), 127.112 (10.10), 133.086 (12.62), 146.081 (3.12), 224.128 (12.15)				
					56.050 (3.02) , 57.071 (21.38), 69.071 (4.10), 71.086 (17.95), 73.066 (6.71), 74.061 (21.54) , 89.060 (38.94), 95.086 (1.56), 102.055 (19.36) , 105.070 (100.00), 106.074 (6.27), 109.102 (4.63), 123.081 (1.93), 127.112 (12.08), 133.086 (19.49), 146.081 (4.58), 224.128 (15.72)				

Fig. S1. Retention times curves for Cx-HSL, oxo-Cx-HSL and OH-Cx-HSL based on 23 AHL standards ($R^2 > 0.99$).

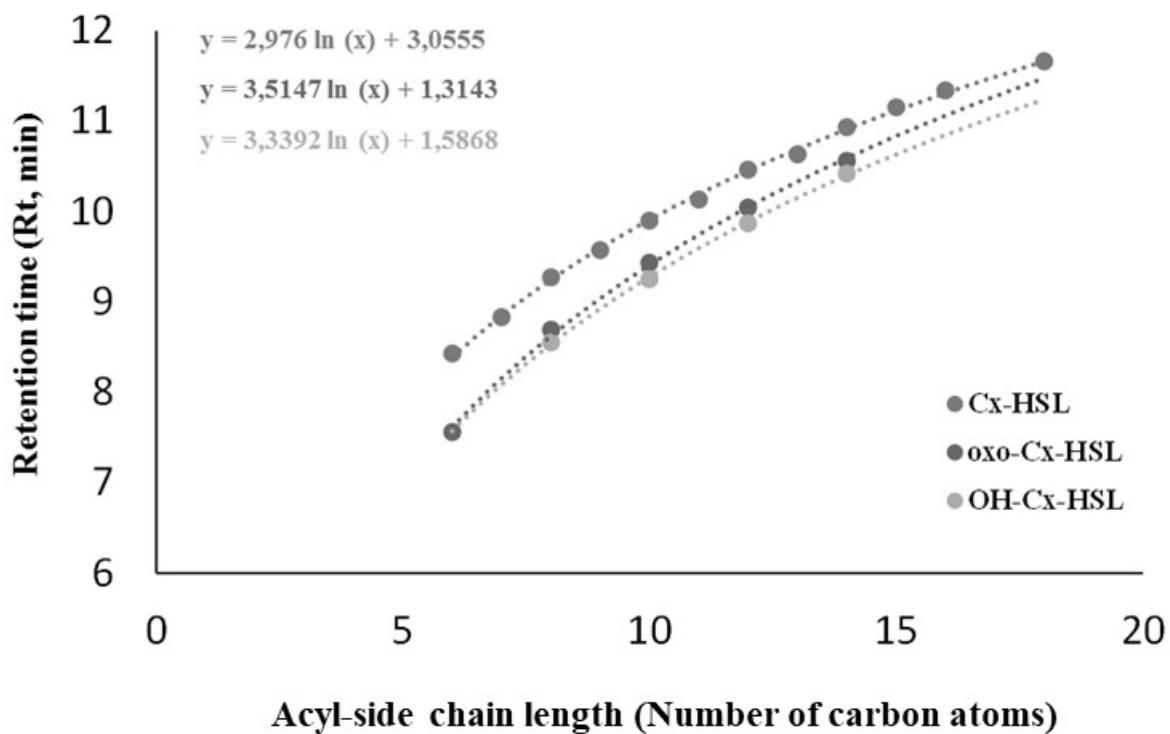


Fig. S2. Neighbour joining tree of 13 symbiotic *V. fischeri* strains using the JTT model (MEGA). Bootstrap values are based on 1000 replicates. A: AinR (623 aa); B: LuxI (190 aa) and C: LuxR (250 aa).

