Whole-cell bacterial bioreporter for actively searching and sensing of alkanes and oil spills

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Summary

Acinetobacter baylyi ADP1 was found to tolerate seawater and have a special ability of adhering to an oil–water interface of 10–80 μm emulsified mineral and crude oil droplets. These properties make ADP1 an ideal bacterial chassis for constructing bioreporters that are able to actively search and sense oil spill in water and soils. Acinetobacter baylyi bioreporter ADPWH_alk was developed and applied to the detection of alkanes and alkenes in water, seawater and soils. Bioreporter ADPWH_alk was able to detect a broad range of alkanes and alkenes with carbon chain length from C7 to C36. So far, ADPWH_alk is the only bioreporter that is able to detect alkane with carbon chain length greater than C18. This bioreporter responded to the alkanes in about 30 min and it was independent to the cell growth phase because of two point mutations in the alkM promoter recognized by alkane regulatory protein ALKR. ADPWH_alk was applied to detect mineral oil, Brent, Chestnut and Sirri crude oils in water and seawater in the range 0.1–100 mg l⁻¹, showing that the bioreporter oil detection was semi-quantitative. This study demonstrates that ADPWH_alk is a rapid, sensitive and semi-quantitative bioreporter that can be useful for environmental monitoring and assessment of oil spills in seawater and soils.

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Introduction

Crude oil spill (such as the recent Mexico Gulf oil spill) and contamination associated with crude oil pumping, process and transportation posed a great threat to the environment and also public health. Alkanes and alkenes with various carbon chains are main components of crude oil. Although many microorganisms were able to aerobically or anaerobically degrade alkanes and alkenes (Van Beilen et al., 1994), biodegradation of long chain alkanes and alkenes was limited owing to their low solubility and accessibility to microorganisms, and therefore these compounds resided a relatively long time in the environment. Consequently, a rapid, cheap and sensitive way to detect alkanes or alkenes present in natural habitats was required for environmental monitoring and risk assessment. In regards to this, whole-cell bioreporters for the detection of alkanes or alkenes are especially useful to environmental management because cell-based bioreporters could indicate the bioavailability portions of alkanes and alkenes in a complex environment which generally could not be addressed using standard chemical analytical techniques. Acinetobacter sp. are ubiquitous bacteria in natural aquatic and soil environment (Young et al., 2005) that are frequently found to be capable to degrade a broad range of carbon chain alkanes and alkenes (Lal and Khanna, 1996; DiCello et al., 1997; Ratajczak et al., 1998a,b; Baldi et al., 1999; Choi et al., 1999; Razak et al., 1999; Koma et al., 2001; Pleshakova et al., 2001; Tani et al., 2001; Throne-Holst et al., 2007; Wentzel et al., 2007; Tanaka et al., 2010). Among these alkane degraders, Acinetobacter baylyi ADP1 is able to utilize alkanes with carbon lengths ranging from 12 up to 36 and the gene regulation for alkane degradation was well characterized (Ratajczak et al., 1998a,b; Throne-Holst et al., 2007). ADP1 has an AraC/XylS-like transcriptional regulatory protein ALKR that regulates alkane hydroxylase gene alkM in ADP1 chromosome to initiate alkane oxidation (Ratajczak et al., 1998a,b).

To date, only a few alkane bioreporters have been developed (Sticher et al., 1997; Alkasrawi et al., 1999; Minak-Bernero et al., 2004), and those bioreporters have been shown to detect short and medium (i.e. C5–C10) carbon chain of alkanes and alkenes. To extend alkane detection spectra, we constructed an alkane/
alkene bioreporter ADPWH_alk that can respond to alkanes and alkenes with various carbon chain lengths ranging from C7 to C36. We found that A. baylyi ADP1 and ADPWH_alk were able to adhere to an interface of oil and water; and to emulsify mineral and crude oils into oil droplets at micrometre level. These special properties enabled ADPWH_alk to overcome alkane’s low solubility and accessibility, and to actively search and sense oil spill in water and soils. The ADPWH_alk was used to determine mineral and crude oils in water, seawater and soils.

