J Ind Microbiol Biotechnol (2003) 30: 636–642
DOI 10.1007/s10295-003-0093-6

ORIGINAL PAPER

S. Ripp · K. A. Daumer · T. McKnight · L. H. Levine
J. L. Garland · M. L. Simpson · G. S. Sayler

# Bioluminescent bioreporter integrated-circuit sensing of microbial volatile organic compounds

Received: 23 April 2003 / Accepted: 28 August 2003 / Published online: 28 October 2003
© Society for Industrial Microbiology 2003

**Abstract** A bioluminescent bioreporter for the detection of the microbial volatile organic compound *p*-cymene was constructed as a model sensor for the detection of metabolic by-products indicative of microbial growth. The bioreporter, designated *Pseudomonas putida* UT93, contains a *Vibrio fischeri luxCDABE* gene fused to a *p*-cymene/*p*-cumate-inducible promoter derived from the *P. putida* F1 *cym* operon. Exposure of strain UT93 to 0.02–850 ppm *p*-cymene produced self-generated bioluminescence in less than 1.5 h. Signals in response to specific volatile organic compounds (VOCs) such as *m*- and *p*-xylene and styrene, also occurred, but at two-fold lower bioluminescent levels. The bioreporter was interfaced with an integrated-circuit microluminometer to create a miniaturized hybrid sensor for remote monitoring of *p*-cymene signatures. This bioluminescent bioreporter integrated-circuit device was capable of detecting fungal presence within approximately 3.5 h of initial exposure to a culture of *p*-cymene-producing *Penicillium roqueforti*.

**Keywords** BBIC · Bioluminescence · Bioreporter · *lux* · MVOC · *p*-cymene

S. Ripp · M. L. Simpson · G. S. Sayler (✉)
Center for Environmental Biotechnology,
University of Tennessee, 676 Dabney Hall,
Knoxville, TN 37996-1605, USA
E-mail: sayler@utk.edu
Tel.: +1-865-9748080
Fax: +1-865-9748086

K. A. Daumer · L. H. Levine · J. L. Garland
Dynamac Corporation, Kennedy Space Center,
FL 32899, USA

T. McKnight · M. L. Simpson
Molecular-Scale Engineering and Nanoscale Technologies
Research Group, Oak Ridge National Laboratory,
Oak Ridge, TN 37831, USA

## Introduction

Microbial contaminants in enclosed spaces have received considerable attention due to their association with "sick building syndrome" epidemics [2]. The dissemination of fungal and bacterial spores through ventilation systems in residential and commercial buildings or other enclosed workspaces (naval vessels, spacecraft) can trigger the onset of allergy-related symptoms, including headaches and eye, ear, throat, and skin irritations. Airborne allergens are also implicated in serious illnesses requiring medical intervention (pneumonitis, hives, bronchopulmonary aspergillosis, contact dermatitis). Musty and moldy odors produced by biocontaminants add to the overall discomfort of building occupants. In addition, microbes can have biocorrosive effects on physical structures, affecting stainless steel, copper, aluminum, concrete, and glass [13, 29]. Microbes also have the capability to destroy wood, fabrics, leather, electrical insulation, and many other commercial products as well. The effectiveness of bound biocides for preventing microbial growth on surfaces is limited, especially in long-term applications in which these biocides have been found to deteriorate with time [28].

Currently, the most frequently cited objective for the detection of microbial contamination is through microbially generated volatile organic compounds (MVOCs) [37]. MVOCs are produced as metabolic by-products of bacteria and fungi and are detectable before any visible signs of microbial growth appear. MVOCs can therefore serve as very early indicators of potential biocontamination problems [1]. Using MVOC analysis, it is also possible to identify specific microorganisms comprising a biomass [7], determine exposure to and assess potential toxicity from individual MVOCs [12, 27], and predict the metabolic production of certain mycotoxins [26].

