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Humic Acid

Hydrated Ferric Oxide

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CONTENTS

Aquatic environment

Removal of total cyanide in coking wastewater during a coagulation process: Significance of organic polymers
Jian Shen, He Zhao, Hongbin Cao, Yi Zhang, Yongsheng Chen ······ 231
Removal of arsenate with hydrous ferric oxide coprecipitation: Effect of humic acid
Jingjing Du, Chuanyong Jing, Jinming Duan, Yongli Zhang, Shan Hu ······ 240
Arsenic removal from groundwater by acclimated sludge under autohydrogenotrophic conditions
Siqing Xia, Shuang Shen, Xiaoyin Xu, Jun Liang, Lijie Zhou ······ 248
Characteristics of greenhouse gas emission in three full-scale wastewater treatment processes
Xu Yan, Lin Li, Junxin Liu ······ 256
Effect of temperature on anoxic metabolism of nitrites to nitrous oxide by polyphosphate accumulating organisms
Zhijia Miao, Wei Zeng, Shuying Wang, Yongzhen Peng, Guihua Cao, Dongchen Weng, Guisong Xue, Qing Yang ······ 264
Efficacy of two chemical coagulants and three different filtration media on removal of *Aspergillus flavus* from surface water
Hamid Mohammad Al-Gabr, Tianling Zheng, Xin Yu ······ 274
Beyond hypoxia: Occurrence and characteristics of black blooms due to the decomposition of the submerged plant *Potamogeton crispus* in a shallow lake
Qiushi Shen, Qilin Zhou, Jingge Shang, Shiguang Shao, Lei Zhang, Chengxin Fan ······ 281
Spatial and temporal variations of two cyanobacteria in the mesotrophic Miyun reservoir, China
Ming Su, Jianwei Yu, Shenling Pan, Wei An, Min Yang ······ 289
Quantification of viable bacteria in wastewater treatment plants by using propidium monoazide combined with quantitative PCR (PMA-qPCR)
Dan Li, Tiezheng Tong, Siyu Zeng, Yiwen Lin, Shuxu Wu, Miao He ······ 299
Antimony(V) removal from water by hydrated ferric oxides supported by calcite sand and polymeric anion exchanger
Yangyang Miao, Feichao Han, Bingcai Pan, Yingjie Niu, Guangze Nie, Lu Lv ······ 307
A comparison on the phytoremediation ability of triazophos by different macrophytes
Zhu Li, Huiping Xiao, Shuiping Cheng, Liping Zhang, Xiaolong Xie, Zhenbin Wu ······ 315
Biostability in distribution systems in one city in southern China: Characteristics, modeling and control strategy
Pinpin Lu, Xiaojian Zhang, Chiqian Zhang, Zhangbin Niu, Shuguang Xie, Chao Chen ······ 323

Atmospheric environment

Characteristics of ozone and ozone precursors (VOCs and NO*x*) around a petroleum refinery in Beijing, China
Wei Wei, Shuiyuan Cheng, Guohao Li, Gang Wang, Haiyang Wang ······ 332
Identification of sources of lead in the atmosphere by chemical speciation using X-ray absorption near-edge structure (XANES) spectroscopy
Kohei Sakata, Aya Sakaguchi, Masaharu Tanimizu, Yuichi Takaku, Yuka Yokoyama, Yoshio Takahashi ······ 343
Online monitoring of water-soluble ionic composition of PM_{10} during early summer over Lanzhou City
Jin Fan, Xiaoying Yue, Yi Jing, Qiang Chen, Shigong Wang ······ 353
Effect of traffic restriction on atmospheric particle concentrations and their size distributions in urban Lanzhou, Northwestern China
Suping Zhao, Ye Yu, Na Liu, Jianjun He, Jinbei Chen ······ 362

Environmental health and toxicology

A review on completing arsenic biogeochemical cycle: Microbial volatilization of arsines in environment
Peipei Wang, Guoxin Sun, Yan Jia, Andrew A Meharg, Yongguan Zhu ······ 371
Alginate modifies the physiological impact of CeO_2 nanoparticles in corn seedlings cultivated in soil
Lijuan Zhao, Jose R. Peralta-Videa, Bo Peng, Susmita Bandyopadhyay, Baltazar Corral-Diaz, Pedro Osuna-Avila, Milka O. Montes, Arturo A. Keller, Jorge L. Gardea-Torresdey ······ 382
Humification characterization of biochar and its potential as a composting amendment
Jining Zhang, Fan Lü, Chenghao Luo, Liming Shao, Pinjing He ······ 390
Immigrant *Pantoea agglomerans* embedded within indigenous microbial aggregates: A novel spatial distribution of epiphytic bacteria
Qing Yu, Anzhou Ma, Mengmeng Cui, Xuliang Zhuang, Guoqiang Zhuang ······ 398
Remediation of nutrient-rich waters using the terrestrial plant, *Pandanus amaryllifolius* Roxb.
Han Ping, Prakash Kumar, Bee-Lian Ong ······ 404

