

**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		
<i>Nitrobacter winogradskyi</i> ATCC 25391	Type strain	ATCC
<i>Escherichia coli</i> BL21(DE3)	F <sup>-</sup> <i>ompT hsdS<sub>B</sub></i> (r <sub>B</sub> <sup>-</sup> m <sub>B</sub> <sup>-</sup> ) <i>dcm gal λ</i> (DE3) Cm <sup>r</sup>	Promega
<i>Agrobacterium tumefaciens</i> KYC55(pJZ372) (pJZ384) (pJZ410)	AHL biosensor strain, Tc <sup>r</sup> , Spe <sup>r</sup> , Gm <sup>r</sup>	<sup>1</sup>
Plasmids		
pGEX-4T-1	<i>E. coli</i> cloning and expression vector	GE
pGEX-nwII	<i>nwII</i> of <i>N. winogradskyi</i> expressed from pGEX-4T-1 <i>tac</i> 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	<i>nxA</i>	5' CACTCCGCACTTTAATCGTA (F) 5' GCGCCAGATGAACCACAT (R)
nwi0775	<i>nX</i>	5' TCAACTACGACGTACCCG (F) 5' TTACCGCCCGAAGATGCT (R)
nwi0776	<i>nxB</i>	5' CGGTTCGCAGGTTTACGC (F) 5' CCGTCCTCACCACGCTTGTA (R)
nwi0777	<i>narJ</i>	5' CGTGATCTGCGAAAGCAA (F) 5' CGTAAAGCATCTAGCACCTGA (R)
nwi0778	<i>nxC</i>	5' GACTCCCACGGCACTTTA (F) 5' CCTGTAAGCCAGCGAAAC (R)
nwi0779	<i>nark</i>	5' CAGCATTCCACTCGGTATTT (F) 5' CCCTGCTTAGACTTCGGATA (R)
nwi0780	C4 dicarboxylic acids/malic acid or tellurium transporter	5' CACGTCGTGGCGACTTAT (F) 5' CATGATCGTCAGGTTAGCG (R)

**A**

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NwiI      MIIHIVTAENIGSYQYEMEQAYRLRHNVFDVEMGWDLRKPDGREDQDFDGRALHMLYIE
BjaI      MIHAI SAVNRHLYEDVLEQHFRLRHDIFVEERHWE TLRRPDGREWDSYDDED TVYLLALE
LasI      -MIVQIGRREEFDKLLGEMHKLRQAQVFKERKGVDS-VIDEMEDGVDALSPYMLIQE
          : . . : : : ** : * . * : * * * : * . : * *

NwiI      ED---RVLGYQRMLPSMRPHLLTEVLPHLCEG-DFFVGPPIWETRYCVTRQHRDRGRIL
BjaI      GR---RVVGGHRLYP TTKPSMMSEVFPHLAAVRGCPSDPLIWEWSRYFVVRDRRD-----
LasI      DTPEAQVFGCWRLDITGPYMLKNTFPPELLHGKEAPCSPHIWELESAFAINSGQKGLG-F
          : * . * * : : * : : . : * . * * * * : * : : : . :

NwiI      SPVGNLLL SAIWEVGLASGVQKIIEMNPLWLLRLVQLNFRVTPGLPQQIGKDSIIAVT
BjaI      GALNLQLMAAVQEFCLDQGIQVSAI METWVLPFRFHEAGFVVTPLGLPALVENAWTMAAT
LasI      SDCTLEAMRALARYSLQNDIQTLVTVTIVGVEKMMIRAGLDVSRFGPHLKIGIERAVALR
          . : * : . * . : : : : : : : : : * : * : : * :

NwiI      AAFDRRTLKKLQEVGRNIVPAIVAQPEADQRLRA-----
BjaI      VDIRRQTL DVLHDIRIGMPSIVQQDGPRLDAVARANL CGLAAAQRKSA
LasI      IELNAKTQIALYGGVLEQRLAVS-----
          : : * *