Results

Genetic structure of alkane bioreporter A. baylyi ADPWH_alk

ADPWH_alk has been constructed by inserting promoter-less luxCDABE cassette into alkM in ADP1 and luxCDABE transcription is controlled by ALKR regulation system (Fig. 1). The vector pAlkRM_lux_km was constructed on ABE transcription is controlled by ALKR regulation system (Fig. 1). The vector pAlkRM_lux_km was constructed on pGEM-T backbone which cannot replicate in ADP1, suggesting that the luxCDABE cassette should be inserted in the chromosome of ADP1. Southern blotting confirmed that a single copy of luxCDABE was inserted into the chromosome by Campbell-like integration. The DNA sequences of colony PCR products, which used ADPWH_alk colony as DNA template and ADP1_alk_for/luxC_rev and alk_P_up/ADP1_alk_rev as primer pairs (Table 1), confirmed the genetic structure of ADPWH_alk construct (Fig. 1A). The DNA sequence also indicated that three point mutations at the promoter region of ADPWH_alk, which were introduced by pAlkRM_lux_km. The mutations were within the intergenic region between alkM1-luxCDABE and alkR (Fig. 1B).

ADPWH_alk actively searching and sensing oils

The solubility of alkane and crude oil in water are usually extremely low; hence alkanes and crude oils are inaccessible to cells, which may hamper oil detections in complex water and soil environments. Acinetobacter baylyi ADP1 and its derivative ADPWH_alk were found to adhere to an oil–water interface and to emulsify oils into small droplets (Fig. 2). In the Escherichia coli DH5α–oil mixture, it was difficult to observe small oil droplets unless vigorous shaking was applied, and E. coli was not associated with oil neither (Fig. 2A). However, in the ADPWH_alk–oil mixture, ADPWH_alk emulsified both mineral and crude oils into 10–80 μm oil droplets and the cells were found attached to the surface of oil droplets but were absent from the water phase (Fig. 2B–D). It indicated that A. baylyi was able to recognize alkanes and twitch their movements to the surface oil droplets. The cell density of ADPWH_alk on the surface of mineral and crude oil droplets was estimated to be 1.4 ± 0.2 × 10^10 cells m⁻², implying that cells occupied a single layer on the oil surface.

Characterization of alkane bioreporter ADPWH_alk

ADPWH_alk induction was conducted by exposing the bioreporters to 100 μM suspension solution of a series of alkanes including: hexane (C6), dodecane (C12), tetradecane (C14), octadecane (C18), tetracosane (C24), triacontane (C30) and tetroctane (C40). The time-course of ADPWH_alk activation and the bioluminescence expression were plotted in Fig. 3A. In the presence of dodecane (C12), tetradecane (C14), octadecane (C18), tetracosane (C24) and triacontane (C30), ADPWH_alk was rapidly induced and expressed bioluminescence within 30 min compared with a background level of bioluminescence in the absence of alkanes (Fig. 3A). The maximum induction by octadecane (C18) was approximately two times higher than other alkanes (Fig. 3A), which was consistent with previous report (Ratajczak et al., 1998a). ADPWH_alk was also rapidly induced by Brent crude oil in pure water and seawater and the ADPWH_alk sensing performance to Brent crude oil was not affected by salt and other impurities in seawater (Fig. 3B).