Construction of a dual fluorescence whole-cell biosensor to detect *N*-acyl homoserine lactones
Xuemei Deng, Guoqiang Zhuang, Anzhou Ma, Qing Yu, Xuliang Zhuang 415
Digestion performance and microbial community in full-scale methane fermentation of stillage from sweet potato-shochu production
Tsutomu Kobayashi, Yueqin Tang, Toyoshi Urakami, Shigeru Morimura, Kenji Kida 423
Health risk assessment of dietary exposure to polycyclic aromatic hydrocarbons in Taiyuan, China
Jing Nie, Jing Shi, Xiaoli Duan, Beibei Wang, Nan Huang, Xiuge Zhao 432
Acute toxicity formation potential of benzophenone-type UV filters in chlorination disinfection process
Qi Liu, Zhenbin Chen, Dongbin Wei, Yuguo Du 440
Exposure measurement, risk assessment and source identification for exposure of traffic assistants to particle-bound PAHs in Tianjin, China
Xiaodan Xue, Yan You, Jianhui Wu, Bin Han, Zhipeng Bai, Naijun Tang, Liwen Zhang 448

Environmental catalysis and materials

Fabrication of Bi_2O_3/TiO_2 nanocomposites and their applications to the degradation of pollutants in air and water under visible-light
Ashok Kumar Chakraborty, Md Emran Hossain, Md Masudur Rhaman, K M A Sobahan 458
Comparison of quartz sand, anthracite, shale and biological ceramsite for adsorptive removal of phosphorus from aqueous solution
Cheng Jiang, Liyue Jia, Bo Zhang, Yiliang He, George Kirumba 466
Catalytic bubble-free hydrogenation reduction of azo dye by porous membranes loaded with palladium nanoparticles
Zhiqian Jia, Huijie Sun, Zhenxia Du, Zhigang Lei 478
Debromination of decabromodiphenyl ether by organo-montmorillonite-supported nanoscale zero-valent iron:
Preparation, characterization and influence factors
Zhihua Pang, Mengyue Yan, Xiaoshan Jia, Zhenxing Wang, Jianyu Chen 483

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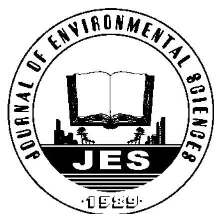

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Construction of a dual fluorescence whole-cell biosensor to detect *N*-acyl homoserine lactones

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ABSTRACT

Detection of *N*-acyl homoserine lactones (AHLs) is useful for understanding quorum sensing (QS) behaviors, including biofilm formation, virulence and metabolism. For detecting AHLs and indicating the host cells *in situ*, we constructed the plasmid pUCGMA2T_{1-4} to make a dual fluorescent whole-cell biosensor based on the AhlI/R AHL system of *Pseudomonas syringae* pv. *syringae* B728a. The plasmid contains three components: constitutively expressed P_{nptII}::*gfp* for indicating host cells, P_{ahlI}::*mcherry* that produces red fluorescence in response to AHL, and the *ahlR* gene that encodes an AHL regulatory protein. Meanwhile, two copies of T_{1-4} (four tandem copies of a transcriptional terminator) were added into the plasmid to reduce background. The results showed that when the plasmid was placed into *Escherichia coli*, the dual fluorescence whole-cell biosensor was able to respond with red fluorescence within 6 hr to 5×10^{-8}–1×10^{-5} mol/L of 3OC6-HSL. Bright green fluorescence indicated the host cells. Furthermore, when the plasmid was transferred to wild-type *Pseudomonas* PhTA125 (an AHL-producing bacterium), it also showed both green and red fluorescence. This result demonstrates that this plasmid can be used to construct whole-cell indicators that can indicate the AHL response and spatial behaviors of microbes in a microenvironmental niche.

Introduction

Quorum sensing (QS) was first described as a way for bacteria to coordinate their behaviors by cell-to-cell communication using chemical signaling molecules (Fuqua et al., 1996). These behaviors have a significant impact on biofilm formation, gene transfer, toxic factors, metabolism and other processes (Llamas et al., 2004; Whitehead et al., 2006). Among chemical signaling molecules, *N*-acyl-homoserine lactones (AHLs) are a highly conserved class of QS signaling molecules used by a wide range of Gram-negative (GN) proteobacteria that inhabit diverse environments (Manefield and Whiteley, 2007). In recent years, AHLs have been found to importantly impact species in microbial communites and the substrate cycle (Keller and Surette, 2006; Lü et al., 2012; von Bodman et al., 2008). To study the function of AHLs in microbial communites and especially microbial behaviors mediated by QS, it is necessary to develop *in situ* monitoring and detection methods.

Because of their integrated nature, whole-cell biosensors are ideal for *in situ* monitoring and detection, as they are not restricted by spatial niches in a microenvironment and provide selective and sensitive rapid responses in real time. To detect bacterial behaviors mediated by QS in a micro-ecosystem, several whole-cell AHL biosensors have been constructed in recent years. The typical construct contains the AHL synthase promoter fused to a reporter gene and also the AHL regulator protein gene (R). With different AHL regulators, whole-cell AHL biosensors respond to different AHLs. For instance, *Escherichia coli* harboring plasmids based on LuxI/R from *Vibrio fischeri*, AhyI/R from *Aeromonas hydrophyla* and RhlI/R from *Pseudomonas aeruginosa* show good sensitivity towards

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C6-3-oxo-AHL, C6-AHL, C8-3-oxo-AHL, and C8-AHL. *E. coli* with the *P. aeruginosa* LasI/R-based plasmid pKDT17 responds well to C12-3-oxo-AHL, C10-3-oxo-AHL and C12-AHL (Steindler and Venturi, 2007). These kinds of biosensors are widely employed for the detection of QS in specific *in situ* environments, such as intact rhizospheres, lake sediments and leaf surfaces (DeAngelis et al., 2007; Dulla and Lindow, 2008; Llamas et al., 2004; Lumjiaktase et al., 2010).

