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Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Phylogenetic analysis of sequences of QS6-1.
A. Midpoint rooted ML tree of homologue sequences of QS6-1-1, transglutaminase-like enzyme.
B. Midpoint rooted ML tree of homologues of QS6-1-7, hypothetical protein.
C. Midpoint rooted ML tree of homologues of QS6-1-4, hypothetical protein.
Tree topologies were determined using phyml version 2.4.4. Numbers at the nodes are bootstrap values obtained from 1000 replicates using PAUP (Version 4.0b) under neighbour-joining algorithm (heuristic search). *A., Alteromonadales; B., Burkholderia; Br., Bradyrhizobium; Ca., Campylobacter; Ce., Cellulophaga; F., Flavobacterium; G., Gramella; H., Hahella; Hs., Halorhodospira; M., Mesorhizobium; Mc., Methylococcus; Ms., Magnetospirillum; P., Polaribacter; Pc., Prosthecochloris; Pf., Psychroflexus; S., Shewanella; X., Xanthomonas; V., Vibrio.*

Fig. S2. Midpoint rooted ML tree of homologues of QS10-1-2, Mg-chelatase related protein. Tree topology was determined using phyml version 2.4.4. Bootstrap values were obtained from using PAUP (Version 4.0b) under neighbour-joining algorithm ('Fast' stepwise-addition). *Ac., Acidovorax; Ae, Aeromonas; B., Burkholderia; C., Comamonas; Cb., Chromobacterium; Ch., Chromohalobacter; Co., Coxiella; D., Delftia; Dc, Dechloromonas; F., Francisella; H., Herminiimonas; J., Janthinobacterium; L., Leptothrix; Mb., Methylobacillus; Mc., Methylococcus; Nc., Nitrococus; Ne., Neisseria; Ns., Nitrosomonas; Nsc. Nitrosococcus; P., Polaromonas; R., Ralstonia; Rf., Rhodofera; T., Thiobacillus; V., Verminephrobacter; X, Xanthomonas.*

Fig. S3. ML tree of homologues of QS10-2-1, Zinc containing alcohol dehydrogenase protein. Tree topology was determined using phyml version 2.4.4. Bootstrap values were obtained using PAUP (Version 4.0b) under neighbour-joining algorithm (full heuristic). *A., Acidovorax; Ag., Agrobacterium; Ar., Azorhizobium; B., Burkholderia; Bt. Bordetella; Co., Comamonas; Cu., Cupriavidus; D., Delftia; G., Granulibacter; H., Herminiimonas; Ho., Hoeflea; J., Janthinobacterium; Jb., Janibacter; Mb., Methylibium; Mr, Mesorhizobium; Ns., Nitrosospira; O., Oceanicola; Pa., Parvibaculum; Pn., Polynucleobacter; Po., Polaromonas; R., Rhizobium; Rb., Roseobacter; Rf., Rhodoferax; Rhb., Rhodobacterales; Rhs., Rhodospirillum; Rt., Ralstonia; S., Serratia; Sb., Solibacter; T., Thermobifida; V., Verminephrobacter; X., Xanthobacter; Y., Yersinia.*

Fig. S4. A, ML tree of homologues of QS10-2-4, phytonyl-CoA dioxygenase related protein. B, ML tree of homologues of QS10-2-5, putative GntR family transcriptional regulator. Tree topologies were determined using phyml version 2.4.4. Bootstrap values were obtained using PAUP (Version 4.0b) under neighbour-joining algorithm (full heuristic). *B., Burkholderia; Bj., Beijerinckia; Bo, Bordetella; Br. Bradyrhizobium; E., Erythrobacter; M., Mesorhizobium; Mb., Mycobacterium; MGP, marine gamma proteobacterium; O., Oceanicaulis; Pb., Parvibaculum; Ps., Pseudomonas; Pa., Pseudoalteromonas; R., Ralstonia; Rh. Rhizobium; Rv., Roseovarius; Sm., Sphingomonas; Sp., Sphingopyxis; St., Streptomyces; T., Thermotoga; V., Verminephrobacter.*

Fig. S5. Midpoint rooted maximum likelihood trees of LuxI (A) and LuxR (B) homologues. Same as Fig. 4 except accession numbers for the sequences were supplied.

Fig. S6. ESI MS and MS/MS results of the standard 3-O-C12 HSL (Cayman chemical) and the active compounds produced by $LuxI_{QS10\text{-}1}$ (at spot I and K in Fig. 6).
A. MS (left) and MS/MS (right) of synthetic 3-O-C12 HSL.