Detection of a broad range of carbon chain alkenes and alkanes (C7–C36)

Figure 4 represented that ADPWH_alk was sensitive to alkanes and alkenes with carbon chain length ranging from C7 to C36. Although A. baylyi could only utilize alkanes with carbon chain longer than C12 (Ratajczak et al., 1998a; Wentzel et al., 2007), ADPWH_alk was able to respond to alkanes with low carbon chain length down to C7, meaning that this bioreporter could cover a broad range of alkenes and alkanes (i.e. C7–C36). Alkanes with shorter (e.g. hexane – C6) or longer carbon chains (e.g. tetroctante – C40) did not activate ADPWH_alk (Figs 3A and 4). ADPWH_alk induction ratio varied to alkanes or

| Table 1 | Primers used in this study. |
|--------|-----------------------------|
| Primers | Sequence (5′→3′) |
| alk_P_up | GTCGTACCATCTCTGTATATGAAATGAG |
| alk EB_rev | CATTGAGATTCCGAATCTGAAAGGCTTTT |
| alk EB_for | GAGCGCCAAATGGAGAAGGATAG |
| alk out_down | TGTATAAGAGAAGGAAATGAA |
| ADP1_alk_for | GAGATCATCATTAAATGGCAG |
| luxC_rev | GCAAAACGAAAGCAGACCTACCC |

a. The underlines indicate EcoRI and BamHI restriction sites.
Fig. 1. A. Schematic outline of construction of alkane bioreporter *Acinetobacter baylyi* ADPWH_alk (DNA lengths are not scaled). The three point mutations were marked as . B. Genetic structure of alkane regulation part of ADPWH_alk. There are three mutation points at promoter region of alkRM. The mutation points were highlighted with bold. The inverted repeat with two mismatches is marked by arrows under the sequence.
alkenes with different lengths of carbon chain, while alkanes or alkenes with the same carbon chain length, such as C12 dodecane/dodecene and C18 octadecane/octadecene, exhibited similar induction ratios (Fig. 4). The induction ratios of C12, C18 and C24 alkanes or alkenes were significantly higher than alkanes/alkenes with other carbon chain length (Fig. 3), suggesting that alkanes or alkenes with specific carbon chain lengths had effects on the complex of ALKR–promoter–RNAP and favoured alkM transcription initiation.

Calibration of ADPWH_alk responding to mineral and crude oils in water, seawater and soil

ADPWH_alk induction ratios increased in response to higher concentrations of mineral, Brent, Chestnut and Sirri crude oils in the range of 0.1–100 mg l\(^{-1}\) in a semi-quantitative manner (Fig. 5), and the detection limit (0.1 mg l\(^{-1}\)) was equivalent to the US EPA crude oil contamination limit (US EPA water standard and gold book). Optimal time of ADPWH_alk for oil quantitative estimation was 200–240 min. The three crude oils tested were typical oils, and Brent crude was a standard crude oil used by world oil trade. Although the mineral oil and crude oils are consist of a mixture of alkanes and alkenes with different compositions, ADPWH_alk induction patterns to different oils were similar (Fig. 5), which suggested that a single general calibration curve was sufficient to estimate the oil concentration in water in practice. ADPWH_alk induction in seawater and in pure water was similar, indicating that seawater had little impact on the bioreporter’s performance and that ADPWH_alk could be applied to detect oil spill in seawater. A curve of ADPWH_alk induction ratio against crude oil contents in a standard soil was plotted in Fig. 6, which shows that the induction ratio increased with higher contents of crude oil in soils. On the other hand, the more soil added into the bioreporter, the more accuracy of the estimation. On the other hand, less amount of soil would be desirable since soil would interfere with the bioreporter induction measurement by blocking the bioluminescence light. Hence, in Fig. 6, each measurement point was present at its optimal condition which was identified as the maximum bioreporter induction ratio with the minimal soil content. This problem can be overcome by applying magnetic functionalized nanoparticles to the bioreporter (Zhang et al., 2011).