However, when *in situ* detection is performed, there are some apparent limitations in current AHL biosensors with single fluorescence labels. For example, when an environment is devoid of AHLs or has an insufficient AHL concentration to elicit a response by the AHL whole-cell biosensor, the sensor host cell cannot be detected or evaluated during its development *in situ*. To overcome this limitation, Lumjiaktase et al. (2010) developed a two-color AHL biosensor reporter system for monitoring AHL-producing bacteria in lake sediments. This system contains one plasmid and a transposon. The transposon contains a promoter fused to a GFP reporter gene that integrates into the genome of host cell to monitor AHLs, and the plasmid contains an RFP gene to mark the host strain. As this system is specially designed to horizontally transfer the transposon into different bacterial species in the environmental niche, it cannot be used to function in a specific bacterium for indication *in situ*. So far, there has not been a combination of these components in an AHL biosensor that can not only detect exogenous or endogenous AHLs but can also mark the development of the host cell in the selected environment. If such a system could be constructed, it could mark strains that do not produce AHLs but that are affected by AHLs and have ecological functions (Chakrabarty et al., 1975; Velicer et al., 2000).

In order to realize this function in an AHL biosensor, we constructed a dual fluorescence biomarker plasmid, pUCGMA2T_{1-4}, which contains three components: P_{nptII}:*gfp*, constitutively expressing GFP as a host cell marker; the *ahlR* gene of *Pseudomonas syringae* pv. *syringae* (*Pss*) B728a, encoding an AHL regulator protein; and a P_{ahlI}: *mcherry* fragment, which responds to AHLs with cherry red fluorescence. The induction experiment demonstrated that this plasmid constitutively expresses AHL-independent bright green fluorescence and sensitively detects 3OC6-HSL intuitionally. This kind of construct not only detects AHLs but also reveals host cell development *in situ*.

1 Materials and methods

1.1 Bacterial strains and culture conditions

The bacterial strains and plasmids used in this study are listed in **Table 1**.

P. syringae pv. *syringae* B728a (*Pss*) was grown on KB medium at 28°C, and *E. coli* DH5α was cultured on LB medium at 37°C; others strains were grown at 30°C. Solid medium contained 15 g/L agar (OXOID, England). Antibiotics were used at the following concentrations when required: ampicillin, 50 mg/L; kanamycin, 50 mg/L; rifampicin, 100 mg/L; spectinomycin, 20 mg/L.

Table 1 Bacterial strains and plasmids used

Strain or plasmid	Relevant genotype and/or characteristics	Source or reference
Escherichia coli DH5α	F'Φ80dlacZ Δ(*lacZYA-argF*)U169 recA1endA1 hsdR17(rKmK+)supE44 λ-thi-1 *gyrA relA*1	Tiangen Biotech (Peking, China)
Pseudomonos syringae pv. *syringae* B728a	Wt AHL−producing bacterium *ahlR* Rif^rpBQ9	Dulla and Lindow, 2008
TA125	*Pseudomonos* sp. AHL−producing bacterium	Lv et al., 2013
pUC19	Cloning vector Amp^r, ColE1-ori	Invitrogen
pBQ9	pPROBE-OT derivative harboring *P. syringae ahlI* promoter upstream of GFP Sp^r	Quinones et al., 2004
pAN583	*mcherry* Amp^r	Nelson et al., 2007
pTS	P_{nptII} Kan^r	Stiner and Halverson, 2002
pWM1009	*cfp* T_{1-4} Kan^r	Monier and Lindow, 2005
pUCNG	P_{nptII}:*gfp* Amp^r	This study
pUCAG	P_{ahlI}:*gfp* Amp^r	This study
pUCAM	P_{ahlI}:*mcherry* Amp^r	This study
pUCGMA	P_{nptII}:*gfp*/*ahlR*/P_{ahlI}:*mcherry* Amp^r	This study
pUCGMAT_{1-4}	P_{nptII}:*gfp*/*ahlR*/T_{1-4}/P_{ahlI}:*mcherry* Amp^r	This study
pUCGMA2T_{1-4}	P_{nptII}:*gfp*/*ahlR*/T_{1-4}/P_{ahlI}:*mcherry*/T_{1-4} Amp^r	This study

Rif^r: rifampicin resistance; Amp^r: ampicillin resistance; Kan^r: kanamycin resistance; Sp^r: spectinomycin resistance. T_{1-4}: four tandem copies of transcriptional terminator derived from the *rrnB* operon of *E. coli*.

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1.2 DNA manipulation

Genomic DNA was isolated with a Genomic DNA Purification Kit (Fermentas, Canada). Plasmid DNA was isolated with an E.Z.N.A. Plasmid Mini Kit (Omega, USA). DNA products from PCR and restriction enzyme-digested fragments were purified with the E.Z.N.A. Cycle-Pure Kit (Omega, USA) or E.Z.N.A. Gel Extraction Kit (Omega, USA). Restriction enzyme digestions and DNA ligations were performed according to the manufacturers' instructions (NEB, USA and Takara, Japan). Transformations of *E. coli* DH5α used for cloning were conducted according to the manufacturers' instructions. DNA sequencing was performed by Biomed (Beijing Office, China). Analysis of nucleotide sequences was performed with DNAMAN software. Primers were designed with primer Premier 5 and synthesized by Invitrogen (Beijing Office, China) (**Table 2**).

