A New Acyl-homoserine Lactone Molecule Generated by *Nitrobacter winogradskyi*

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Supplementary Table S1. Pharmacokinetic parameters of the decrease in the nitrite concentration decrease in the control and C10:1-HSL groups

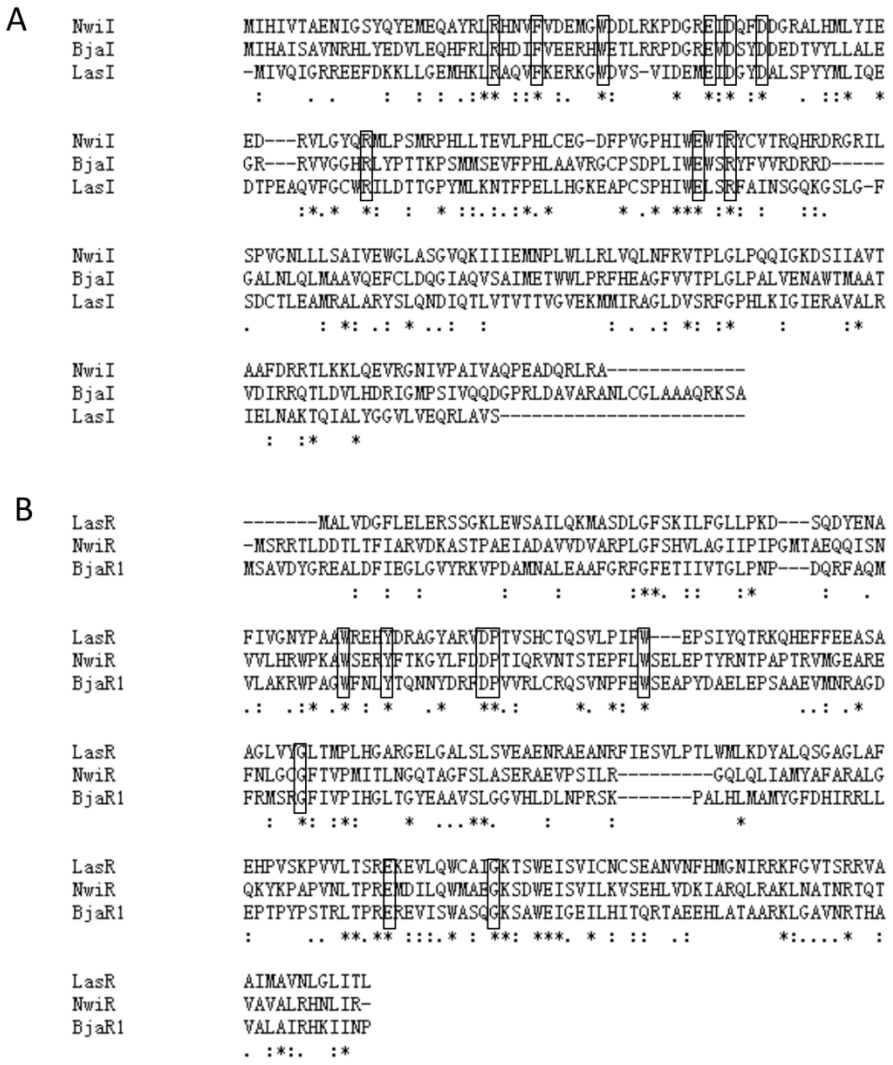
Pharmacokinetic parameters	Control group	C10:1-HSL group
First-order kinetic equation	y=-0.1336x+5.1022	y=-0.1442x+5.1077
R^2	0.95705	0.97785
k	0.1336	0.1442
$t_{1/2}$	5.19	4.81