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**B**

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LasR      -----MALVDGFLELERSSGKLEWSAILQKMASDLGFSKILFGLLPKD---SQDYENA
NwiR      -MSRRITLDDTLTFIARVDKASTPAEIAADAVVDVARPLGFSHVLG IIPIPGMTAEQQISN
BjaR1     MSAVDYGREALDFIEGLGVYRKVPDAMNLEAAFGRFGPETIIIVTGLPNP---DQRFAQM
          : : : : : : : : : * * . : : * :

LasR      FIVGNYPAAVREHIDRAGYARVDFIVSHCTQSVLPIN---EPSIYQTRKQHEFFEEASA
NwiR      VVLRHWPKAWSERYFTKG YLFDDFTIQRVNTSTEPFLWSELEPTYRNT PAPTRVMGEARE
BjaR1     VLAKRWPAGVFNLYTQNNYDRFDHVVRLCRQSVNPFVWSEAPYDAELEPSAAEVMMNRAGD
          . : . : * * : * . * * * : * . * * * . . : . *

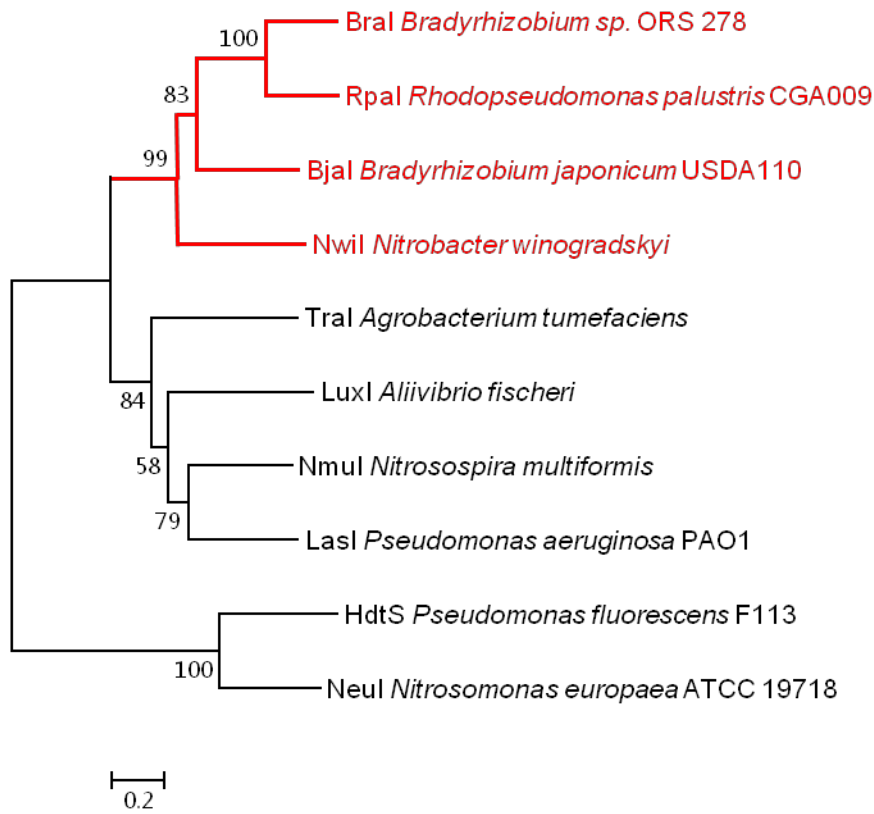
LasR      AGLVYQLT MPLHGARGELGALSLSVEAENRAEANRFIESVLP TLWMLKDYALQSGAGLAF
NwiR      FNLGCGFTVPMITLNGQTAGFSLASERA E VPSILR-----GQLQLIAMYAFARALG
BjaR1     FRMSRQFIVPIHGLTG YEAAVSLGGVHLDL NPRSK-----PALHLMAMYGFDIRRL
          : * : : * * . . * * . : : : : *

LasR      EHPVSKPVVLTSRKEVLQWCAIQKTSWEISVICNCSEANVNFHMGNIRRRKFGVTSRRVA
NwiR      QKYKPA PVNLTPREMDILQWMAECKSDWEISVILKVSEHLVDKIARQLRAKLNATNRQT
BjaR1     EPYPYSTRLTPREVISWASQCKSAWEIGEILHITQRTAEHLATAARKLGAVNRTHA
          : . . * * . * : : . * : * * * . * : : : . : * : . . * :