1.3 Construction of the transcriptional fusion of plasmid pUCGMA2T_{1-4}

We first amplified a fragment of *rbs*-*gfp* derived from pBQ9 containing an AGGAGG sequence and spacer sequence (Quinones et al., 2004) with the primers *rbs*-*gfp*-*Pst*I-F and *rbs*-*gfp*-*Sph*I-R. The PCR product was cloned into the *Sph*I and *Pst*I sites of pUC19. Then the promoter of *nptII* was amplified from pTS using the primers *nptII*-*Sal*I-F and *nptII*-*Pst*I-R (Stiner and Halverson, 2002). The PCR fragment was inserted into the *Sal*I and *Pst*I sites of pUC19-*rbs*-*gfp* to yield pUC19-*nptII*-*rbs*-*gfp*, also named pUCNG.

Pss B728a was cultured overnight on KB medium at 28°C with 100 mg/L rifampicin. Its genomic DNA was isolated according to the instructions of the Genomic DNA Purification Kit. The complete 1.14 kb *ahlR* gene including the *ahlR* promoter (P_{ahlR}) (GenBank: AJ566343.1) was amplified from the *Pss* B728a genomic DNA with primers *ahlR*-*Xba*I-F and *ahlR*-*Sal*I-R.

The plasmid pUCAM, which responds to AHL using the AHL-responsive promoter of *ahlI* (P_{ahlI}) and the reporter gene *mcherry*, was constructed as follows. First, the P_{ahlI}:*gfp* fragment was amplified from pBQ9 with the primers *ahlI*-*gfp*-*Xba*I-F and *ahlI*-*gfp*-*Xma*I-R and cloned into pUC19, creating pUCAG. Second, the *mcherry* coding sequence was amplified from pAN583 with the primers *mcherry*-*Nde*I-F and *mcherry*-*Hpa*I-R (Nelson et al., 2007). The PCR fragment was inserted into the *Nde*I and *Hpa*I sites of pUCAG, replacing most of the *gfp* gene present. The P_{ahlI}:*mcherry* fusion was re-amplified with the primers *ahlI*-*gfp*-*Xba*I-F and *mcherry*-*Xma*I-R, digested with *Xba*I and *Xma*I, and cloned into pUC19, forming pUCAM.

The 1.123 kb P_{ahlI}:*mcherry* fragment was purified and inserted into the *Xba*I and *Xma*I sites of pUCNG, resulting in pUCGM. Plasmids pUCGM and pUC-*ahlR* were cut with *Sal*I and *Xba*I. The products were purified and cloned into digested pUCGM, resulting in pUCGMA. To lower background expression from pUCGMA, we inserted the transcriptional terminator T_{1-4} (four tandem copies of the T_1 terminator (Brosius et al., 1981) into pUCGMA using three steps. First, we obtained T_{1-4} by PCR using pWM1009 (Monier and Lindow, 2005) as a template and two primer sets, T_{1-4}-*Xma*I-F/T_{1-4}-*Xma*I-R and T_{1-4}-*Xba*I-F/T_{1-4}-*Xba*I-R. Second, the purified T_{1-4}PCR product containing *Xba*I sites and pUCGMA were each cut with *Xba*I. The T_{1-4} fragment was ligated to digested pUCGMA, generating pUCGMAT_{1-4}. Finally, the T_{1-4} PCR product containing *Xma*I sites and pUCGMAT_{1-4} were each cut with *Xma*I. The T_{1-4} fragment was ligated to digested pUCGMAT_{1-4}, generating pUCGMA2T_{1-4}. The

Table 2 Primer sequences

Primer name	Primer sequence (5′⟶3′)	Temperature (°C)	Restriction site
*rbs-gfp-Pst*I-F	AAAACTGCAGAAGGAGG AAAAACATATGAG	46	*Pst*I
*rbs-gfp-Sph*I-R	ACATGCATGC CTATTTGTATAGTTCATCCATGC	46	*Sph*I
*nptII-Sal*I-F	ACATGTCGACGTCAGGCTGTAACAGCTCAGA	59	*Sal*I
*nptII-Pst*I-R	AAAACTGCAGATCCTGTCTCTTGATCAGATCTTG	63	*Pst*I
*ahlI-gfp-Xba*I-F	TGCTCTAGACTCTGATCCTGGTGCGTGTGG	63	*Xba*I
*ahlI-gfp-Xma*I-R	TCCCCCGGGCTATTTGTATAGTTCATCCATGCCA	63	*Xma*I
*mcherry-Nde*I-F	GGAATTCCATATGATGGTGAGCAAGGGCGAG	53	*Nde*I
*mcherry-Hpa*I-R	GCGGCCGTTAACTTAAGATCTGTACAGCTCGT	51	*Hpa*I
*mcherry-Xma*I-R	TCCCCCGGGATTAATTTAAGATCTGTACAGCTCGTCCAT	63	*Xma*I
*ahlR-Xba*I-F	TGCTCTAGACCACGCAGCGCAAGCCCCG	63	*Xba*I
*ahlR-Sal*I-R	ACGCGTCGACAGCCTGAGTCAGACCATGCCC	63	*Sal*I
T_{1-4}-X*ma*I-F	TCCCCCCGGGGGCCGCAATTCCCAATTCCA	65	*Xma*I
T_{1-4}-X*ma*I-R	TCCCCCCGGGATGCAAGCTTCCGATCCCCAAT	64	*Xma*I
T_{1-4}-X*ba*I-F	CTAG TCTAGAGGCCGCAATTCCCAATTCCA	65	*Xba*I
T_{1-4}-X*ba*I-R	CTAG TCTAGAATGCAAGCTTCCGATCCCCAAT	64	*Xba*I

Underlined sequences represent restriction sites.

