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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** ***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 alkenes and alkanes (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 oxidization (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 promoterless *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 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 at ADPWH_alk (data not shown). ADPWH_alk was able to grow on LB agar plate with 300 μg ml^{-1} ampicillin, indicating that the whole vector pAlkRM_lux_km had been 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 vagarious 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 $1.4 \pm 0.2 \times 10^{10}$ cells m^{-2}, implying that cells occupied a single layer on the oil surface.

Table 1. Primers used in this study.

Primers	Sequence (5′→3′)
alk_P_up	GTCGTACCATCCTGTAATGTGAAATGAG
alk_EB_rev[a]	CATCCAGAATTCGGATCCTAAGTAACGTTT
alk_EB_for[a]	AAACGTTACTTAGGATCCGAATTCTGGATG
alk_out_down	CGAGCGCCAAATGAGACAGGATATGA
ADP1_alk_for	TTGATTAAGCGGATGGCTGGCATATA
luxC_rev	GAGAGTCATTCAATATTGGCAGG
ADP1_alk_rev	CGAACTATGAAAAGCAGCACTCATCC

a. The underlines indicate EcoRI and BamHI restriction sites.

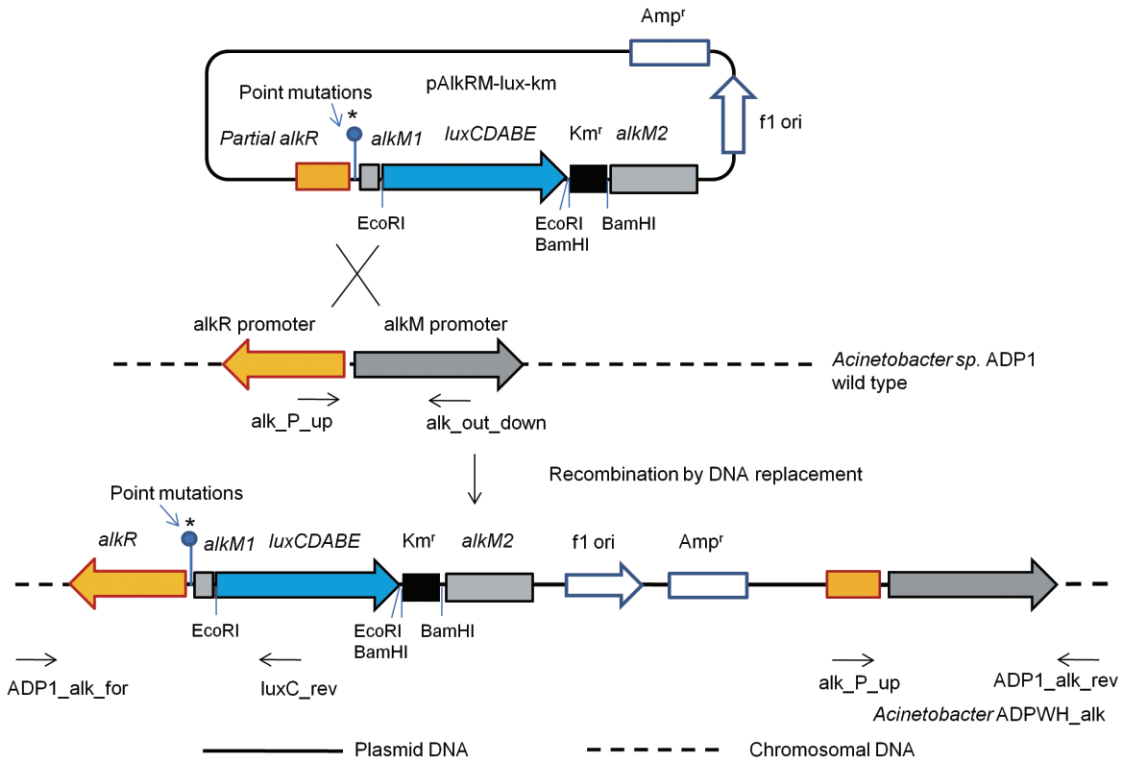
Characterization of alkane bioreporter ADPWH_alk

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 tetracontane (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. tetracontane – C40) did not activate ADPWH_alk (Figs 3A and 4). ADPWH_alk induction ratio varied to alkanes or