B. MS (left) and MS/MS (right) of the active compounds at spot I. The fragmentation pattern is the same as synthetic 3-O-C12 HSL except there is an extra peak at m/z 175, which is most probably from host contamination.
C. MS (left) and MS/MS (right) of control for spot I, which is extracted from the same position of a TLC plate loaded with extracts from an *E. coli* BL21(DE3) cell culture. The MS/MS for the ion at m/z 199 showed a major fragmentation peak at m/z175, which confirms that the peak 175 in lane b is a contaminant from the host cells.
D. MS (left) and MS/MS (right) of the active compounds at spot K.

Fig. S7. ESI MS and MS/MS results of the standard C8-HSL and the AHLs produced by $LuxI_{QS10\text{-}2}$ (at spot M and N in Fig. 6).
A. MS (left) and MS/MS (right) of synthetic C8 HSL.
B. MS (left) and MS/MS (right) of the compounds at spot M.
C. MS (left) and MS/MS (right) of the compounds at spot N.

Table S1. Bacterial strains and plasmids used in this study.

Table S2. Primers used to amplify *luxI* and *luxR* homologues.

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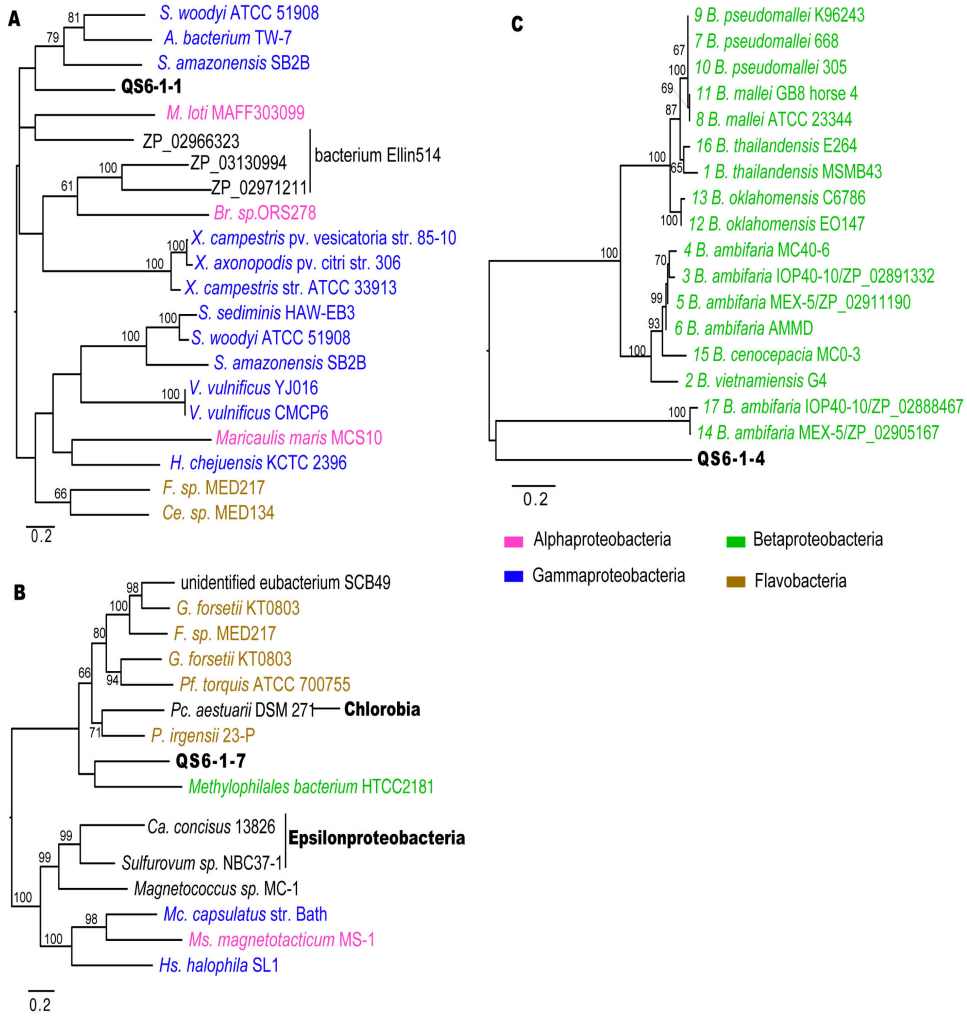
Supporting Information