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T_{1-4} section of pUCGMA2T_{1-4}, verified by PCR and cut by *Xba*I and *Xma*I, was chosen.

1.4 Induction of *E. coli* AHL biosensors

To characterize the response of the biosensor plasmid to AHLs, overnight cultures of *E. coli* DH5α (pUCGMA2T_{1-4}) were inoculated (10%, *V*/*V*) in flasks with fresh LB medium containing 50 mg/L ampicillin at 37°C and grown exponentially for 3 hr to an OD_{600} of approximately 0.6. Next, 3OC6-HSL dissolved in acetonitrile (UV grade) was added to the cultures so that they contained 0 or 10^{-6} mol/L 3OC6-HSL. The cultures were further incubated at 37°C. Culture samples (180 µL) were added to a 96-well microtiter plate (flat-bottom, COSTAR, USA) at various time intervals (0, 3, 6, 9, 12 and 24 hr), and the relative fluorescence units (RFU) of green and cherry red fluorescence were respectively measured at 510/610 nm with excitation wavelength 488/543 nm (Andersen et al., 2001) and with a Multimode Microplate Spectrophotometer (VarioSkan Flash dispenser, Thermo, USA). For each sample, the measured fluorescence value was normalized to 1 mL of culture. Simultaneously, we withdrew 10 µL samples of each culture to make slides that we then imaged using the green and red channels of a fluorescence microscope (AxioSkop 40FL, Zeiss, Germany). Every experiment did three parallel tests.

The AHL dose response of *E. coli* DH5α (pUCGMA2T_{1-4}) was analyzed. Overnight biosensor cultures were inoculated as described above. When the OD_{600} reached approximately 0.6, cultures were divided into six subcultures. Every subculture was used for three parallel tests. 3OC6-HSL solutions were added into each subculture to final concentrations of 0, 10^{-8}, 5×10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} mol/L. The cultures were incubated at 37°C, and samples were withdrawn at 0, 3, 6, 9, 12, 16, 20 and 24 hr. Measurement of green and cherry red fluorescence and microscopic imaging were performed as described above. The measured fluorescence values were normalized to 1 mL of culture.

1.5 Transformation of plasmid pUCGMA2T_{1-4} into *Pseudomonas syringae* TA125

Electrocompetent *P. syringae* TA125, isolated from laboratory environmental *Nicotiana tabacum* and capable of producing short-chain AHLs (Lv et al., 2013) was prepared according to the method described by Swingle et al. (2010). In each experiment, 50 ng of plasmid pUCGMA2T_{1-4} DNA was added to 60 µL electrocompetent cells. The mixture was then maintained statically for 1 min and transformed by electroporation in a chilled 0.2 cm cuvette using a Gene pulser in mode two (Bio-Rad Laboratories, Hercules, CA). After the electroporation, 1 mL of room temperature LB medium was immediately added. The mixture was then added to 4 mL LB medium and shaken at 30°C for 4 hr to allow *P. syringae* TA125 to recover and express ampicillin resistance. Finally, 1 mL of the recovery culture was centrifuged for 1–3 min at 4000 r/min at room temperature, and the cell pellet was resuspended in 200 µL LB medium. Of this, 100 µL was serially diluted to a 10^3 factor, spread on selective LB agar plates with 50 mg/L ampicillin and incubated at 30°C until colonies appeared (usually with 24 hr). We also transformed pBQ9 into *P. syringae* TA125 as a control. For image analysis, we inoculated one clone into a test tube containing 5 mL LB medium with 50 mg/L ampicillin. When it matured, we withdrew 10 µL samples to make slides and imaged the cells with a fluorescence microscope.

2 Results and discussion

2.1 Construction of a dual fluorescence biosensor plasmid

The plasmid pUCGMA2T_{1-4}, with a dual fluorescent AHL biosensor, was constructed to respond to AHLs using the AhlI/R AHL system of *Pss* B728a and to indicate the host cells using constitutively produced GFP. A schematic outline of the construct is presented in **Fig. 1**. To achieve a strong signal indicating the host cells, P_{nptII} and *rbs-gfp* from plasmids pTS and pBQ9 were ligated into pUC19 to form the plasmid pUCNG. This strategy can achieve much higher green fluorescence than *gfp* alone (Andersen et al., 2001). Meanwhile, an *mcherry* fragment from plasmid pAN583 and the P_{ahlI} from plasmid pBQ9 were fused and integrated into pUC19 to form a fragment for responding to 3OC6-HSL with cherry red fluorescence. By *Xma*I and *Xba*I digestion of plasmids pUCAM and pUCNG, we constructed plasmid pUCGM, which contains the cherry red unit for the AHL response and the green fluorescent unit to indicate host cells. Next, the *ahlR* gene was integrated between *Sal*I and *Xba*I sites to construct plasmid pUCGMA, which contains three components: P_{nptII}:*gfp* for constitutively expressing GFP in the host cell, P_{ahlI}:*mcherry* to produce cherry red fluorescence in response to AHL and the *ahlR* gene encoding the AHL regulator protein. Previous studies showed that background expression can be decreased by 94% by using one copy of the terminator; the background can be lowered an additional twofold (97% inhibition) with four tandem copies (Miller et al., 2000). To achieve lower background and to prevent dual fluorescence interference, four tandem copies of the T_1 from the *E. coli rrnB1* operon were added in pUCGMA with P_{ahlI}:*mcherry* to construct the final plasmid pUCGMA2T_{1-4} (Brosius et al., 1981).