Supplementary Table S2. Bacterial strains and plasmids used in this study

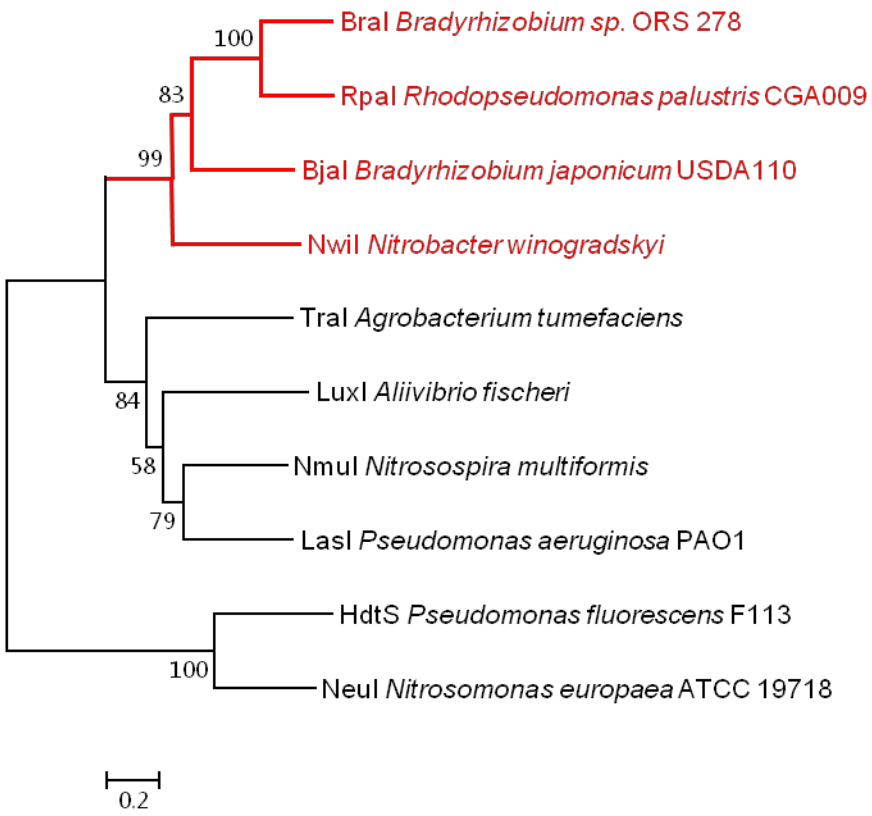
Strain or plasmid	Description	Reference
Strains		
Nitrobacter winogradskyi ATCC 25391	Type strain	ATCC
Escherichia coli BL21(DE3)	F^- *omp*T *hsd*S_B (r_B^- m_B^-) *dcm gal* λ (DE3) Cm^r	Promega
Agrobacterium tumefaciens KYC55(pJZ372) (pJZ384) (pJZ410)	AHL biosensor strain, Tc^r, Spe^r, Gm^r	[1]
Plasmids		
pGEX-4T-1	*E. coli* cloning and expression vector	GE
pGEX-nwiI	*nwiI* of *N. winogradskyi* expressed from pGEX-4T-1 *tac* promoter	This study

Supplementary Table S3. Primers used in the quantitative PCR

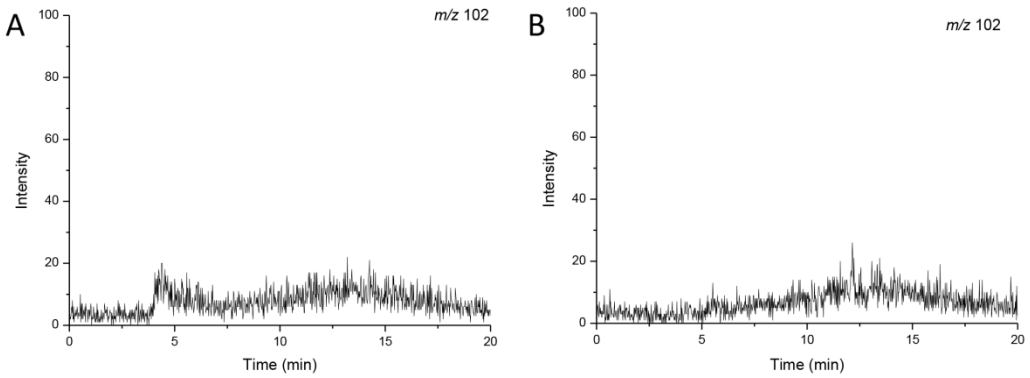
Locus tag	Gene name/description	Primers
nwi0773	cyt. C class 1	5’ TAAGGGTGATGCTGAGAACG (F)
		5’ CTTCATGGTCGGAGTGGG (R)
nwi0774	*nxr*A	5’ CACTCCGCACTTTAATCGTA (F)
		5’ GCGCCAGATGAACCACAT (R)
nwi0775	*nxr*X	5’ TCAACTACGACGTACCCG (F)
		5’ TTACCGCCCGAAGATGCT (R)
nwi0776	*nxr*B	5’ CGGTTCGCAGGTTTACGC (F)
		5’ CCGTCCTCACCACGCTTGTA (R)
nwi0777	*nar*J	5’ CGTGATCTGCGAAAGCAA (F)
		5’ CGTAAAGCATCTAGCACCTGA (R)
nwi0778	*nxr*C	5’ GACTCCCACGGCACTTTA (F)
		5’ CCTGTAAGCCAGCGAAAC (R)
nwi0779	*nar*k	5’ CAGCATTCCACTCGGTATTT (F)
		5’ CCCTGCTTAGACTTCGGATA (R)
nwi0780	C4 dicarboxylic	5’ CACGTCGTGGCGACTTAT (F)
	acids/malic acid or	5’ CATGATCGTCAGGTTAGCG (R)
	tellurium transporter	