A



B

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ADP1_wt   → ttttactaag tgcatccata gcgcgttaaa aattcttatt tgagcaagag tatagaatga
ADPWH_alk → ttttactaag tgcatccata gcgcgttaaa aattcttatt tgagcaagag tatagaatga
              alkR start code ←
ADP1_wt   → gattgtagct tatacatgtg attttaggac attgacatgg ttgaaaatgc caaagacttt
ADPWH_alk → ggttgtagct tatacatgtg attttaggac attgacatgg ttgaaaatgc caaagacttt
                                                              ←
ADP1_wt   → gacataggtt ttttggacga tcacaactgt ttttaatata taagatggat gattatgagc
ADPWH_alk → gacataggtt ttttggacga taacgactgt ttttaatata taagatggat gattatgagc
                  →                  -35                          -10
ADP1_wt   → agtatgaata gaggataaca agaaaggatt cactatgaat gcacctgtac atgtcgatca
ADPWH_alk → agtatgaata gaggataaca agaaaggatt cactatgaat gcacctgtac atgtcgatca
               +1                   RBS        → alkM start code
ADP1_wt   → aaactttgaa gaagtgatca atgctgcaag atcaatgcgt gaaattgatc gtaaacgtta
ADPWH_alk → aaactttgaa gaagtgatca atgctgcaag atcaatgcgt gaaattgatc gtaaacgtta

ADP1_wt   → cttatggatg attagtcctg cattaccagt cattgggatc gggattttgg caggttatca
ADPWH_alk → cttaGAATTC AGGCTTGGAG GATACGTATG ACTAAAAAAA TTTCATTCAT TATTAACGGC
              EcoRI                  luxC start code →
              site for inserting promoterless luxCDABE
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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.

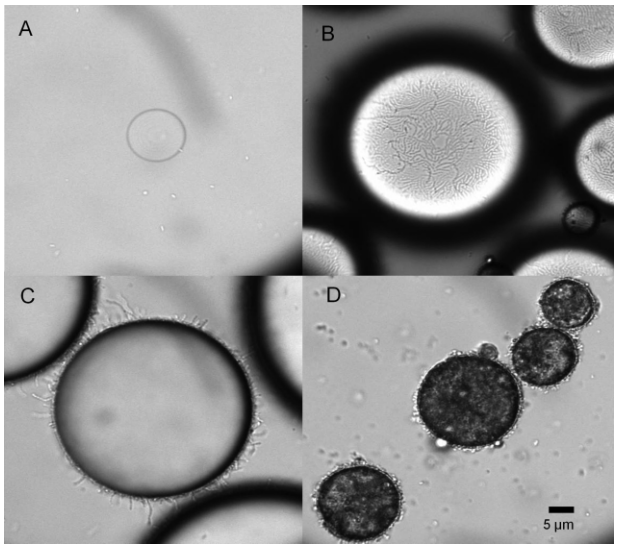

Fig. 2. *Acinetobacter baylyi* ADPWH_alk natural affinity to oil droplets. Unlike *E. coli* which was unable to emulsify and attach to oil droplets (A), ADPWH_alk, a subclone from ADP1, was able to emulsify and attached to the surface of mineral oil (B, C) and crude oil (D) droplets. The scale bar is 5 μm.

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 crudes 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 one 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