2.2 A dual fluorescence biosensor using *E. coli* as the host cell

In terms of AHL signals, there are three types of Gram-

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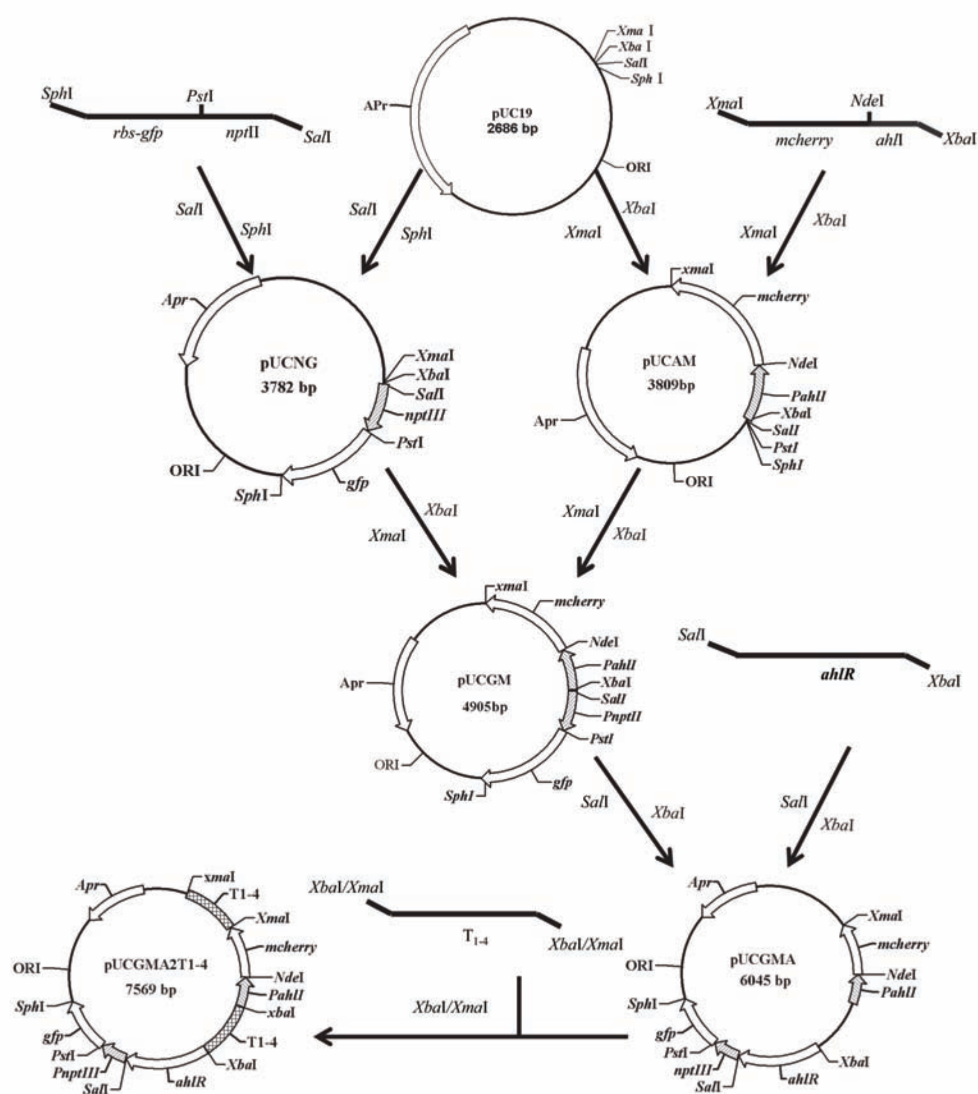

Fig. 1 Construction of *N*-acyl-homoserine lactone (AHL) sensor plasmids using a pUC19 plasmid backbone. Plasmid pUCGMA2T$_{1-4}$ contains a fusion of P_{ahlI}:*mcherry*, which responds to AHL, and a constitutively expressed P_{nptII}:*gfp* marker gene to facilitate identification of the bioreporter cells. It also contains *ahlR*, the AHL regulator gene, and two T$_{1-4}$ (four tandem copies of transcriptional terminator) sequences. Arrows indicate directionality of transcription.