MVOC detection methods typically rely on cumbersome and expensive combinations of gas chromatography/mass spectrometry (GC/MS), HPLC, and

solid-phase microextraction techniques. To provide an easier and faster method of MVOC analysis, a prototype bioluminescent bioreporter was constructed in this study for specific application towards the sensing and monitoring of a model fungal MVOC, *p*-cymene (4-isopropyltoluene). *p*-cymene has been shown to affect air quality [25, 34, 35], to be an eye and respiratory irritant [9], and, upon long-term exposure in rats, is neurotoxic [21]. Bioluminescent bioreporters generate visible light in response to specific chemical or physical agents in their environment due to transcriptional activation of a genetically incorporated *luxCDABE* cassette [24], and they have been used on-line to generate real-time assessments of chemical exposure bioavailability [30]. Recent advances in miniaturized electro-optics have produced integrated-circuit luminometers that can be directly interfaced with bioluminescent bioreporters for remote sentinel detection of target analytes [32, 33]. These devices, denoted as BBICs (bioluminescent bioreporter integrated circuits), have proven sensitive enough to detect chemicals when using as few as 5,000 maximally induced bioreporter cells [6]. It was the purpose of this study to develop a bioluminescent bioreporter for the detection of *p*-cymene and test its functionality for remote sensing of biocontaminants on the BBIC platform.

## Materials and methods

### Strain construction

The *cym* operon from *Pseudomonas putida* F1 encodes the enzymes required for the conversion of *p*-cymene to *p*-cumate [15]. The operon consists of six genes in the order *cymBCAaAbDE* and is controlled by the *cymR* repressor (GenBank accession no. U24215). Downstream of the *cym* operon is the *cmt* operon, which further catabolizes *p*-cumate to isobutyrate, pyruvate, and acetyl-CoA [14]. The *cym* and *cmt* operons are inducible by *p*-cymene via *p*-cumate-induced de-repression of the *cymR* repressor. Therefore, in the presence of *p*-cymene and/or *p*-cumate, transcription of the *cym* operon is initiated from *cymB*. The region surrounding the 34-bp promoter sequence of *cymB* was amplified from *P. putida* F1 genomic DNA by PCR. The forward primer *GCGGCCGCA*GCCTCGCAGGGACGGTACCTCCG contained an introduced *Not*I restriction site (*italics*) targeting the region just upstream of the 3′ end of *cymB* while the reverse primer, *ACTAGT*GCGACTTTGTCTTTCAGTCTCATGGC, contained an introduced *Spe*I site (*italics*) targeting the region just downstream of the 5′ end of *cymB*. The PCR protocol consisted of an initial 5-min denaturation at 94 °C, 28 cycles of a 30-s denaturation at 94 °C, 30-s annealing at 68 °C, a 30-s extension at 72 °C, and a final cycle for 10 min at 72 °C. PCR was done in an MJ Research PTC-225 Peltier Thermal Cycler (Watertown, Mass., USA). The resulting amplicon was cloned into a pCR2.1-TOPO vector (Invitrogen, Carlsbad, Calif., USA) to form plasmid pCYM1. Digesting with *Not*I and *Spe*I yielded a 179-bp fragment containing the 34-bp *cymB* promoter which was then ligated into *Not*I- and *Xba*I-compatible sites in a mini-Tn5 vector designated pUTK215, which contains a promoterless *luxCDABE* cassette from *Vibrio fischeri* (Fig. 1) [4]. The ligation mix was electroporated into *Escherichia coli* SV17 (λpir) and resulting transformants selected on Luria-Bertani (LB) plates containing 50 μg kanamycin/ml (Kn). Plasmid DNA was isolated and cleaved with *Bam*HI and *Not*I to confirm the presence of the *cymB* promoter region to yield the plasmid $pUT_{cym}93$. *E. coli* ($pUT_{cym}93$) was mated with *P. putida* F1 in the presence of toluene vapor to maintain selective pressure and plated on *Pseudomonas* isolation agar plus Kn. Resulting colonies were replica plated onto minimal salts media (2 g $NaNO_3$, 0.75 g $KH_2PO_4$, 0.003 g $FeCl_3$, 0.1 g $MgSO_4$, 0.005 g $CaCl_2$, and 0.25 g $Na_2HPO_4$ per liter) containing 0.5 mM *p*-cymene and Kn. Several colonies produced enough light to be seen in a darkened room. One of these, designated UT93, was chosen for further analysis.