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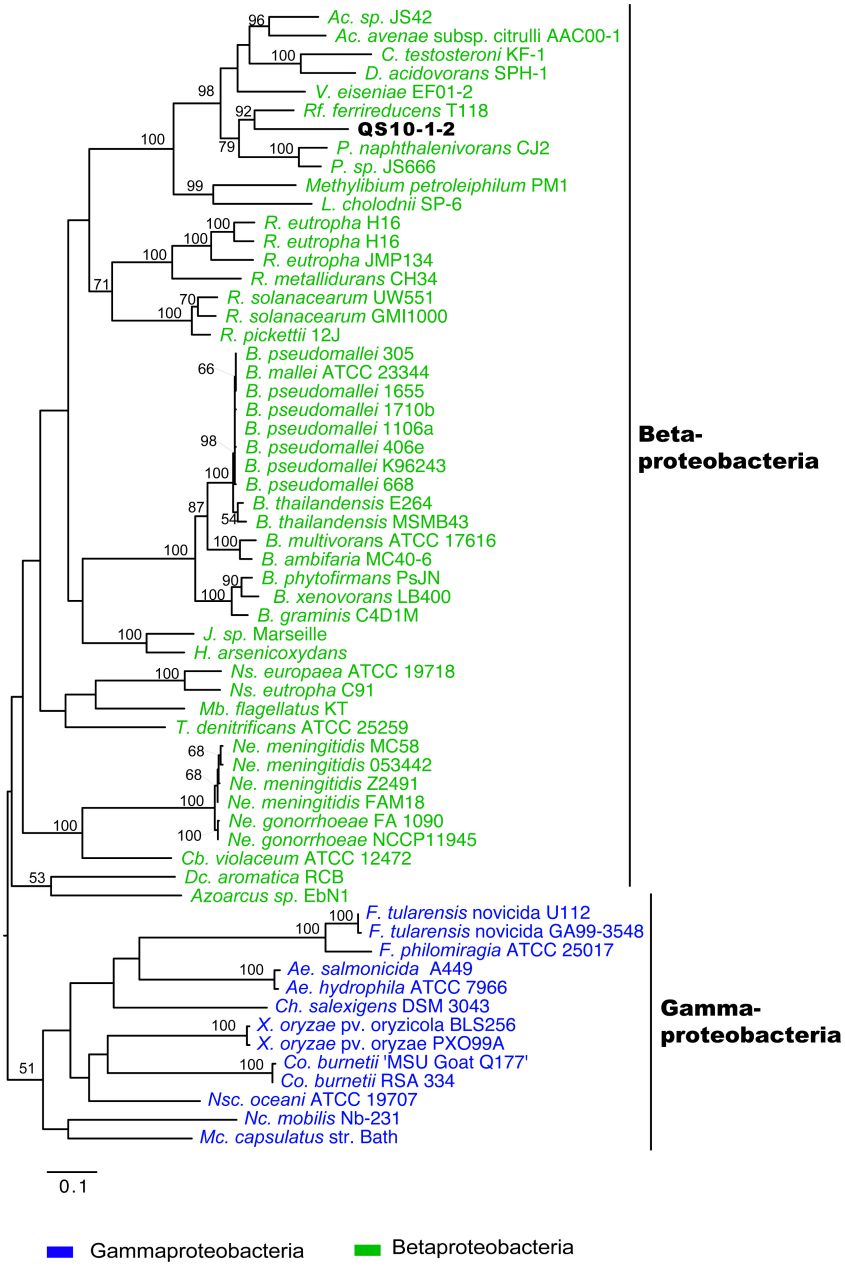


Fig S2

Midpoint rooted ML tree of homologs of QS10-1-2, Mg-chelatase related protein. Tree topology was determined using phyml version 2.4.4. Bootstrap values were obtained from using PAUP (Version 4.0 b) under neighbour joining algorithm ("Fast" stepwise-addition). Abbreviations: *Ac., Acidovorax; Ae, Aeromonas; B ., Burkholderia; C., Comamonas; Cb., Chromobacterium; Ch., Chromohalobacter; Co., Coxiella; D., Delftia; Dc, Dechloromonas; F., Francisella; H., Herminiimonas; J., Janthinobacterium; L., Leptothrix; Mb., Methylobacillus; Mc., Methylococcus; Nc., Nitrococus; Ne., Neisseria; Ns., Nitrosomonas; Nsc. Nitrosococcus; P., Polaromonas; R., Ralstonia; Rf., Rhodofera; T., Thiobacillus; V., Verminephrobacter; X, Xanthomonas.*