negative bacteria, namely bacteria with an AHL synthase and its regulation unit, bacteria with an orphan regulation unit but no AHL synthase and bacteria lacking both. *E. coli* has an orphan LuxR family protein SdiA that can activate the *rhlI* promoter, but it does not have the cognate SdiA LuxI-family synthase to synthesize AHLs, so *E. coli* is available for AHL biosensor host. Although it is known that this LuxR family protein can become activated to produce extracellular enzymes that break down antimicrobials, its biological significance remains unclear. Thus, early AHL biosensors could be easily constructed to detect and measure AHLs by introducing LuxI-family synthase gene fused to a reporter gene (Steindler and Venturi, 2007). However, this strategy is limited to hosts with an AHL regulation system. When the dual fluorescence plasmid pUCGMA2T$_{1-4}$ was transformed into *E. coli* DH5α, the dual fluorescence whole-cell biosensor *E. coli* DH5α (pUCGMA2T$_{1-4}$) showed a maximal response after 9 hr with 10^{-6} mol/L 3OC6-HSL (**Fig. 2A**), but red fluorescence was easily observed by fluorescence microscopy after 3–6 hours. At 6 hours, the cultures clearly showed cherry red that was visible by eye (**Fig. 2B-f**), and very bright red fluorescence above background was observed with fluorescence microscopy (**Fig. 2B-e, C**). Meanwhile, the intensity of the green fluorescent host cell indicator increased with host cell growth and was not affected by the presence or absence of 3OC6-HSL (**Fig. 2A**). Furthermore, fluorescence microscopic imaging demonstrated that *E. coli* DH5α (pUCGMA2T$_{1-4}$) constitutively expressed green fluorescence as an AHL-independent marker. At the

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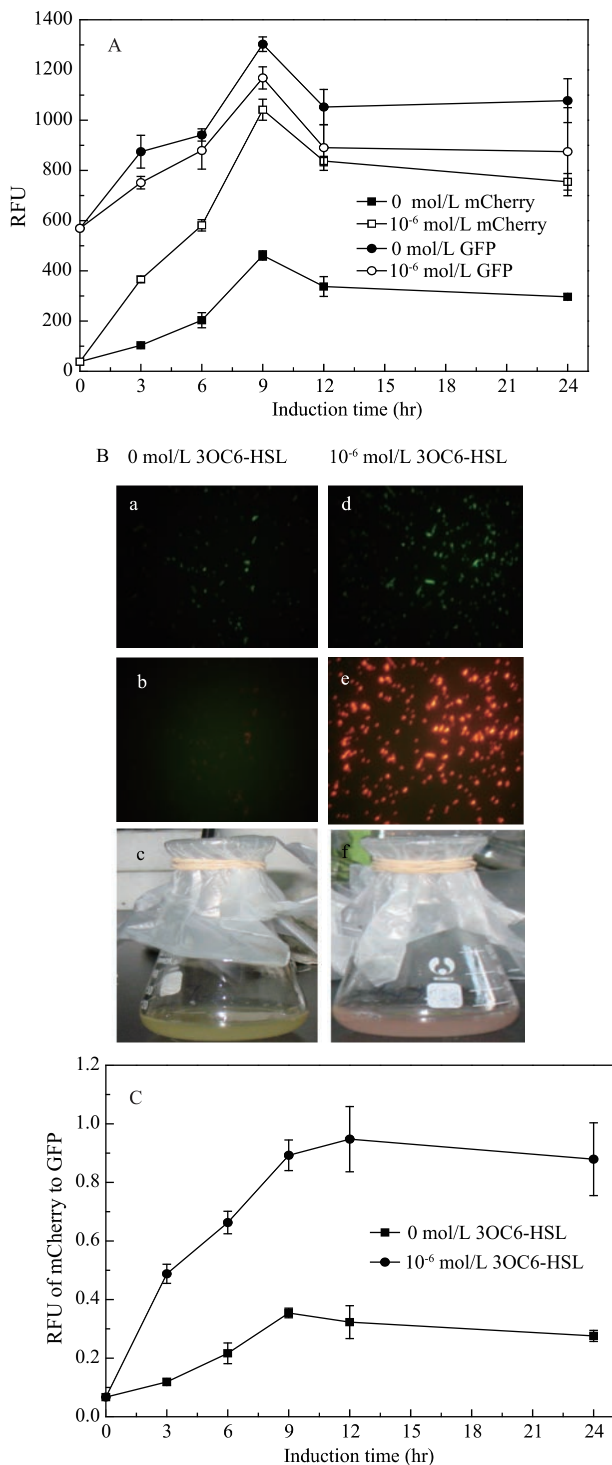

Fig. 2 *E. coli* DH5a (pUCGMA2T$_{1-4}$) biosensor was induced with 0 or 10^{-6} mol/L 3OC6-HSL in LB medium. (A) RFU of the mCherry and GFP fluorescence with 0 and 10^{-6} mol/L 3OC6-HSL, (B) fluorescence microscope images of the green (a, d) and red (c, e) channels and images taken with an ordinary camera (c, f) after 6 hr of induction by 0 (a–c) or 10^{-6}(d–f) mol/L 3OC6-HSL, (C) RFU ratio of mCherry to GFP fluorescence with 0 and 10^{-6} mol/L 3OC6-HSL. Error bars represent the standard error of the mean for three biological replicates at each concentration.

same time, the cells effectively respond to AHL (**Fig. 2**).