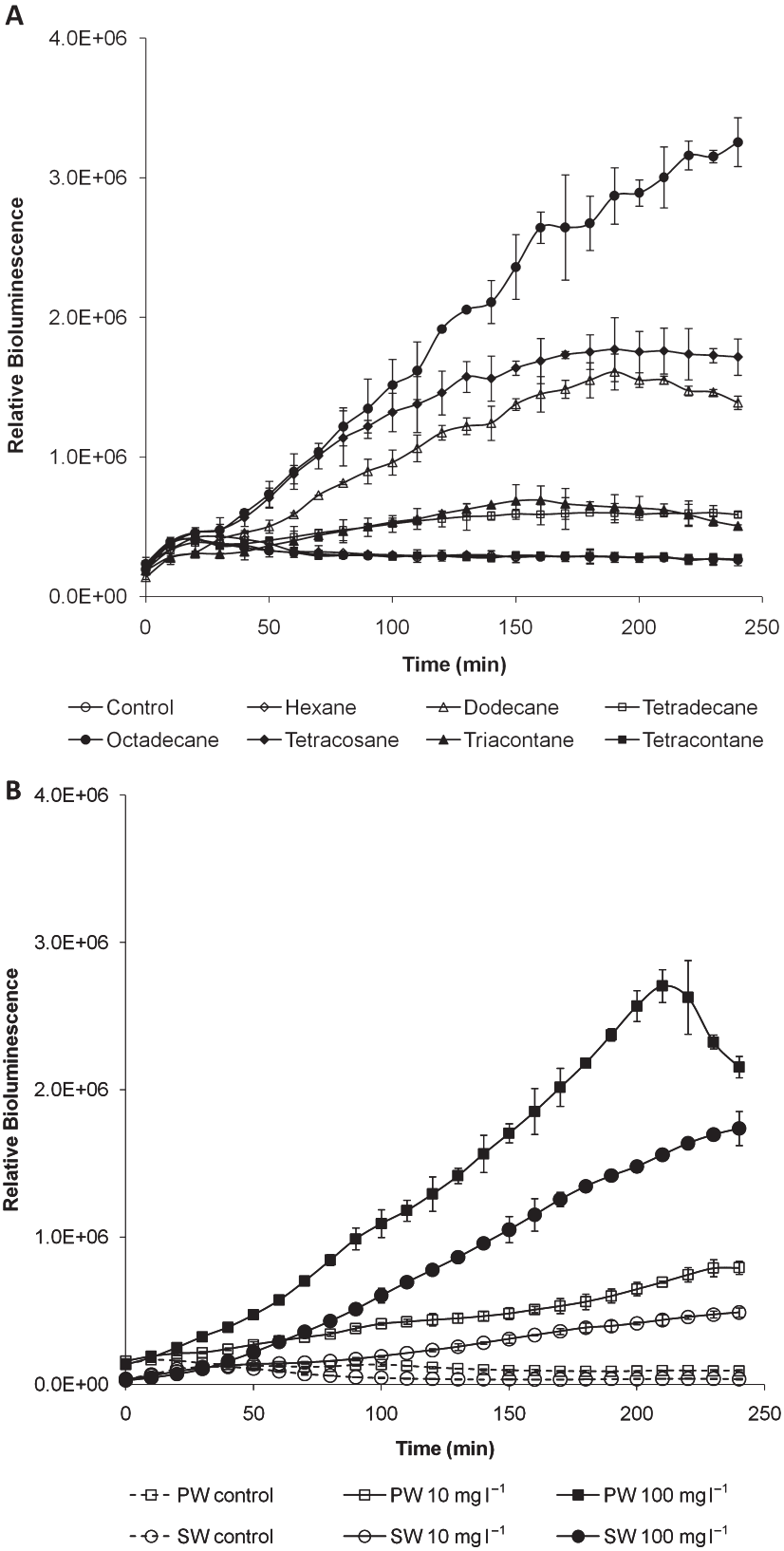

Fig. 3. Dynamic ADPWH_alk response to alkanes with 100 μM of each compound (A); and 100 mg l^{-1} Brent crude oil in pure water (PW) and seawater (SW) (B).