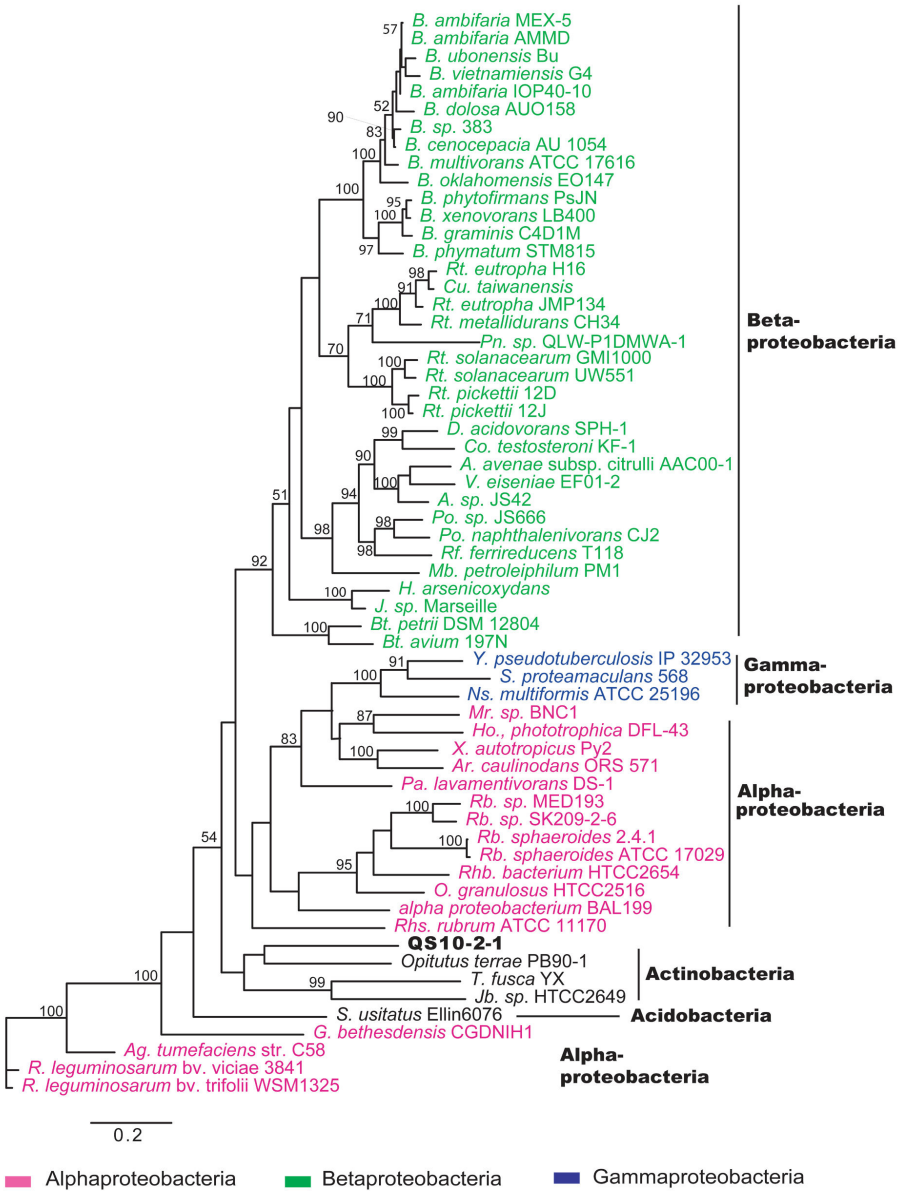
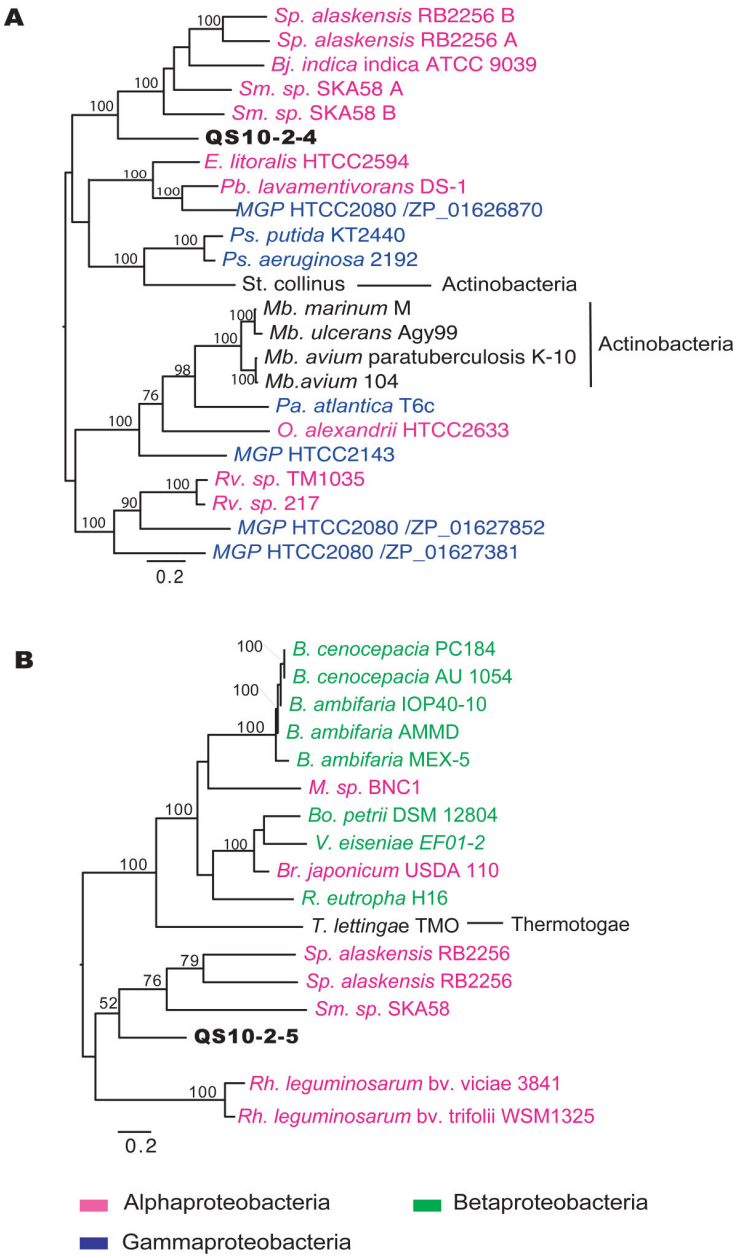