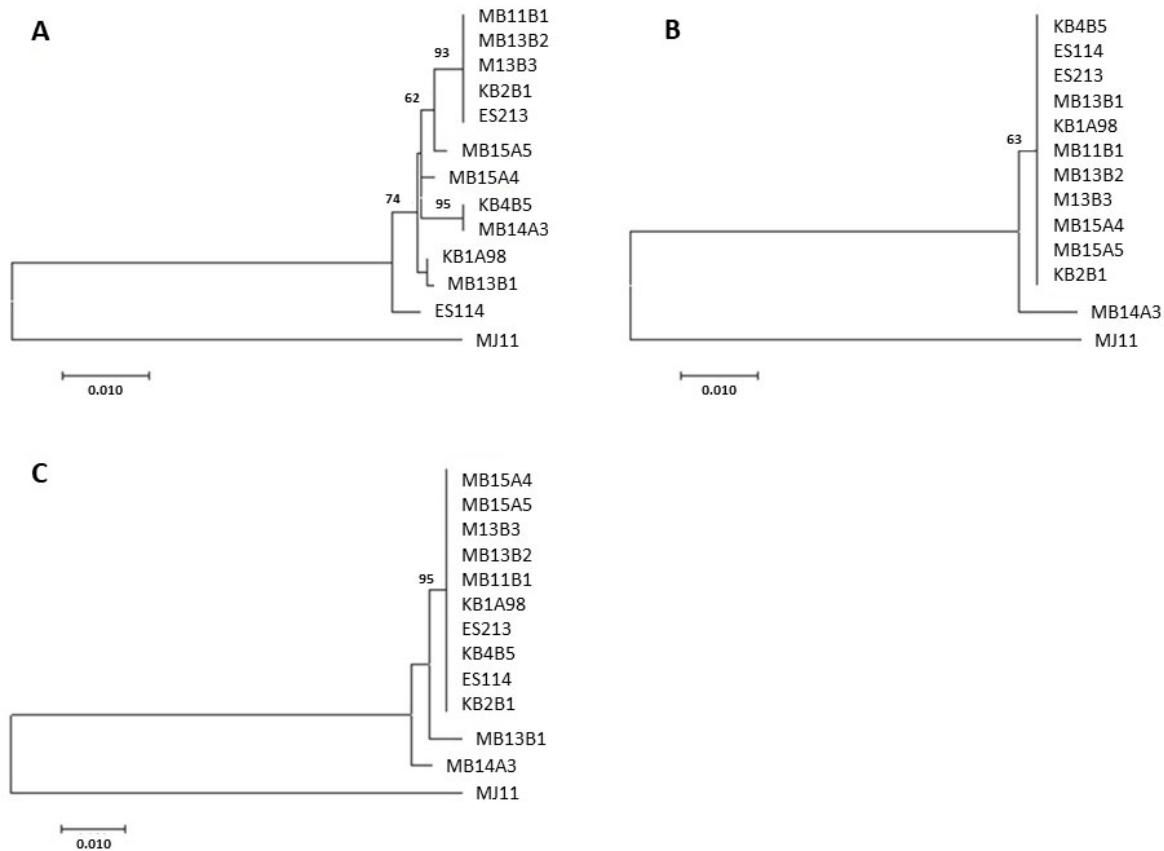


Fig. S3. Alignment of LuxR proteins from the 5 *V. fischeri* strains (250 aa). Asterisks are indicating the amino acid changes in MB13B1 described in Table 2.