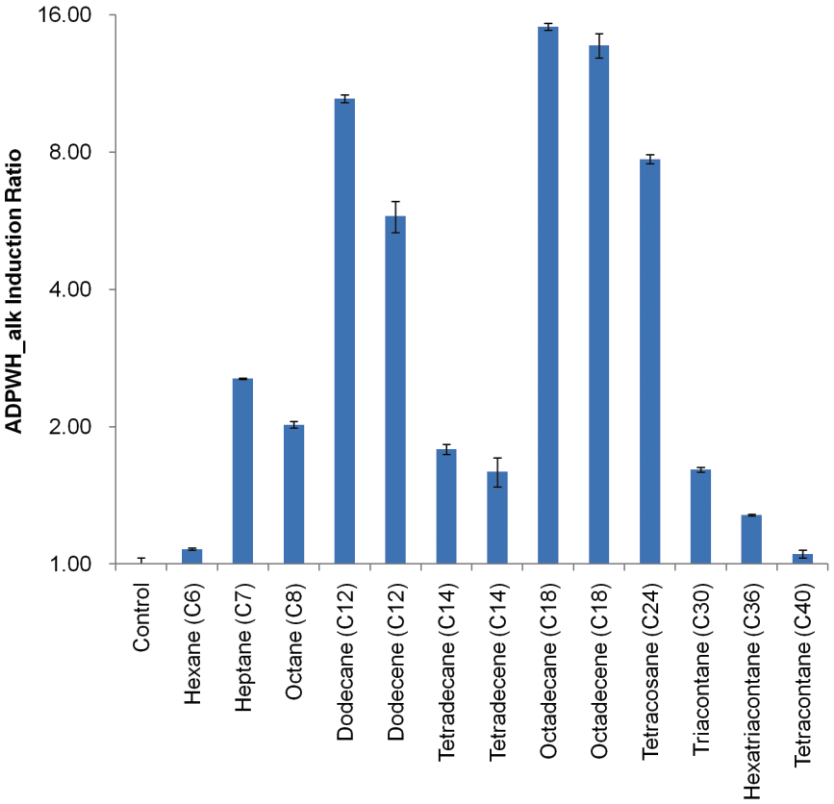

Fig. 4. ADPWH_alk response to alkanes and alkenes with various carbon chain length from C6 to C40. The alkanes or alkenes were emulsified by ultrasound and the final concentration of each alkane or alkene was 100 μM. The *P*-values of induction ratios to C7–C36 < 0.001 (against control).

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 dimmers. There were two point mutations between –35 region and the inverted repeat in ADPWH_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 ADPWH_alk is the only bioreporter that is able to detect alkane with carbon chain length greater than C18.

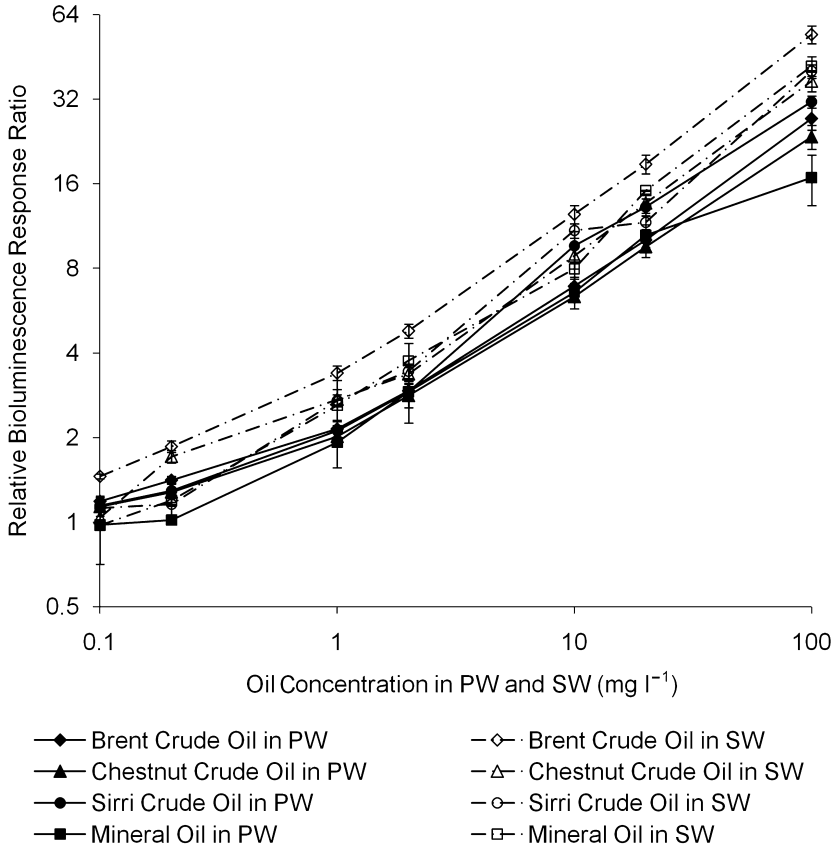

Fig. 5. ADPWH_alk response to different concentrations of mineral oil and Brent, Chestnut and Sirri crude oils in pure water and seawater.