Fig S3

ML tree of homologs of QS10-2-1, Zinc containing alcohol dehydrogenase protein. Tree topology was determined using phyml version 2.4.4. Bootstrap values were obtained using PAUP (Version 4.0 b) under neighbour joining algorithm (full heuristic). Abbreviations: *A., Acidovorax; Ag., Agrobacterium; Ar., Azorhizobium; B., Burkholderia; Bt. Bordetella; Co., Comamonas; Cu., Cupriavidus; D., Delftia; G., Granulibacter; H., Herminiimonas; Ho., Hoeflea; J., Janthinobacterium; Jb., Janibacter; Mb., Methylibium; Mr., Mesorhizobium; Ns., Nitrosospira; O., Oceanicola; Pa., Parvibaculum; Pn., Polynucleobacter; Po., Polaromonas; R., Rhizobium; Rb., Roseobacter; Rf., Rhodoferax; Rhb., Rhodobacterales; Rhs., Rhodospirillum; Rt., Ralstonia; S., Serratia; Sb., Solibacter; T., Thermobifida; V., Verminephrobacter; X., Xanthobacter; Y., Yersinia.*



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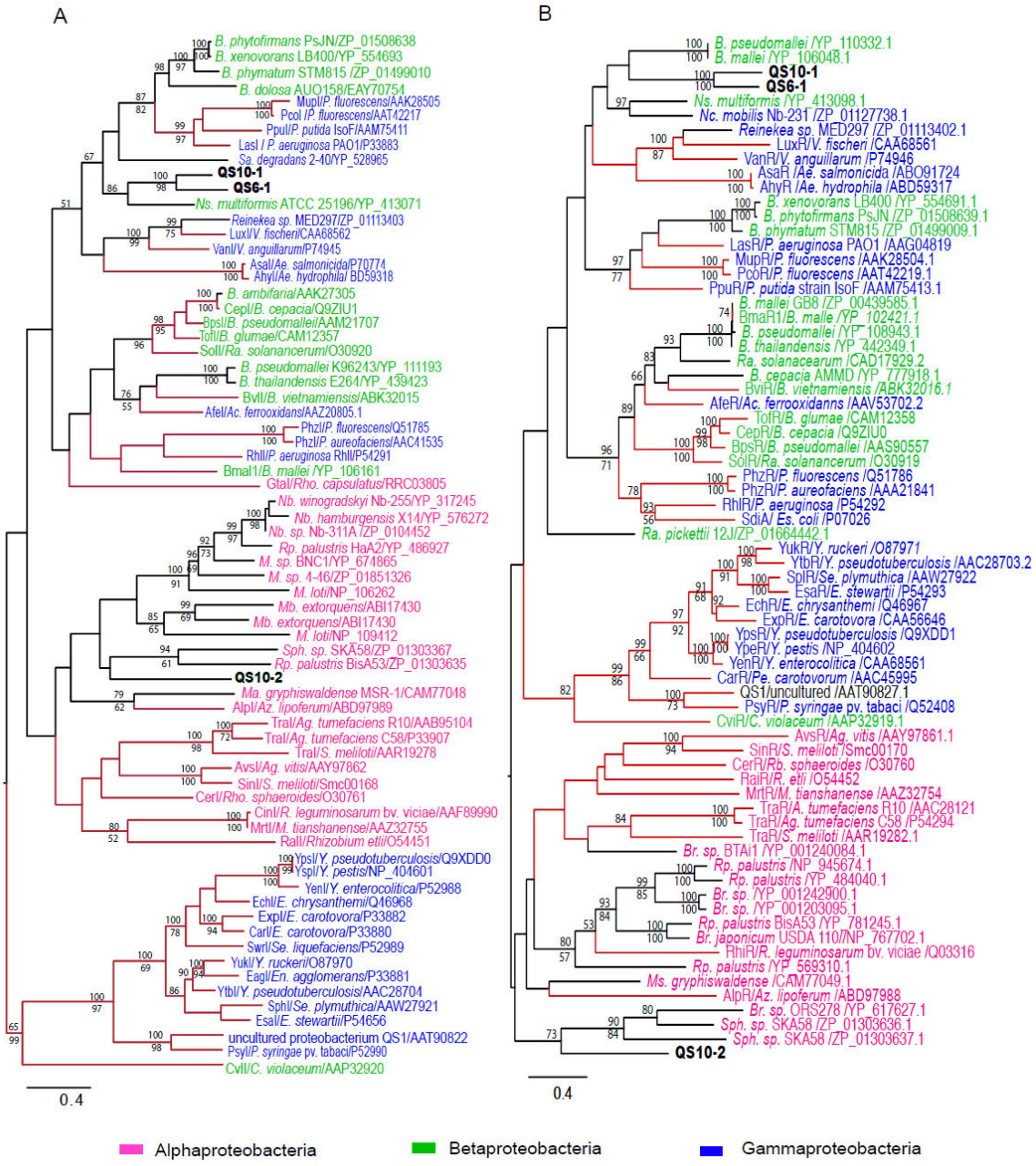

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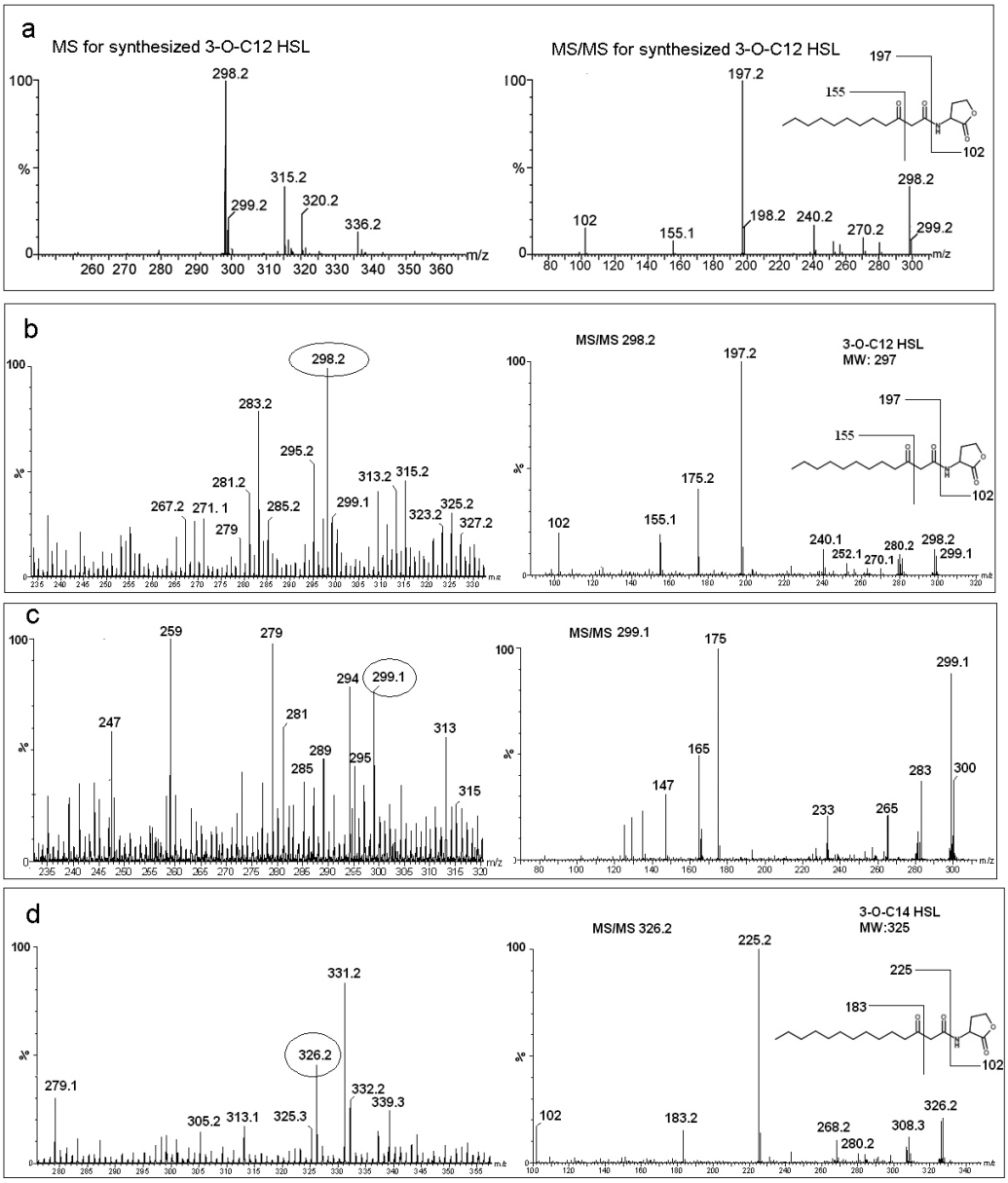