	10	20	30	40	50	60	70
ES114	MNIKNINANEKIIDKIKTCNNNKDINQCLSEIAKIIHCEYYLFIAIYPHSIIIPDPDVSIIDNYPEKWRKYYDD						
KB2B1	MNIKNINANEKIIDKIKTCNNNKDINQCLSEIAKIIHCEYYLFIAIYPHSIIIPDPDVSIIDNYPEKWRKYYDD						
ES213	MNIKNINANEKIIDKIKTCNNNKDINQCLSEIAKIIHCEYYLFIAIYPHSIIIPDPDVSIIDNYPEKWRKYYDD						
KB4B5	MNIKNINANEKIIDKIKTCNNNKDINQCLSEIAKIIHCEYYLFIAIYPHSIIIPDPDVSIIDNYPEKWRKYYDD						
MB13B1	MNIKNINANEKIIDKIKTCNNNKDINQCLSEIAKIIHCEYYLFIAIYPHSIIIPDPDVSIIDNYPEKWRKYYDD						
	80	90	100	110	120	130	140
ES114	AGLLEYDPVVVDYSKSHHSPINWNVFEKKTIKKESPNVIKEAQESGLITGFSFFIHTASNGFGMLSFAHSKD						
KB2B1	AGLLEYDPVVVDYSKSHHSPINWNVFEKKTIKKESPNVIKEAQESGLITGFSFFIHTASNGFGMLSFAHSKD						
ES213	AGLLEYDPVVVDYSKSHHSPINWNVFEKKTIKKESPNVIKEAQESGLITGFSFFIHTASNGFGMLSFAHSKD						
KB4B5	AGLLEYDPVVVDYSKSHHSPINWNVFEKKTIKKESPNVIKEAQESGLITGFSFFIHTASNGFGMLSFAHSKD						
MB13B1	AGLLEYDPVVVDYSKSHHSPINWNVFEKKTIKKESPNVIKEAQESGLITGFSFFIHTASNGFGMLSFAHSKD	*					*
	150	160	170	180	190	200	210
ES114	IYTDSLFLHASTNVPLMLPSLVNDNYQKINTTRKKSDSILTKREKECLAWASEGKSTWDISKILGCSERTVTFH						
KB2B1	IYTDSLFLHASTNVPLMLPSLVNDNYQKINTTRKKSDSILTKREKECLAWASEGKSTWDISKILGCSERTVTFH						
ES213	IYTDSLFLHASTNVPLMLPSLVNDNYQKINTTRKKSDSILTKREKECLAWASEGKSTWDISKILGCSERTVTFH						
KB4B5	IYTDSLFLHASTNVPLMLPSLVNDNYQKINTTRKKSDSILTKREKECLAWASEGKSTWDISKILGCSERTVTFH						
MB13B1	IYTDSLFLHASTNVPLMLPSLVNDNYQKINTTRKKSDSILTKREKECLAWASEGKSTWDISKILGCSERTVTFH	*					
	220	230	240	25			
ES114	LTNTQMQLNTTNRCQSISKAILTGAINCPYLN			•			
KB2B1	LTNTQMQLNTTNRCQSISKAILTGAINCPYLN						
ES213	LTNTQMQLNTTNRCQSISKAILTGAINCPYLN						
KB4B5	LTNTQMQLNTTNRCQSISKAILTGAINCPYLN						
MB13B1	LTNTQMQLNTTNRCQSISKAILTGAINCPYLN						

Fig. S4. Alignment of AinS proteins from the 5 *V. fischeri* strains (355 aa). Asterisks are indicating the amino acid changes in MB13B1 described in Table 2. Accession numbers: ES114: WP_011261670.1; ES213/KB2B1: WP_081248857.1; KB4B5: WP_081249183.1; MB13B1: WP_081249608.1.