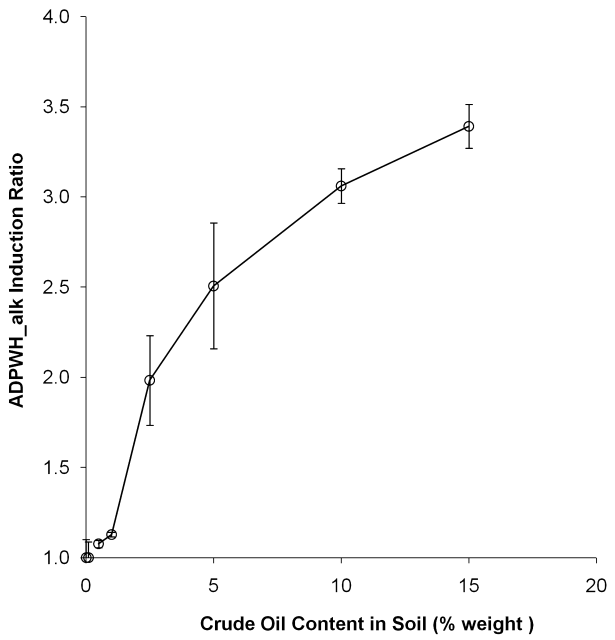

Fig. 6. ADPWH_alk response to crude oil in soils.

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

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}[a]	C6–C10	Jaspers *et al.* (2001)
E. coli DH5α	*alkB-alkS*	*luxAB*	1.0–2.0 h	0.011 mg l^{-1}[a]	C6–C10	Sticher *et al.* (1997), Tecon *et al.* (2010)
E. coli DH5α	*alkBFGHJT*	*luxAB*	–	0.78 mg l^{-1}	C6–C10	Minak-Bernero *et al.* (2004)
Y. lipolytica	–	OD_{600}	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}[b]	C7–C36	Zhang *et al.* (2011) and this study

a. The detection limit for octane only.
b. The detection limit for octadecane is 8.6×10^{-4} mg l^{-1}.

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 Na_2HPO_4, 2.5 g of KH_2PO_4, 1.0 g of NH_4Cl, 0.1 g of $MgSO_4{\cdot}7H_2O$, 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} and 50 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.

DNA manipulation and cloning alkane bioreporter ADPWH_alk

First, a DNA fragment *alkRM* was amplified by PCR from wild-type ADP1, as shown in Fig. S1. Briefly, PCR reactions were made by using a colony of wild-type ADP1 as DNA template and primer pairs of alk_P_up/alk_EB_rev and alk_EB_for/alk_out_down (Table 1). The two PCR products were fused by overlap extension PCR (Huang *et al.*, 2005) using a primer pair of alk_P_up and alk_out_down to create EcoRI and BamHI restriction sites between *alkR* and *alkM* fragments. The PCR product *alkRM* with EcoRI/BamHI (878 bp) was gel purified and cloned into pGEM-T (Promega, UK) vector to create pAlkRM_EB (Table 3). Second, the Km gene from mini-Tn*5* 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		
ADP1(BD413)	Wild type	Juni and Janik (1969)
ADPWH_lux	Bioreporter of salicylate. Promoterless *luxCDABE* from pSB417 were inserted between *salA* and *salR* genes in the chromosome of ADP1	Huang *et al.* (2005)
ADPWH_Tol (ADPWH-Pu-lux-xylR)	Bioreporter of toluene. Promoterless *luxCDABE* from pSB417 were fused with Pu promoter and inserted between *salA* and *salR* genes, and *xylR* gene was inserted at *salA* in the chromosome of ADP1	Huang *et al.* (2008)
ADPWH_recA	Bioreporter of genotoxicity. Promoterless *luxCDABE* from pSB417 were inserted in *recA* gene in the chromosome of ADP1	Song *et al.* (2009)
ADPWH_alk	Bioreporter of alkanes. Promoterless *luxCDABE* were inserted in *alkM* gene in the chromosome of ADP1	This study
Escherichia coli		
JM109	High efficiency competent cells	Promega
DH5α	F^-endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169, hsdR17($r_K^-m_K^+$), λ^-	Grant *et al.* (1990)
Plasmids		
pGEM-T	Amp^r, T7 and SP6 promoters, *lacZ*, vector	Promega
pSB417	*luxCDABE* source plasmid, *luxCDABE* from *Photorhabdus* (*Xenorhabdus*) *luminescens* ATCC2999 (Hb strain)	Winson *et al.* (1998)
pUTKm1	Source of kanamycin resistance gene	Delorenzo *et al.* (1990)
pAlkRM_EB	*alkRM* with EcoRI/BamHI (878 bp) inserted into pGEM-T	This study
pAlkRM_lux_km	*luxCDABE* (5846 bp) from pSB417 and *Km* (1708 bp) from pUTKm1 were inserted at EcoRI and BamHI site of pAlKRM_EB	This study

