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New naphthalene whole-cell bioreporter for measuring and assessing naphthalene in polycyclic aromatic hydrocarbons contaminated site

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HIGHLIGHTS

- We constructed a novel bioreporter, for rapid detecting naphthalene.
- It suggested a new concept for multiple PAHs whole-cell bioreporter construction.
- The bioreporter achieved rapid evaluation of naphthalene in real site.

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ABSTRACT

A new naphthalene bioreporter was designed and constructed in this work. A new vector, pWH1274_Nah, was constructed by the Gibson isothermal assembly fused with a 9 kb naphthalene-degrading gene nahAD (*nahAa nahAb nahAc nahAd nahB nahF nahC nahQ nahE nahD*) and cloned into *Acinetobacter* ADPWH_lux as the host, capable of responding to salicylate (the central metabolite of naphthalene). The ADPWH_Nah bioreporter could effectively metabolize naphthalene and evaluate the naphthalene in natural water and soil samples. This whole-cell bioreporter did not respond to other polycyclic aromatic hydrocarbons (PAHs; pyrene, anthracene, and phenanthrene) and demonstrated a positive response in the presence of 0.01 μM naphthalene, showing high specificity and sensitivity. The bioluminescent response was quantitatively measured after a 4 h exposure to naphthalene, and the model simulation further proved the naphthalene metabolism dynamics and the salicylate-activation mechanisms. The ADPWH_Nah bioreporter also achieved a rapid evaluation of the naphthalene in the PAH-contaminated site after chemical spill accidents, showing high consistency with chemical analysis. The engineered *Acinetobacter* variant had significant advantages in rapid naphthalene detection in the laboratory and potential *in situ* detection. The state-of-the-art concept of cloning PAHs-degrading pathway in salicylate bioreporter hosts led to the construction and assembly of high-throughput PAH bioreporter array, capable of crude oil contamination assessment and risk management.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), a group of persistent organic pollutants, exist extensively in subsurface environments (Wilcke, 2000). PAHs have been designated by the US Environmental Protection Agency (US EPA) as priority pollutants because of their possible carcinogenicity and toxicity to humans and animals (Boffetta et al., 1997). Naphthalene, a classical PAH with possible carcinogenicity, has drawn considerable concerns because of its high water solubility and high volatility (Valdman et al., 2004b). As one of the most widespread xenobiotic pollutants, the detection and natural attenuation of naphthalene are the main challenges in PAH contamination (Valdman et al., 2004a).

Chemical analysis is the main approach for the detection of naphthalene in environmental samples. Despite their cost and

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laborious pre-treatment, gas chromatography/mass spectrometry (GC/MS) (Potter and Pawliszyn, 1994) and high-performance liquid chromatography (Oliferova et al., 2005) are the most commonly applied techniques in environmental monitoring (Zhang et al., 2013). Recently, the increasing attention on genetically engineered whole-cell bioreporters attributed to their high sensitivity, low cost, time efficiency, and ease of operation (Song et al., 2009). More importantly, whole-cell bioreporters can assess the bioavailability and toxicity of contaminants in their natural environments (Deepthike et al., 2009; Kohlmeier et al., 2008; Tecon et al., 2009). They could further be used for *in situ* or online measurement of contaminants and evaluation of their ecological impacts (Chen et al., 2013; Elad et al., 2011). Whole-cell bioreporters are viewed as supplementary techniques to chemical analysis for environmental risk assessment. Numerous whole-cell bioreporters have been reported to sense crude oil and PAHs, such as *n*-alkane (Zhang et al., 2012a), benzene, toluene, ethylbenzene, and xylene (Keane et al., 2008; Kuncova et al., 2011), naphthalene (King et al., 1990; Trogl et al., 2012), and phenanthrene (Shin et al., 2011) (Appendix 1). Particularly, for naphthalene, *Pseudomonas fluorescens* HK44 is the most commonly investigated naphthalene bioreporter (King et al., 1990; Trogl et al., 2007). *P. fluorescens* HK44 can not only be directly applied in wastewater monitoring (Valdman et al., 2004b) but can also achieve online naphthalene detection (Valdman et al., 2004a), PAH degradation assessment (Paton et al., 2009), or immobilized in gel to retain its long storage time (Trogl et al., 2005). The bacterial biosensor HK44 has been shown to respond to sensitively and quantitatively to naphthlene (Paton et al., 2009). Indeed, while the biosensor in the study of polar organic contaminants in soil has been reported, their application in soil is less common (Semple et al., 2003). Currently, the biosensors need to interface with such target pollutants either following a suitable organic solvent extraction step or pioneering a technique directly via the gas phase (Heitzer et al., 1994). Most biosensor of naphthalene applications have remained in research lab for many reasons, some of which relate to a lack of standardization, the difficulty in maintaining living microbes, and also the poor analytical quality of the assays, poor specificities of detection, and the high detection limits (Werlen et al., 2004).