Discussion

Actively searching and sensing alkanes and oils

It is crucial that bacteria should access to insoluble alkanes or oils before they can detect them. Oil emulsification would significantly increase the oil surface area and provide a much larger interface for biochemical reactions. Alkane chemotaxis provides another advantage of oil-degrading bacteria. Acinetobacter baylyi is able to carry out both alkane emulsification and chemotaxis, which make ADPWH_alk a desirable oil-detecting bioreporter as it actively searches and senses oils in water and soils. We observed that it took ADPWH_alk ~20 min to emulsify and bind alkane droplets. Given that the cells take ~10 min to carry out signal transport, regulation process and gene expression, the minimal response time for the alkane bioreporter would be about 30 min. Additionally, it was observed that ADPWH_alk could also be activated by alkanes within 40 min after stored in water at 4°C for more than 1 month. Although the alkM–lux fusion and the wild-type alkM gene in ADPWH_alk may suffer a second cross-over event to remove luxCDABE cassette, ADPWH_alk unstability was not observed in our lab even after 200 continuous generations. In practice, negative and positive controls are always required to rule out possible false-negative results.

The transformant ADPWH_alk was able to respond to alkanes with various lengths of carbon chains. To rule out the possibility that activation of ADPWH_alk was due to alkanes being served as substrates of luciferase LuxAB instead of ALKR-based transcriptional regulation, a series of controls and tests were carried out. The results showed that ADPWH_alk was induced by octadecane while other bioreporters such as ADPWH_lux (salicylate) (Huang et al., 2005), ADPWH_Tol (toluene) (Huang et al., 2008) and ADPWH_recA (toxicity) (Song et al., 2009), which also contained luxCDABE as reporter as well, could not
Fig. 3. Dynamic ADPWH_alk response to alkanes with 100 µM of each compound (A); and 100 mg l⁻¹ Brent crude oil in pure water (PW) and seawater (SW) (B).
be activated by octadecane and the addition of octadecane did not increase bioluminescence expression of those bioreporters (Fig. S2).

**Mutation in alkM shortens response time to alkanes and oils**

Those point mutations affected at alkM promoter alkR regulatory binding and RNA transcription in response to alkanes. ADPWH_alk responded to alkanes in about 30 min and was independent of the growth phases (Fig. 3A and Fig. S3), while previous report showed that strain WH405 (alkM::lacZ) was silenced for about 600 min (10 h) until the strain reached a transition period between exponential and stationary phases (Ratajczak et al., 1998a). Such differences might be associated with the point mutations in alkM promoter within alkR–alkM intergenic region which controls the reporter genes luxCDABE. ALKR is a transcriptional regulator belonging to AraC/XylS family and is constitutively transcribed at low level in different growth phases (Ratajczak et al., 1998a), which was observed as constant bioluminescent background of the controls in Fig. 3. ALKR regulatory protein includes C-terminal DNA-binding domain (CTD) for promoter binding and N-terminal domain (NTD) for inducer recognition (Gallegos et al., 1997; Tropel and van der Meer, 2004; Dominguez-Cuevas et al., 2010). Alkanes were presumably recognized by ALKR regulatory protein and their interaction triggered a conformation change of ALKR dimers which isomerizes the promoter–RNAP complex and led to the activation of alkM gene. The inverted repeat region shown in Fig. 1B was presumptively the binding site of ALKR dimers. There were two point mutations between −35 region and the inverted repeat in ADP-WH_alk (Fig. 1A), which might affect binding and interaction between ALKR dimers and alkM promoter. It was assumed that this change enabled cells to overcome some limiting factors related to growth phases.