LasR      AIMAVNLGLITL
NwiR      VAVLRHNLIR-
BjaR1     VALAIRHKIINP
          . : * . : *


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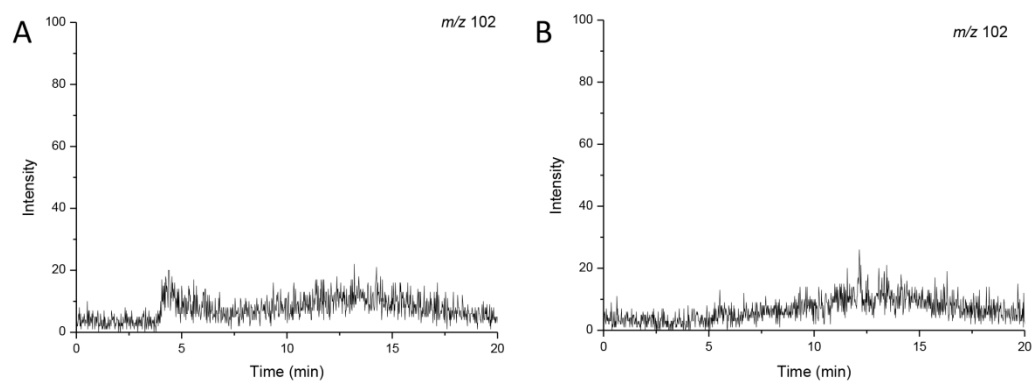
**Supplementary Figure S1. Sequence alignment of NwiI and NwiR.** The amino acid sequences of NwiI/R were compared to BjaI/R and LsaI/R, respectively. Identical and similar amino acid residues in the three proteins are indicated by asterisks and colons, respectively, and nine conserved residues in the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



**Supplementary Figure S2. Phylogenetic tree of LuxI and HdtS family members.**

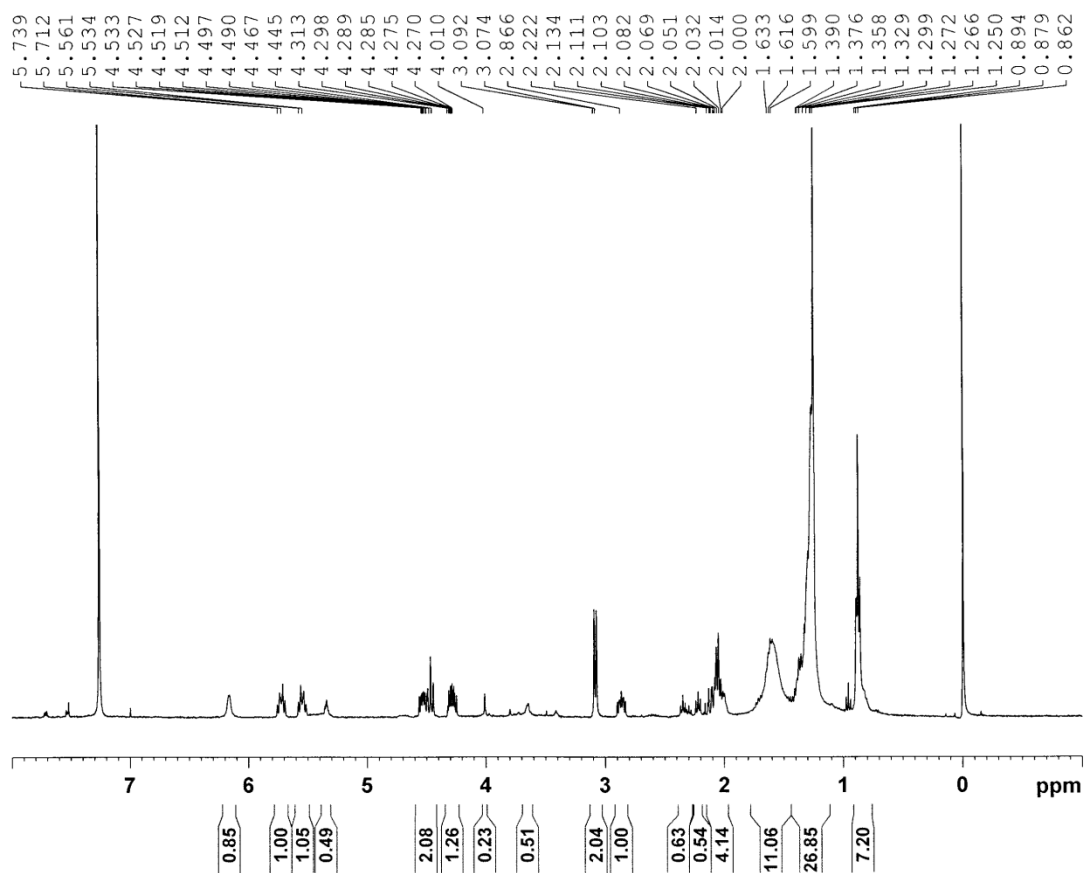
The scale bar indicates the number of substitutions per residue. Bootstrap values as the percentage of 500 samplings are shown for nodes with values of 50% or greater.