### Alginate encapsulation

Strain UT93 was grown overnight at 30 °C in yeast extract/peptone-glucose (YEPG) medium (0.2 g yeast extract, 2.0 g polypeptone, 1.0 g glucose per liter) supplemented with 10% phosphate buffer (0.05 M $K_2HPO_4/NaH_2PO_4$) and Kn. The next day, a 1:10 inoculum of the overnight culture was transferred to fresh YEPG/phosphate media without Kn and grown to an optical density at 546 nm ($OD_{546}$) of 0.8. The culture was washed three times with sterile 0.85% NaCl by centrifugation at 8,000 rpm for 10 min and resuspended in an equal volume of 0.85% NaCl. Cells were immobilized by combining the washed culture with low-viscosity 3% alginic acid (w/v in $H_2O$) in a 1:2 ratio [36]. Beads of approximately 2 mm diameter were formed by dropwise addition into sterile 0.1 M $SrCl_2$ through a 26-gauge syringe needle. After 30 min, the $SrCl_2$ was decanted and the beads removed and stored at 4 °C for up to 2 weeks. The approximate number of cells/bead was determined by dissolving the beads in 50 mM sodium hexametaphosphate, plating on YEPG + Kn, and incubating overnight at 30 °C.

### Vapor-phase test system

Detection limits of the UT93 bioreporter were determined by exposure to vapor phase *p*-cymene (99 + %, Acros Organics, N.J., USA) in a flow-through chamber consisting of a diluting airflow of ultra-pure air and an airflow of pure *p*-cymene. *p*-Cymene vapor-phase concentrations ranging from approximately 0.01 to 1000 ppm (v/v) were obtained by adjusting the flow rates via two flowmeters. The mixed flow was transferred into a 900-$cm^3$ test chamber through 6.0-mm diameter Teflon tubing. A monolayer of 34 alginate beads in a porous plastic screen was suspended 4.0 mm beneath a liquid light cable. The light cable was connected to an Oriel 7070 photomultiplier tube (PMT) (Oriel, Stratford, Conn., USA) attached to a multifunction optical power meter (OPM) that displayed the bioluminescence output in nanoAmps (nAmp). An RS-232 cable connected the OPM to a laptop computer installed with a data-logging program that recorded nAmp/min for each trial. To allow comparisons to be made among trials, data acquired in nAmps were normalized to cell density and converted into arbitrary units, referred to as relative bioluminescence units (RBUs). Alginate beads were added to the test chamber only after the *p*-cymene concentration had stabilized (within approximately 2 h). In order to limit desiccation of the beads, 100 ml deionized water were added to the chamber. All tests were carried out at 25 °C.

### Induction of *P. putida* UT93 by non-biological sources

Sources of sick-building volatiles include non-biological VOCs associated with building materials, paint, furnishings, carpeting, cleaning supplies, and appliances. Ethylbenzene, toluene, acetone, *m*- and *p*-xylene, and styrene are some of the most common VOCs encountered [18]. These compounds were therefore tested to determine whether they could induce *P. putida* UT93 bioluminescence, leading to potential false-positive signaling. Due to the volatility of these substances, a single-use, disposable test system was necessary to prevent cross-contamination between samples. Therefore, a simple static vial set-up was used rather