Nevertheless, many other types of PAHs are still undetectable by living organisms. The fusing of reporter genes to the promoters of degradation genes and the use of biological signaling chains coupled to fluorescent or bioluminescent proteins are attributed to the construction principle (Belkin, 2003; van der Meer et al., 2004). For instance, the conventional methods for naphthalene bioreporter construction followed the fusion of *lux* or *gfp* reporter gene in the operon (like *nahR*) encoding naphthalene metabolism (Shin, 2010), hosted by indigenous naphthalene-degrading microbes (e.g., *P. fluorescens*) (King et al., 1990) or genetically engineered model strains (e.g., *Escherichia coli*) (Mitchell and Gu, 2005), thereby allowing the expression of biological signals during naphthalene degradation (Ripp et al., 2000). The construction of each bioreporter is unique and laborious for specific PAH molecules. Most PAH metabolism occurs via the salicylate pathway, including naphthalene (Chen and Aitken, 1999; Harwood and Parales, 1996; Johri et al., 1999; Loh and Yu, 2000; Yen and Serdar, 1988), and salicylate behaved as a significant signaling metabolite for whole-cell bioreporters. By cloning naphthalene-degrading operons into the salicylate bioreporter host, whole-cell bioreporters detect metabolic salicylate and quantify the existence of the parent naphthalene in natural environment. This technique is also applied for the construction of series PAHs whole-cell bioreporters for multi sensing array.

In this study, a new type of naphthalene bioreporter was constructed and applied in groundwater and soil naphthalene contamination measurement. A Gibson isothermal assembly (Gibson et al., 2009) was introduced for the construction of a recombinant naphthalene-degrading plasmids, pWH1274_Nah, with the capability of transferring naphthalene to the central metabolite salicylate. The new bioreporter, ADPWH_Nah, was constructed by cloning the pWH1274_Nah vector in the host *Acinetobacter* ADPWH_lux (Huang et al., 2005) and the *salAR* and *luxCDABE* operons, which were inducible by salicylate. Converting naphthalene to salicylate by the expression of *nahAD* operon on the vector, the ADPWH_Nah bioreporter was able to quantitatively respond to naphthalene and assess naphthalene contamination in natural water and soil. The naphthalene-degrading pWH1274_Nah vector could be replaced by other plasmids with respective PAH metabolic operons to achieve the biological detection of targeting PAHs. Our work presented a routine and simple method for the construction of various bioreporters responsive to different PAH molecules, exhibiting its extensive application possibilities in water and soil monitoring and assessment.

2. Materials and methods

2.1. Bacterial strains, plasmids, and culture media

The bacterial strains and plasmids are listed in Table 1. Unless otherwise stated, all the chemicals are analytical-grade reagents. Luria–Bertani (LB) was used as the cultivation medium for ADPWH_lux (Thermo Scientific, USA). ADPWH_Nah and

Table 1
Bacterial strains and plasmids used in this study.

Strain/plasmid	Description	Reference
Bacteria		
Escherichia coli DH5α	High efficient competent cells.	Tiangen, China
Pseudomonas putida NCIB9816	Naphthalene degrader with NahAD operon (9 kb) for naphthalene metabolization.	(Cane and Williams, 1982)
Escherichia coli DH5α_pWH1274_Nah	*Escherichia* cells with pWH1274_Nah vector.	This study
ADPWH_lux	*Acinetobacter* bioreporter responsive to salicylate. A promoterless *luxCDABE* from pSB417 was inserted between *salA* and *salR* genes in the chromosome of ADP1.	(Huang et al., 2005)
ADPWH_Nah	*Acinetobacter* bioreporter responsive to naphthalene. The pWH1274_Nah vector exited in ADPWH_lux.	This study
ADPWH_1274	*Acinetobacter* bioreporter as the negative control of ADPWH_Nah. The pWH1274 vector exited in ADPWH_lux.	This study
Plasmids		
pDTG1	Plasmid with the NahAD operon (9 kb) from *Pseudomonas putida* NCIB9816.	(Dennis and Zylstra, 2004)
pWH1274	*Escherichia coli* and *Acinetobacter baylyi* shuttle plasmid (6 kb), containing P_{tet} constitutive promoter and *Eco*RV restriction site for cloning. Ampicillin is used as antibiotic selection.	(Hunger et al., 1990)
pWH1274_Nah	NahAD operon cloned into the *Eco*RV site of pWH1274 vector.	This study