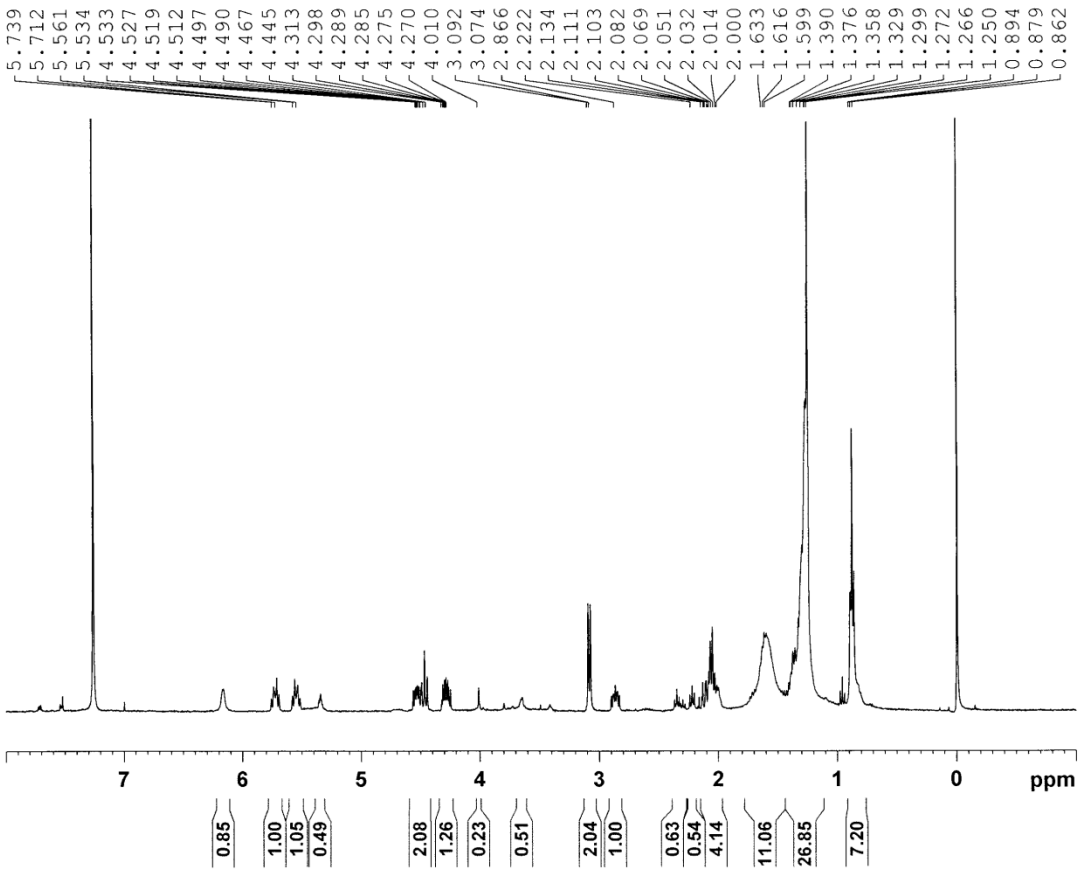
Supplementary Figure S1. Sequence alignment of NwiI and NwiR. The amino acid sequences of NwiI/R were compared to BjaI/R and LsaI/R, respectively. Identical and similar amino acid residues in the three proteins are indicated by asterisks and colons, respectively, and nine conserved residues in the LuxI/R family are highlighted in black boxes.