To determine the dose response of the *E. coli* DH5α (pUCGMA2T$_{1-4}$) biosensor, the induction time and dose were varied as shown in **Fig. 3**. We observed rapid expression from 0–6 hr, with an expression peak at 9 hr with different concentrations of 3OC6-HSL. There was no apparent increase in expression at concentrations below 5×10^{-8} mol/L, which indicates that the detection limit of this biosensor is 5×10^{-8} mol/L. Compared with conventional two-component constructs, which showed bright red fluorescence above background with concentrations of 3OC6-HSL greater than 10^{-7} mol/L (Dulla and Lindow, 2008), our construct is much more sensitive due to the presence of the *ahlR* gene in the plasmid. Furthermore, the use of *mcherry* as the indicator in our construct also has the advantage of a rapid response time. Cherry red was observed by eyes with our biosensor within 6 hr when concentrations of 3OC6-HSL were $\geq 5\times10^{-8}$ mol/L (**Fig. 3b**). When the conventional red fluorescent protein mRFP1 is used as an indicator, red fluorescence can be observed by fluorescent microscopy from 5 to 16 hr. This may be mainly due to the more complete maturation of mCherry and its greater tolerance for N-terminal fusions than mRFP1. The photostability of mCherry is over tenfold greater than mRFP1 (Shaner et al., 2004).

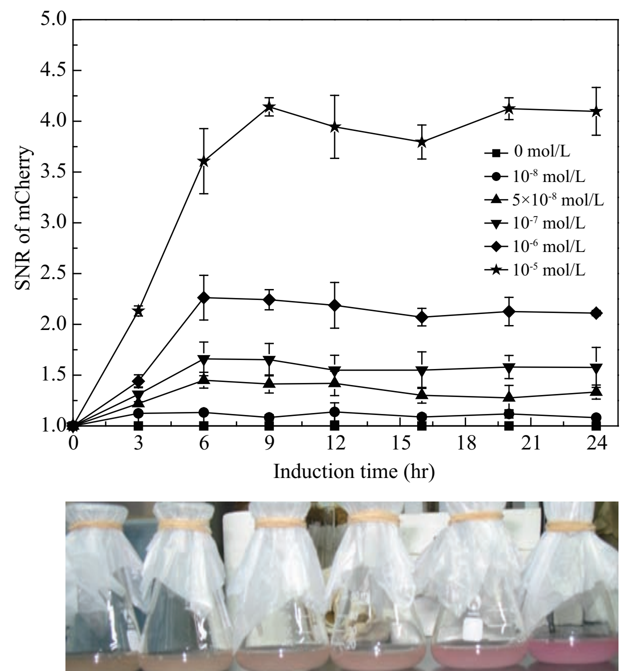

Fig. 3 Response of the *E. coli* DH5a (pUCGMA2T$_{1-4}$) biosensor to a range of 3OC6-HSL doses in LB medium. (a) SNR (signal-to-noise ratio) of cherry red fluorescence. Error bars represent 1 standard error of the mean for three biological replicates at each concentration. (b) an ordinary photograph of the biosensor with each signal concentration after 6 hr of induction; from left to right, the concentrations of 3OC6-HSL are 0, 10^{-8}, 5×10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} mol/L.

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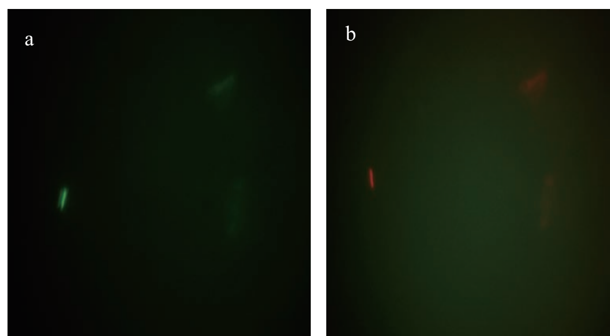

Fig. 4 Fluorescence micrographs of *P. syringae* TA125 harboring plasmid pUCGMA2T$_{1-4}$. (a) green channel; (b) red channel.

2.3 A dual fluorescence biosensor using wild-type *P. syringae* TA125 as the host cell

Usually, constructing a biosensor is relatively easy when typical lab strains such as *E. coli* are used as hosts. In contrast, it can be difficult to obtain expression when wild-type strains are used as host cells, even if a construct expresses very well in typical lab strains. To characterize the responsiveness of plasmid pUCGMA2T$_{1-4}$ to AHL in a wild-type strain, it was introduced to *P. syringae* TA125, a strain isolated from tobacco leaves. The dual fluorescence biosensor in *P. syringae* TA125 exhibited both the constitutive green fluorescent marker and cherry red fluorescence in response to endogenous bacterial AHLs (**Fig. 4**) The function of this biosensor was similar to the typical phyllosphere biosensor pBQ9 (gift of Prof. Lindow, University of California Berkeley; data not shown). This result demonstrated that plasmid pUCGMA2T$_{1-4}$ is a practical vector that is capable of detecting environmental AHLs and bacterial behavior. Repeatability of the biosensor with *E. coli* and wild-type *P. syringae* TA125 as the host cell, respectively, showed that the dual fluorescence whole-cell AHL biosensor was constructed successfully. Therefore, our dual fluorescence whole-cell AHL biosensor is expected to be useful for understanding the mechanisms of bacterial behaviors and for practical applications.

3 Conclusions

The construction of a plasmid for a dual fluorescence whole-cell AHL biosensor is described. The biosensor harbored within this plasmid has the advantages of rapid response time, visible cherry red for easier observation and higher sensitivity in response to AHLs. It also constitutively expresses bright green fluorescence as a marker to highlight the biosensor cells and distinguish them from other surrounding bacteria. Therefore, the AHL biosensor reported here may offer an approach for the construction of other biosensors.

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