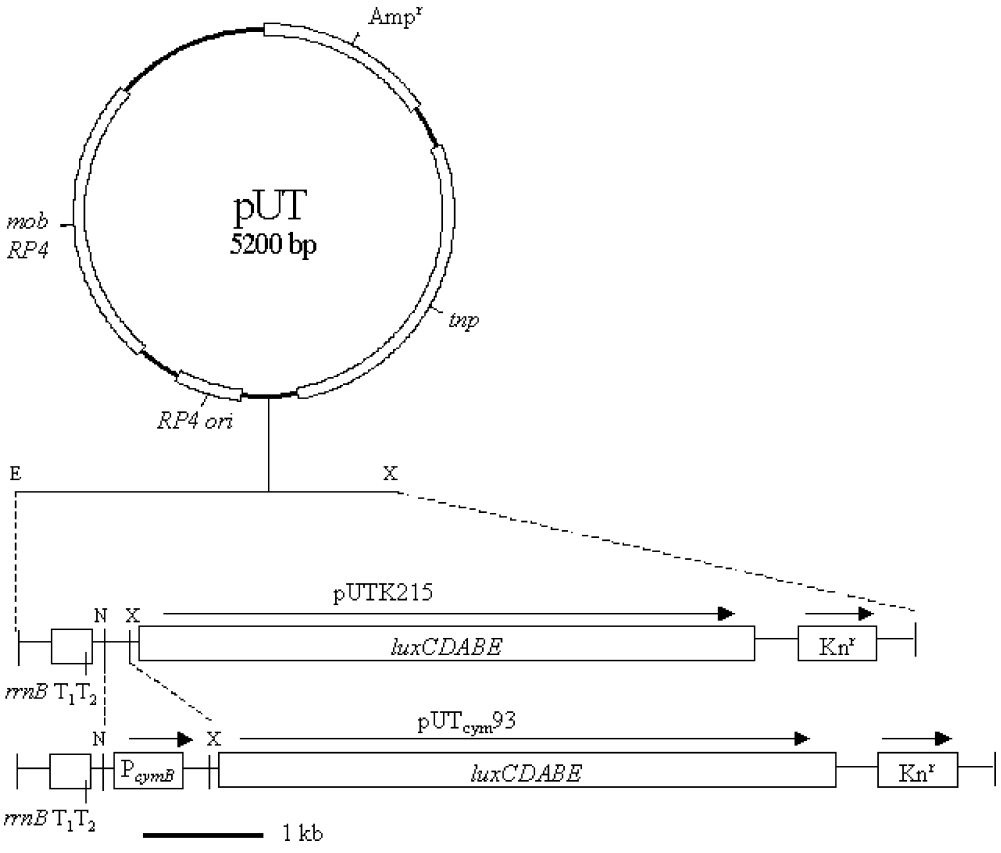


**Fig. 1** Genetic construction of the *Pseudomonas putida* UT93 bioreporter. For descriptions of the suicide vectors pUT and pUTK215, see de Lorenzo et al. [11] and Applegate et al. [3], respectively. pUT$_{cym}$93 contains the promoter region of *cymB* from *P. putida* F1 ligated to the *Vibrio fischeri luxCDABE* cassette. E *Eco*RI, X *Xba*I, N *Not*I, *rrnB* $T_1T_2$ ribosomal terminator site, Amp$^r$ ampicillin resistance, Kn$^r$ kanamycin resistance

than the more elaborate vapor-phase test system described above. An 8-ml vial containing the inducer substrate at approximately 0.05 ppm was inserted into a 40-ml vial (Pierce, Rockford, Ill., USA). A layer of 34 alginate beads was placed in the bottom of the 40-ml vial, which was then sealed with a Teflon-lined septum and screw-cap lid and incubated at 25 °C. Bioluminescence was monitored with a liquid light pipe as described above, except the light pipe was situated directly against the bottom outside portion of the 40-ml vial. At the completion of the experiment, beads were dissolved and plated on YEPG + Kn to determine viable cell numbers.

## Remote BBIC detection of fungal growth

UT93 bioreporter cells were prepared as for the alginate encapsulation experiments above, except encapsulation occurred in a 0.6% agar matrix instead of alginate. A 20-µl drop of agar-encapsulated cells was deposited on the integrated-circuit surface, which was previously coated in a thin layer (~2 mm) of polydimethylsiloxane to protect its circuitry [6].