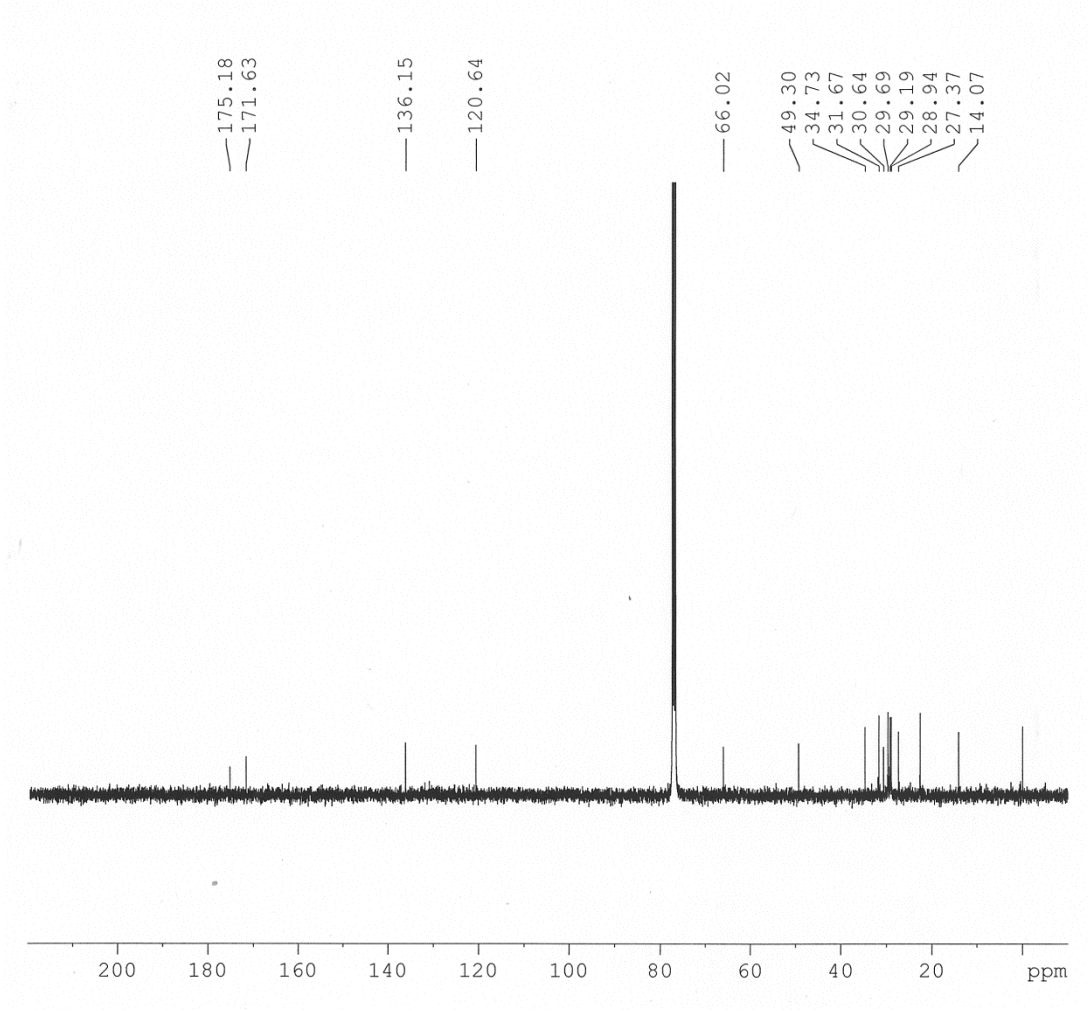
Supplementary Figure S2. Phylogenetic tree of LuxI and HdtS family members. The scale bar indicates the number of substitutions per residue. Bootstrap values as the percentage of 500 samplings are shown for nodes with values of 50% or greater. The subfamily tree of AHL synthases containing BraI, RapI, BjaI and NwiI is highlighted in red.