The subfamily tree of AHL synthases containing BraI, RapI, BjaI and NwiI is highlighted in red.



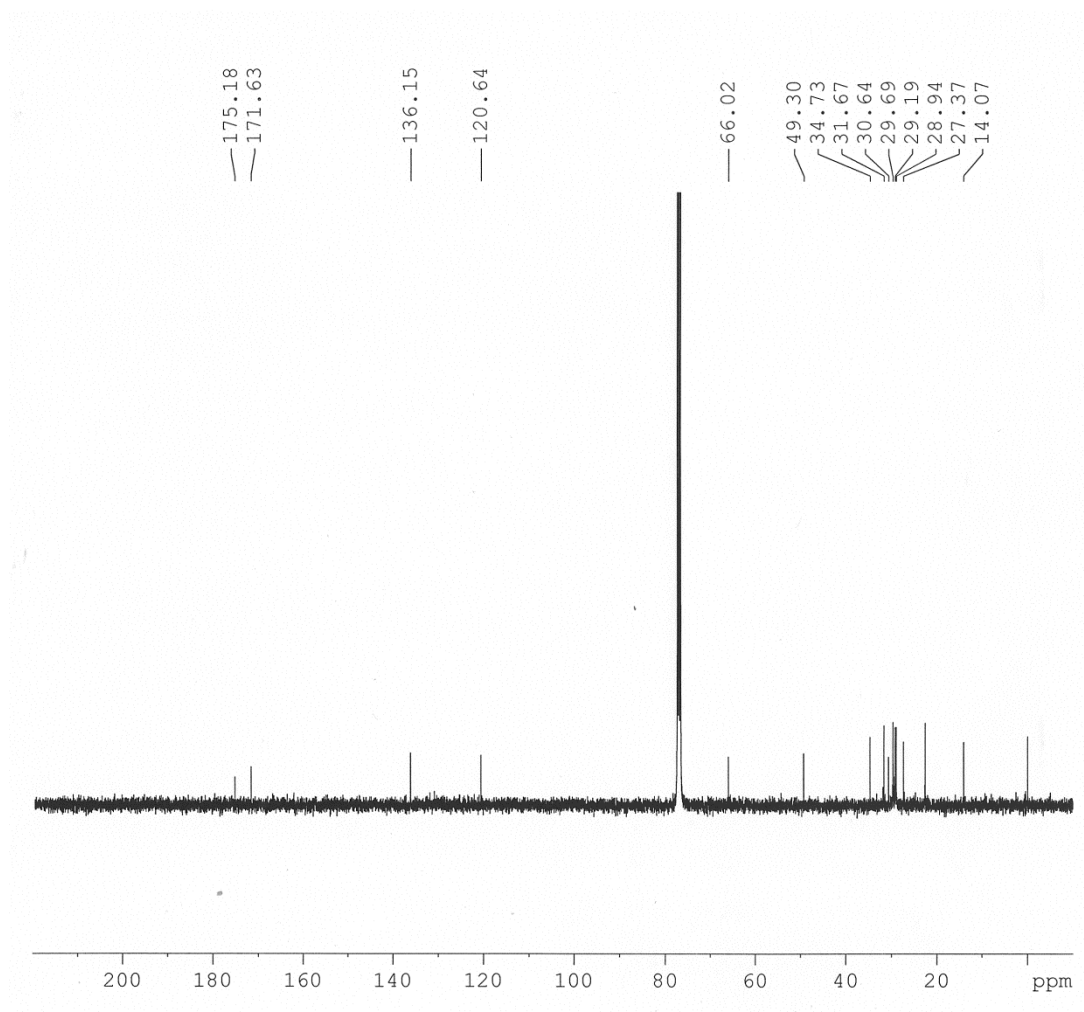
**Supplementary Figure S3. LC-MS chromatograms of LB and 756 medium. (A)**