_lux (Winson *et al*., 1998; Huang *et al*., 2005) were separately cloned into BamHI and EcoRI site of pAlkRM-EB (Fig. 1). The *lux*CDABE-km cassette was flanked by 452 and 415 bp homologous DNA fragments. The resulting pAlkRM-lux-km plasmid was used as transformation carrier to introduce *luxCDABE* and Km genes into the *alkM* in the chromosome of ADP1 (Fig. 1). The transformation was carried out as previous description (Huang *et al*., 2005; 2008). The transformants were selected in LB agar with 10 μg ml^{-1} kanamycin and 1 μl ml^{-1} liquid paraffin. Colonies with strong bioluminescence were selected and designated as ADPWH_alk.

To confirm the integration of *luxCDABE* in the chromosome, PCR was performed using a primer pairs ADP1_alk_for/luxC_rev and alk_P_up/ADP1_alk_rev (Table 1), in which the primers ADP1_alk_for and ADP1_alk_rev were excluded in the vector pAlkRM_lux_km (Fig. 1A). The resultant PCR products were purified, cloned into pGEM-T vector and sequenced.

Quantifying oil adhesiveness to ADPWH_alk

Bacterial cells were harvested by 3000 r.p.m. centrifugation after grown in LB liquid medium overnight at 30°C (ADPWH_alk) or 37°C (*E. coli* DH5α). The cells were then resuspended in mineral medium with 20 mM sodium succinate as sole carbon source. Ten microlitres of mineral oil or Brent crude oil was added into 1 ml of bacteria solution (~10^8 cells ml^{-1}), gently mixed and incubated at 30°C for 30 min. The emulsified bacteria/oil mixture was applied for microscopy examination. The cells found on 30 oil droplets were counted and the diameters of oil droplets were measured for oil surface calculation. The cell density on oil surface was then calculated as number of cells per m^2 surface.

Preparation of alkane bioreporter ADPWH_alk

Luria–Bertani and MMS were used for the cultivation of bioreporters. After grown in LB medium at 30°C overnight, ADPWH_alk cells were harvested by centrifugation at 3000 r.p.m. for 10 min at 4°C, and subsequently washed twice and resuspended in MM medium with the same volume. Subsequently, ADPWH_alk bioreporters were cultured at 30°C for 2 h, followed by centrifugation at 3000 r.p.m. for 10 min at 4°C, and resuspended in water with the same volume as bioreporter stock solution. ADPWH_alk bioreporters were stored at 4°C and were ready for use, which be maintained for more than 1 month without changing performance and sensitivity. This is consistent with a previous report of *A. baylyi*-based toxicity biosensor (Song *et al*., 2009). The other three bioreporter strains including ADPWH_lux, ADPWH_Tol and ADPWH_recA (Table 3) were prepared using the same method as described above.

Induction of bioreporter ADPWH_alk

Before induction experiments, all the bioreporter stock solutions, including ADPWH_alk, ADPWH_lux, ADPWH_Tol and ADPWH_recA, were centrifuged at 3000 r.p.m. for 5 min and then resuspended in the same volume of MMS medium. This treatment would optimize the bioreporter's response.

Different types of inducers: hexane (13.1 μl), heptanes (14.7 μl), octane (16.2 μl), dodecane (22.7 μl), tetradecane (26.0 μl), 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, 20 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, 100 and 150 mg ml^{-1} stock solutions. Ten millilitres of oil–chloroform mixtures were added into 10 g of soils respectively and volatilized at 30°C for 24 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 OD_{600} 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 OD_{600}. 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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