In summary, we developed a bioreporter ADPWH_alk which is able to detect alkanes and oils in seawater and soils. It provides a rapid, sensitive and quantitative tool for environmental monitoring and assessment for oil spills in seawater and soils. The bioreporter was constructed in the chromosome of an environmental bacterium – *A. baylyi*, which is able to actively search and sense oils. This bioreporter is more robust in terms of sensing performance (e.g. responding time, tolerance of seawater) and long-term storage. In comparison with previously reported alkane bioreporters, ADPWH_alk detects a broader carbon chain of alkanes/alkenes, responds in shorter time and its detection limit can be extremely low to some alkanes (e.g. octadecane) (Table 2). To our knowledge, so far ADP-WH_alk is the only bioreporter that is able to detect alkane with carbon chain length greater than C18.
Bioreporters employ live cells (usually genetically engineered bacteria) as detection elements and they can be a complementary tool to chemical analysis. Bioreporters have a few advantages: rapid, simple, cheap, in situ and importantly providing information of bioavailability. Bioavailability is one of main concerns of environmental risk management since these two factors directly link environmental contamination to human health risk. Most environmental samples are mixtures of complex contaminants. The additive, antagonistic and synergistic effects caused by complex physical or chemical interactions would make the risk assessment unpredictable if only chemical analysis (e.g. GC-MS or HPLC) was applied to the estimation of bioavailability of contaminated samples. Chemical analysis of contaminated soil and water samples usually requires sample pre-treatment and extraction, which makes inert or active portions of contaminants indistinguishable, while risk assessment is concerned to the active portion (bioavailability). In contrast, bioreporters detect contaminants’ active or bioavailable portions, and it directly links contamination to biological effects and make bioavailability assessment more relevant to human health risk. It is a challenge to construct bioreporters with high reliability, robustness and reproducibility. With recent advances in molecular cell biology, synthetic biology and

![Fig. 5. ADPWH_alk response to different concentrations of mineral oil and Brent, Chestnut and Sirri crude oils in pure water and seawater.](image)

![Fig. 6. ADPWH_alk response to crude oil in soils.](image)
nanotechnology, bioreporters can be endowed with new functions which enhanced the sensing performance (Zhang et al., 2011). It could reduce the gap between laboratory work and real-world application.

**Experimental procedures**

**Culture media and strains**

The bacterial strains and plasmids used in this study are listed in Table 3. *Acinetobacter baylyi* ADP1 and its mutants were incubated at 30°C, and *E. coli* at 37°C. All chemicals were obtained from Sigma-Aldrich and were analytical grade reagents. Luria–Bertani (LB) medium or minimal medium (MM) (Huang et al., 2005) was used for the cultivation of bacteria as appropriate. One litre of MM contains 2.5 g of NaH_{2}PO_{4}, 2.5 g of KH_{2}PO_{4}, 1.0 g of NH_{4}Cl, 0.1 g of MgSO_{4}·7H_{2}O, 10 μl of saturated CaCl_{2} solution, 10 μl of saturated FeSO_{4} solution and 1 ml of Bauchop & Elsden solution. MM succinate (MMS) was prepared by adding 20 mM succinate to MM. Ampicillin (Amp) at 100 μg ml^{-1} kanamycin (Km) was used for *E. coli*, and 300 μg ml^{-1} Amp and 10 μg ml^{-1} Km for *A. baylyi* ADP1 or its mutants when required.

**Table 2.** Comparison of different alkane bioreporters.

| Host            | Regulation system | Reporter   | Response time | Detection limit | Alkane detection range | Reference |
|-----------------|-------------------|------------|---------------|-----------------|------------------------|-----------|
| *E. coli* DH5α  | alkB-alkS         | gfp        | 2.5 h         | 0.011 mg l^{-1} | C6–C10                 | Zhang et al. (2001) |
| *E. coli* DH5α  | alkB-alkS         | luxAB      | 1.0–2.0 h     | 0.011 mg l^{-1} | C6–C10                 | Sticher et al. (1997), Tecon et al. (2010) |
| *E. coli* DH5α  | alkBFGHUT         | luxAB      | –             | 0.78 mg l^{-1}  | C6–C10                 | Minak-Bernero (2004) |
| Y. lipolytica   | OD<sub>600</sub>  | luxAB      | 24 h          | 0.85 mg l^{-1}  | C9–C12                 | Alkasrawi et al. (1999) |
| A. baylyi ADP1  | alkM-alkR         | lacZ       | 10 h          | –               | C7–C18                 | Ratajczak et al. (1998a) |
| A. baylyi ADP1  | alkM-alkR         | luxCDABE   | 0.5 h         | 0.1 mg l^{-1}   | C7–C36                 | This study |