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b, MS (left) and MS/MS (right) of the active compounds at spot I. The fragmentation pattern is the same as synthetic 3-O-C12 HSL except there is an extra peak at m/z 175, which is most probably from host contamination.

c, MS (left) and MS/MS (right) of control for spot I, which is extracted from the same position of a TLC plate loaded with extracts from an *E. coli* BL21(DE3) cell culture. The MS/MS for the ion at m/z 199 showed a major fragmentation peak at m/z175, which confirms that the peak 175 in lane b is a contaminant from the host cells.

d, MS (left) and MS/MS (right) of the active compounds at spot K.

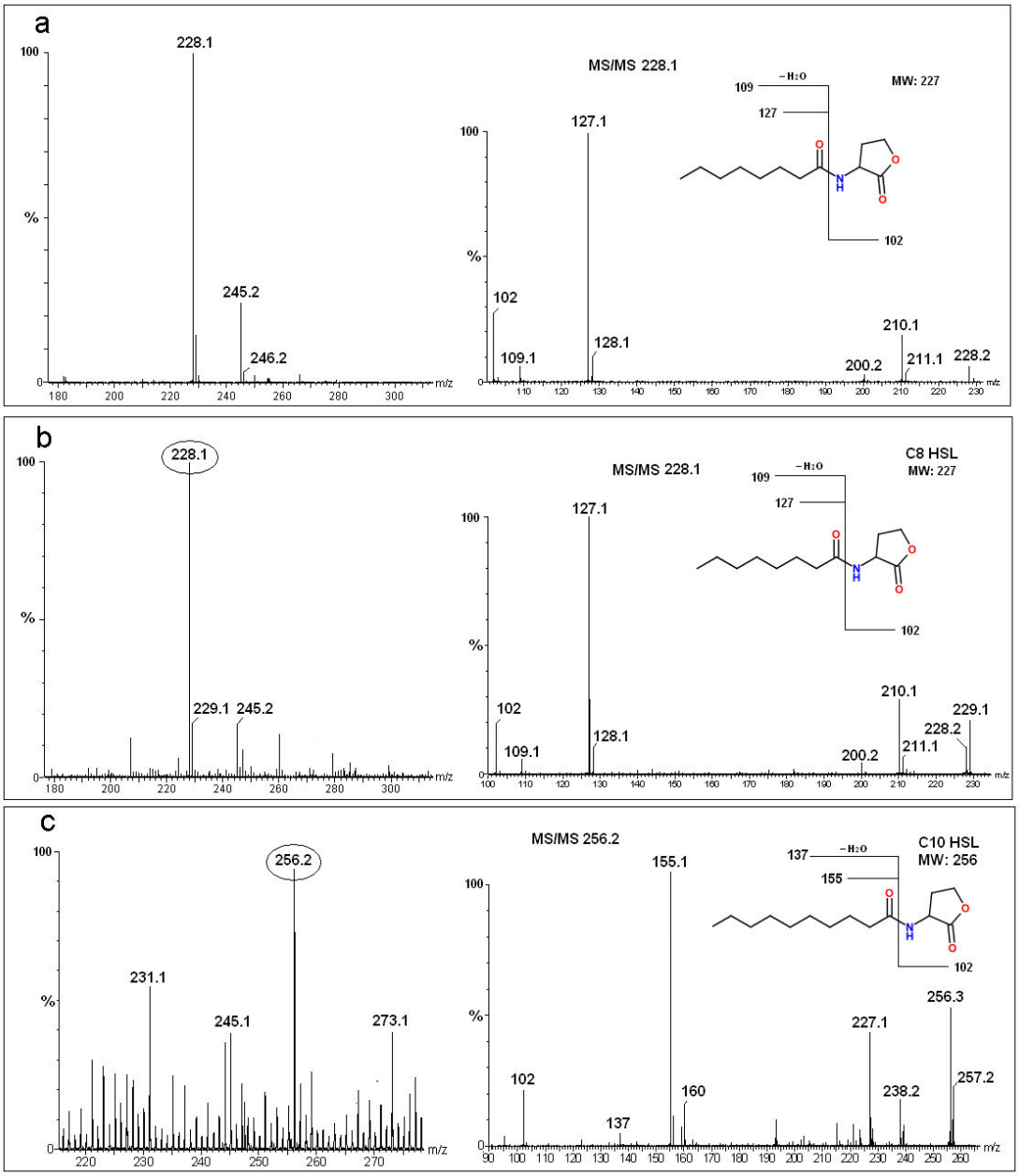

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Table legends

Table S1. Bacterial strains and plasmids used in this study.

Strains or plasmids	Relevant characteristics	Reference or source
Strains		
Agrobacterium tumefaciens		
HC103(pJZ381)	HC103 is R10 based strain, the Ti plasmid contains a traC-lacZ translational fusion and a traI nopolar deletion. In pJZ381, traR is under lac promoter of pBBR1MCS5. Km^r and Gm^r	This study
C58	Wild type strain	(Goodner et al., 2001), (Wood et al., 2001)
Escherichia Coli		
DH5α	recA1 and endA1 cloning strain	Lab stock