LB medium. (B) 756 medium.

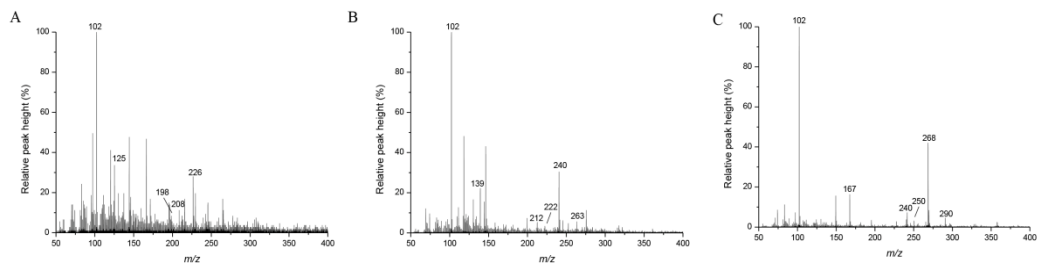


**Supplementary Figure S4.**  $^1\text{H}$  NMR purified from the extracts of recombinant *E. coli* strain contain *nwiI* gene.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ , 400 MHz) spectrum:  $\delta_{\text{H}}$  0.88(3H, t, a), 1.29(8H, m, b), 2.01(2H, m, c), 2.11(1H, m,  $\beta$ ), 2.86(1H, m,  $\beta$ ), 3.08(2H, d, e), 4.28(1H, m,  $\gamma$ ), 4.47(1H, t,  $\alpha$ ), 4.53(1H, m,  $\gamma$ ), 5.54(1H, m, d), 5.73(1H, m, d), 6.17(1H, m, f).



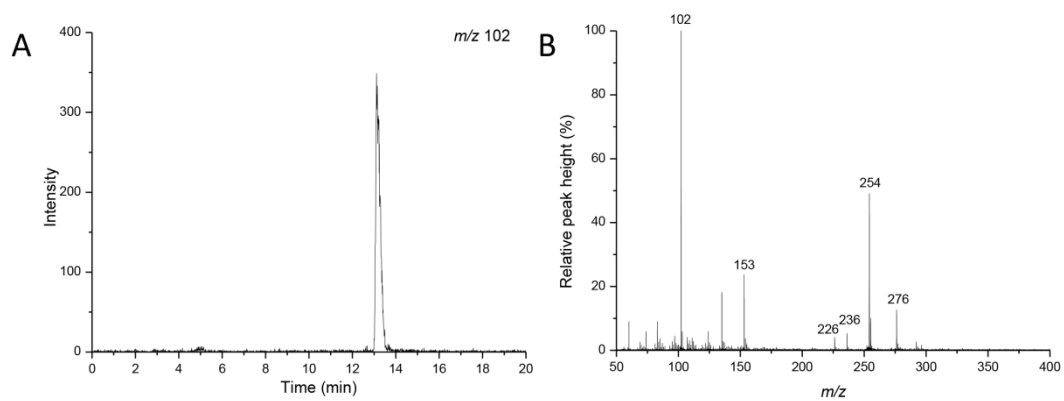


**Supplementary Figure S5. <sup>13</sup>C NMR purified from the extracts of recombinant *E. coli* strain contain *nwiI* gene. <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>): δ 14.07, 27.37, 28.94, 29.19, 29.69, 30.64, 31.67, 34.73, 49.30, 66.02, 120.64, 136.15, 171.63, 175.18.**



**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).