DNA manipulation and cloning alkane bioreporter ADPWH<sub>alk</sub>

First, a DNA fragment alkRM was amplified by PCR from wild-type ADP1, as shown in Fig. S1. Briefly, PCR reactions were made using a colony of wild-type ADP1 as DNA template and primer pairs of alk<sub>P</sub>_up/alk<sub>EB</sub>_rev and alk<sub>EB</sub>_for/alk<sub>out</sub>_down (Table 1). The two PCR products were fused by overlap extension PCR (Huang et al., 2005) using a primer pair of alk<sub>P</sub>_up and alk<sub>out</sub>_down to create EcoRI and BamHI restriction sites between alk<sub>R</sub> and alk<sub>M</sub> fragments. The PCR product alkRM with EcoRI/BamHI (878 bp) was gel purified and cloned into pGEM-T (Promega, UK) vector to create pAlkRM<sub>EB</sub> (Table 3). Second, the Km gene from mini-Tn<sub>5</sub> plasmid pUTKm1 (Delorenzo et al., 1990) and a promoterless luxCDABE cassette from pSalAR

**Table 3.** Strains and plasmids used in this study.

| Bacteria and plasmids | Description | Reference |
|-----------------------|-------------|-----------|
| *Acinetobacter baylyi* strains | Wild type | Juni and Janik (1969) |
| ADPWH<sub>lux</sub> | Bioreporter of salicylate. Promoterless luxCDABE from pSB417 were inserted between saIA and saIR genes in the chromosome of ADP1 | Huang et al. (2005) |
| ADPWH<sub>Tol</sub>–<sub>lux</sub> | Bioreporter of tolune. Promoterless luxCDABE from pSB417 were fused with Pu promoter and inserted between saIA and saIR genes, and xylR gene was inserted at saIA in the chromosome of ADP1 | Huang et al. (2008) |
| ADPWH<sub>recA</sub> | Bioreporter of genotoxicity. Promoterless luxCDABE were inserted in recA gene in the chromosome of ADP1 | Song et al. (2009) |
| ADPWH<sub>alk</sub> | Bioreporter of alkane. Promoterless luxCDABE were inserted in alkM gene in the chromosome of ADP1 | This study |
| *Escherichia coli* | High efficiency competent cells | Promega |
| JM109 | F<sup>−</sup>endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG φ80d lacZΔM15 Δ(lacZYA-argF)U169, hsdR17(k<sub>2</sub>, m<sub>1</sub>, λ) | Grant et al. (1990) |
| DH5<sup>x</sup> | F<sup>−</sup> endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG φ80d lacZΔM15 Δ(lacZYA-argF)U169, hsdR17(k<sub>2</sub>, m<sub>1</sub>, λ) | Grant et al. (1990) |
| Plasmids | Source of kanamycin resistance gene | Delorenzo et al. (1990) |
| pGEM-T | Amp<sup>+</sup>, T7 and SP6 promoters, lacZ, vector | Promega |
| pSB417 | luxCDABE source plasmid, luxCDABE from Photorhabdus (Xenorhabdus) | Winson et al. (1998) |
| pUTKm1 | Source of kanamycin resistance gene | Delorenzo et al. (1990) |
| pAlkRM<sub>EB</sub> | alkRM with EcoRI/BamHI (878 bp) inserted into pGEM-T | This study |
| pAlkRM<sub>lux_km</sub> | luxCDABE (5846 bp) from pSB417 and Km (1708 bp) from pUTKm1 were inserted at EcoRI and BamHI site of pAlkRM<sub>EB</sub> | This study |

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Bioreporter for rapid alkane detection