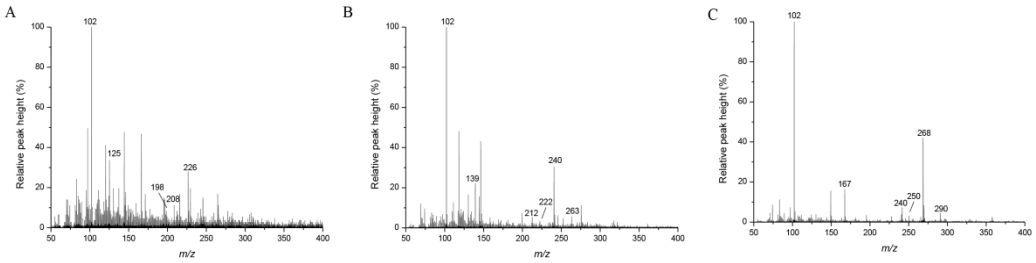
Supplementary Figure S3. LC-MS chromatograms of LB and 756 medium. (A) LB medium. (B) 756 medium.



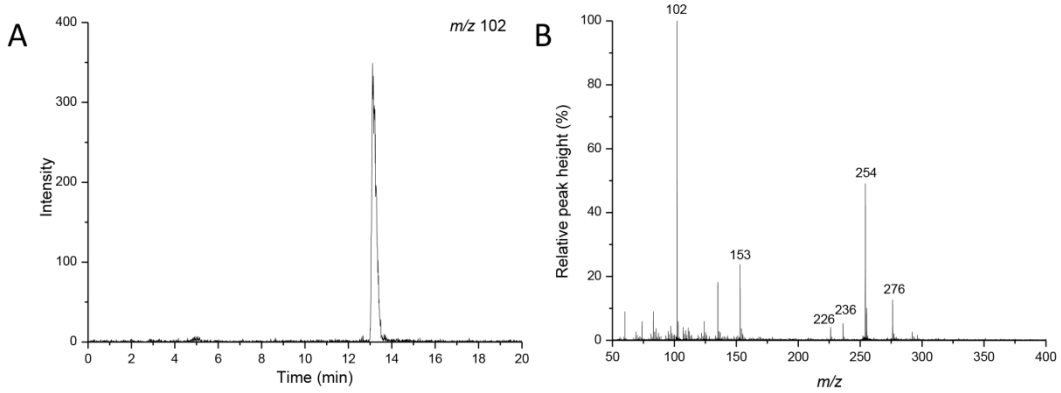
Supplementary Figure S4. ^{1}H NMR purified from the extracts of recombinant *E. coli* strain contain *nwiI* gene. ^{1}H-NMR ($CDCl_3$, 400 MHz) sprctrum: δ_H 0.88(3H, t, a,), 1.29(8H, m, b)， 2.01(2H, m, c), 2.11(1H, m, β), 2.86(1H, m, β), 3.08(2H, d, e), 4.28(1H, m, γ), 4.47(1H, t, α), 4.53(1H, m, γ), 5.54(1H, m, d), 5.73(1H, m, d), 6.17(1H, m, f).



Supplementary Figure S5. ^{13}C NMR purified from the extracts of recombinant *E. coli* strain contain *nwiI* gene. ^{13}C NMR (100MHz, $CDCl_3$): δ 14.07, 27.37, 28.94, 29.19, 29.69, 30.64, 31.67, 34.73, 49.30, 66.02, 120.64, 136.15, 171.63, 175.18.



Supplementary Figure S6. LC-MS chromatograms of putative AHLs molecules in *E. coli* containing pGEX-nwiI. (A) C8:1-HSL, (B) C9:1-HSL, (C) C11:1-HSL.



Supplementary Figure S7. LC-MS chromatograms of C10:1-HSL obtained by preparative HPLC. (A) Chromatogram of the lactone moiety at *m/z* 102. (B) The mass spectra reveal molecular ion [M+H] of *m/z* 254. The major fragmentation products with their respective *m/z* are labeled.

Reference

1 Zhu, J., Chai, Y., Zhong, Z., Li, S. & Winans, S. C. *Agrobacterium* bioassay strain for ultrasensitive detection of *N*-acylhomoserine lactone-type quorum-sensing molecules: detection of autoinducers in *Mesorhizobium huakuii*. *Appl. Environ. Microbiol.* **69**, 6949-6953 (2003).