	10	20	30	40	50	60	70	
ES114	-----LYDYCSQTQNFNLLQQLINARERIILNKFEGGTIGNLEYL	FSTSEAAEILGKQPCSSLPK						
KB2B1	-----LYDYCSQTQNFNLLQQLINARERIILNKFEGGTIGNLEYL	FSTSEAAEILGKQPCSSLPK						
ES213	-----	LNKFEGGTIGNLEYL	FSTSEAAEILGKQPCSSLPK					
KB4B5	-----LYDYCSQTQNFNLLQQLINARERIILNKFEGGTIGNLEYL	FSTSEAAEILGKQPCSSLPK						
MB13B1	-----LYDYCSQTQNFNLLQQLINARERIILNKFEGGTIGNLEYL	FSTSEAAEILGKQPCSSLPK	*					
	80	90	100	110	120	130	140	150
ES114	VYFHIEQMAIKVFGCLLKCSTE	FEMFKLIHKPMSVLHRHLDVTKKNSTS	AIIDEKYHYELVQDISTDERYF	HTEFS				
KB2B1	VYFHIEQMAIKVFGCLLKCSTE	FEMFKLIHKPMSVLHRHLDITKKNSTS	AIIDEKYHYELVQDISTDERYF	HTEFS				
ES213	VYFHIEQMAIKVFGCLLKCSTE	FEMFKLIHKPMSVLHRHLDITKKNSTS	AIIDEKYHYELVQDISTDERYF	HTEFS				
KB4B5	VYFHIEQMAIKVFGCLLKCSTE	FEMFKLIHKPMSVLHRHLDVTKKNSTS	AIIDEKYHYELVQDISTDERYF	HTEFS				
MB13B1	VYFHIEQMAIKVFGCLLKCSTE	FEMFKLIHKPMSVLHRHLDVTKKNSTS	AIIDEKYHYELVQDISTDERYF	HTEFS	*			
	160	170	180	190	200	210	220	
ES114	EHPLPLSDAIVLINIATFIKEHKWYEMLNHLEISSKG	EHFILEHYQYTDNNIYPTI	SSALIQTTSNKHDLWLFDTFFQ					
KB2B1	EHPLPLSDAIVLINIATFIKEHKWYEMLNHLEISSKG	EHFILEHYQYTDNNIYPTI	SSALIQTTSNKHDLWLFDTFFQ					
ES213	EHPLPLSDAIVLINIATFIKEHKWYEMLNHLEISSKG	EHFILEHYQYTDNNIYPTI	SSALIQTTSNKHDLWLFDTFFQ					
KB4B5	EHPLPLSDAIVLINIATFIKEHKWYEMLNHLEISSKG	EHFILEHYQYTDNNIYPTI	SSALIQTTSNKHDLWLFDTFFQ					
MB13B1	EHPLPLSDAIVLINIATFIKEHKWYEMLNHLEISSKG	EHFILEHYQYTDNNIYPTI	SSALIQTTSNKHDLWLFDTFFQ	*				
	230	240	250	260	270	280	290	300
ES114	NTKWKPNLNADGLLSILSHATFMSPLARSHFVDKSSTEIENDLFYSILD	TKACEAIRLTISGEKS	KANYHLYLAQKG					
KB2B1	NTKWKPNLNADGLLSILSHATSMSPLARSQFVDKSSTEIENDLFYSILD	TKACEAIRLTISGEKS	KANYHLYLAQKG					
ES213	NTKWKPNLNADGLLSILSHATSMSPLARSQFVDKSSTEIENDLFYSILD	TKACEAIRLTISGEKS	KANYHLYLAQKG					
KB4B5	NTKWKPNLNADGLLSILSHATSMSPLARSQFVDKSSTEIENDLFYSILD	TKACEAIRLTISGEKS	KANYHLYLAQKG					
MB13B1	NTKWKPNLNADGLLSILSHATSMSPLTRSQFVDKSSTEIENDLFYSILD	TKACEAIRLTISGEKS	KANYHLYLAQKG	*				
	310	320	330	340	350	360	370	380
ES114	LAMALKESGRDIVFTIIEKPAMVLFYQSMNLLPTQSPYVFTSE	QDVNKSGIITYKG	IWLKNASSAFNQYSFKAYNA					
KB2B1	LAMALKESGRDIVFTIIEKPAMVLFYQSMNLLPTQSPYVFTSE	QDVNKSGIITYKG	IWLKNASSAFNQYSFKAYNA					
ES213	LAMALKESGRDIVFTIIEKPAMVLFYQSMNLLPTQSPYVFTSE	QDVNKSGIITYKG	IWLKNASSAFNQYSFKAYNA					
KB4B5	LAMALKESGRDIVFTIIEKPAMVLFYQSMNLLPTQSPYVFTSE	QDVNKSGIITYKG	IWLKNASSAFNQYNFKAYNA					
MB13B1	LAMALKESGRDIVFTIIEKPAMVLFYKAMNLAP	TQSPYVFTSE	QDVNKSGIITYKG	IWLKNASSAFNQYSFKAYNA	**	*		
	390							
ES114	NIISLRKKLNRQ							
KB2B1	NIISLRKKLNRQ							
ES213	NIISLRKKLNRQ							
KB4B5	NIISLRKKLNRQ							
MB13B1	NIISLRKKLNRQ							