Different types of inducers: hexane (13.1 μM), heptanes (14.7 μM), octane (16.2 μM), dodecane (22.7 μM), tetradecane (26.0 μM), octadecane (25.5 mg), tetracosane (33.9 mg), triacontane (42.3 mg), hexatriacontane (50.7 mg) and tetracontane (56.3 mg), were dissolved in 100 ml of deionized water respectively. After homogenization using a 40 kHz ultrasound for 30 s, the n-alkanes emulsion stock solutions were obtained with final concentrations of 1.0 mM. The emulsion treatment enabled alkanes to homologically distribute in water although they were insoluble.

For oil and crude oils pre-treatment, 12.5 μl of mineral oil (Sigma) or crude oils including Brent crude oil (from Brent Reservoir, North Sea, UK), Chestnuts crude oil (from Chestnuts Reservoir, North Sea, UK) and Sirri crude oil (from Sirri Island, Iran) were dissolved in 100 ml of deionized water respectively. The mixtures were then homogenized using a 40 kHz ultrasound for 30 s to make up approximately 100 mg l⁻¹ oil stock solution. The stock solutions were diluted to the final series concentrations of 0.1, 0.2, 1.0, 2.0, 10 and 100 mg l⁻¹ with deionized water or seawater.

Applying ADPWH_alk for the detection of oils in soils

Standard crude oil was added into chloroform to make 0, 1, 2, 5, 10, 25, 50 and 100 mg ml⁻¹ stock solutions. Ten milliliters of oil–chloroform mixtures were added into 10 g of soils respectively and volatilized at 30°C for 2 h to remove any trace of chloroform. The final crude oil contents in the soil were 0.0%, 0.1%, 0.25%, 0.5%, 1.0%, 2.5%, 5.0%, 10% and 15% (weight). For soil sample pre-treatment and detection, 5–200 mg of soil samples with different oil contents were transferred into tubes with eight replicates for each sample. Each tube was added into 5 ml of deionized water and exposed to 40 kHz ultrasound for 300 s for homogenization. After static settlement for 10 min, the supernatants of soil/water mixtures were carefully taken out for ADPWH_alk detection.

Bioluminescence detection

Application of bioreporters for various detections were all carried out in MMS medium. For all water sample detection, 180 μl of bioreporters in MMS medium were added into each well of a black clear-bottom 96-well microplate (Corning Costa, USA). Then, 20 μl of each water sample was added into relative wells. For all soil sample detection, 180 μl of supernatants of soil/water mixtures were mixed with 20 μl of ADPWH_alk in MMS medium. In total 200 μl of sample–bioreporter mixture was added into a well of 96-well microplate for measurement. At least three biological replicates and three measurement replicates were carried out for each sample.

The microplates were loaded into Synergy 2 Multi-mode Microplate Reader (BioTek Instruments, UK) equipped with Gen5 analysis software. The microplates were incubated at 30°C during the measurements. The bioluminescence and OD₅₆₀ were measured every 10 min. Before each measurement, 30 s of vertical shaking was used for oil inducer dispersion. Relative bioluminescence was calculated by dividing induced bioluminescence by OD₅₆₀. Bioluminescence induc-
tion ratio was evaluated by dividing relative bioluminescence of samples by relative bioluminescence of controls (non-induced samples).

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Supporting information

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

Fig. S1. Overlap extension PCR to create EcoRI/BamHI sites at alkR–alkM DNA fragment.

Fig. S2. The effect of alkanes on bioluminescence expression. ADPWH_alk was activated by octadecane while the bioreporters ADPWH_lux (salicylate), ADPWH_Tol (toluene), ADPWH_recA (toxicity) cannot be activated by octadecane and the addition of octadecane did not increase bioluminescence expression.

Fig. S3. Biosensor ADPWH_lux growth curve in LB supplemented with 100 μM alkane with different carbon chain lengths.

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