*Penicillium roqueforti* strain IBT 3877 (Technical University of Denmark) was used as the test organism for BBIC studies since it yields a relatively diverse MVOC profile that includes *p*-cymene [22]. *P. roqueforti* was grown on malt extract agar (MEA, Difco, Detroit, Mich., USA) plates incubated for 7 days at 25 °C in the dark. Spores were gently scraped from the plates using a sterile water solution containing 0.2% agarose, and 1 ml of the resulting diluent was plated on SYES plates (20 g yeast extract, 150 g sucrose, 20 g agar, 5 ppm $CuSO_4$, 10 ppm $ZnSO_4$ per liter) [23]. A single uncovered plate was immediately placed 1 cm below the suspended BBIC probe within a light-tight 2,000-cm$^3$ test chamber that was then sealed. The chamber was maintained at a constant 25 °C. Data were collected every 36 s through a remote linkage to a laptop computer. Similar experiments were carried out in a duplicate chamber using an LB plate inoculated with *E. coli* K12 (ATCC no. 29425) instead of the *P. roqueforti* plate to determine potential induction effects from a bacterial culture.

## Chemical analysis

Gas sampling was used to determine *p*-cymene and non-biological VOC concentrations in the vapor phase. For the flow-through experiments, 0.5- or 1.0-ml headspace samples (depending on the experimental concentrations of *p*-cymene being used) were obtained every hour using a 1.0-ml gas tight syringe. Samples were analyzed with a Shimadzu 17A GC fitted with a Heliflex AT-1000 column (60 m×0.25 mm i.d., 0.20-µm film thickness, Alltech, Deerfield, Ill., USA) and equipped with a mass spectrometer (QP5000). The vial experiments were sampled similarly, but only three consecutive 1.0-ml headspace samples were used to quantify the non-biological VOC compounds.

Initial attempts at detecting fungal volatiles in the BBIC experiments failed due to the large headspace volume of the BBIC test chamber. Since the bioreporter cells were placed directly above the growing fungal culture, sampling of the entire chamber's headspace was considered unrealistic. MVOC profiles of *P. roqueforti* were therefore determined from cultures growing in septum-sealed 300-ml flasks. Although this was unrepresentative of the actual experimental set-up, it provided a necessary indication of what volatiles were being produced. The bottom diameter of these flasks closely approximated the diameter of the fungal growth plates (~100 mm) to which the BBIC was exposed, and each flask contained the same 20-ml volume of SYES agar as the plates and were similarly subcultured with *P. roqueforti*. Headspace samples were collected every 2 h in a 1-ml gas-tight syringe and analyzed as described above.

## Results

### Flow-through detection of *p*-cymene

The flow-through system was used to establish a response profile of strain UT93 to *p*-cymene under controlled exposure. A significant bioluminescent signal (analysis of variance, $p = 0.05$) was produced in response to *p*-cymene concentrations ranging from 0.02 to 850 ppm. Concentrations greater than this became toxic to the bioreporter, as determined by a loss in cell viability. Since the purpose of this bioreporter system is to detect low-level exposure events, a more detailed response analysis was obtained only at the lower detection limits, where a linear profile ($R^2 = 0.91$) was obtained within a *p*-cymene concentration range from 0.03 to 35.67 ppm (Fig. 2A). Within this range, bioluminescence was initiated within 1–1.5 h of exposure with peak bioluminescence occurring 0.5–1 h thereafter (Fig. 2B).