DH5α(pRK600)	DH5α strain containing helper plasmid pRK600, Cm^r	(Finan et al., 1986)
BL21(DE3)	(λDE3) F^- ompT $hsdS_B(r_B^-m_B^-)$ dcm gal	Novagen
Plasmids		
pBluescript II SK(+)	Cloning vector, Amp^r	(Alting-Mees and Short, 1989), (Alting-Mees et al., 1992)
pRK415	Broad host cloning vector, Tc^r	(Keen et al., 1988)
pET30(a+)	IPTG-inducible expression vector; Km^r	Novagen
pET30(b+)	IPTG-inducible expression vector; Km^r	Novagen
pRK6-1LuxI	pRK415 derivative, carrying full-length PCR amplified $LuxI_{QS6-1}$ under the control of the lac promoter of pRK415, Tc^r	This study
pET6-1LuxI	pET30(a+) derivative, carrying full-length $luxI_{QS6-1}$ under the T7 promoter, Km^r	This study
pET10-1LuxI	pET30(a+) derivative, carrying full-length $luxI_{QS10-1}$ under the T7 promoter, Km^r	This study
pET10-2LuxI	pET30(b+) derivative, carrying full-length $luxI_{QS10-2}$ under the T7 promoter, Km^r	This study

Table S2. Primers used to amplify *luxI* and *luxR* homologs.

Name of primers	Sequences
$luxI_{QS6\text{-}1}$ F	ATGCATCACCAGATTTTTACG
$luxI_{QS6\text{-}1}$ R	GGCTAGGCCGTTGCGTCC
$luxI_{QS10\text{-}1}$ F	AAAGTTGTTACACATTCGCTCAGG
$luxI_{QS10\text{-}1}$ R	GCAAATCCGGCTGTACTCCCT
$luxI_{QS10\text{-}2}$ F	GCCGATGATTCTGATCATCAACGC
$luxI_{QS10\text{-}2}$ R	TGTTTCTTTACGCGGCGATCTTT