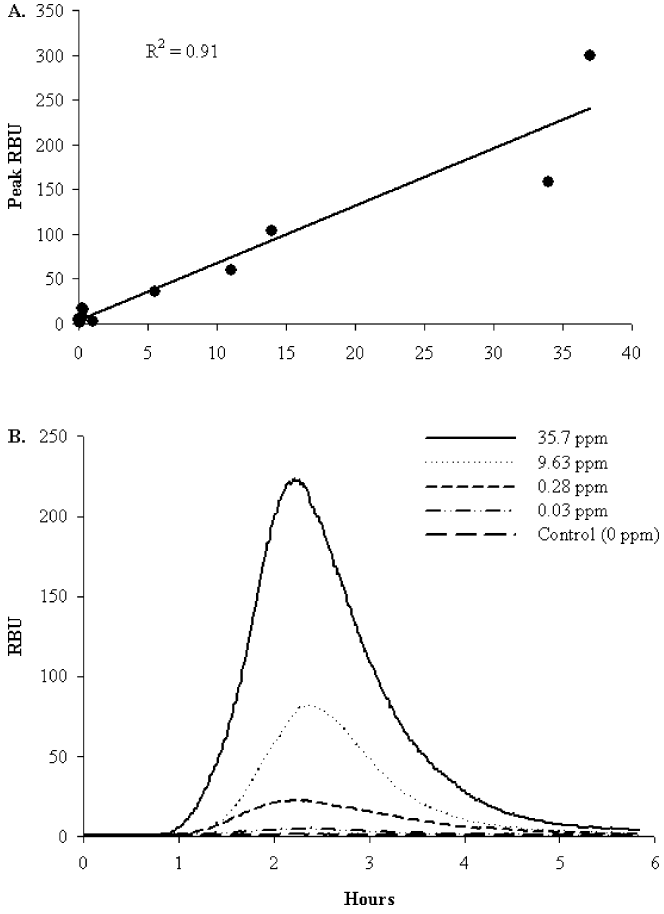


**Fig. 2** **A** Linearity of the bioluminescent response of *P. putida* UT93 in the flow-through chamber at *p*-cymene concentrations ranging from approximately 0.03–35 ppm. **B** Bioluminescent profile of *P. putida* UT93 exposed to low-level *p*-cymene concentrations in the flow-through chamber ($n = 3$). *RBU* Relative bioluminescence units

### Non-biological induction of *P. putida* UT93

Alginate-encapsulated *P. putida* UT93 was statically exposed to ethylbenzene, toluene, acetone, *m*- and *p*-xylene, and styrene to assess the effects of potential non-biological VOC sources on bioluminescence induction. Inducer concentrations were maintained at approximately 0.05 ppm, which would be considered an upper limit of exposure in residential complexes [5, 17, 19, 31]. *m*- and *p*-Xylene and styrene were significant inducers (analysis of variance, $p = 0.05$), typically initiating a bioluminescent response within 1.5–2 h after exposure (Table 1). No significant reduction in cell viability was observed as compared to growth in unexposed cultures, indicating that exposure to these chemicals at 0.05 ppm was not toxic.

### Remote BBIC detection of *P. roqueforti* growth

The BBIC provides a sensitive measure of bioluminescence by integrating the photo-induced current of an on-board n-well/p-substrate photodiode and converting this measurement in seconds into a digital pulse interval that is inversely proportional to the amount of collected light. Providing this digital output facilitates integration of the BBIC sensor with a wide array of low-cost digital electronics. For this work, an inexpensive ($< \$2$) microcontroller with a 16-bit timer/counter input was used to measure the BBIC digital pulse output and to serially transmit the data to a remote computer using a commercially available spread-spectrum radio telemetry system (Adcon Telemetry, Boca Raton, Fla., USA) (Fig. 3A). The lower line in Fig. 3B depicts results obtained after exposing the BBIC/UT93 hybrid sensor to a growing culture of *P. roqueforti*. The upper line denotes the baseline output of a BBIC treated exactly as the experimental one but with a Petri plate devoid of *P. roqueforti* growth (negative control). Significant differences between baseline and experimental bioluminescence occurred after 3.5 h (*t* test, $p = 0.05$) and persisted for approximately 35 h. (*P. roqueforti* growth, after transfer from MEA to SYES agar, was visible to the naked eye after approximately 7 h.) Resuspension of the agar matrix containing the bioreporter cells and plating

**Table 1** Induction of bioluminescence from *Pseudomonas putida* UT93 in response to non-biological volatile organic compounds (VOCs) exposure at 0.05 ppm ($n = 3$). *RBU* Relative bioluminescence units, *nsi* no significant induction

| Inducer | Peak RBU | Average minimum induction time (h) |
|---|---|---|
| *m*-Xylene | $2.24 \pm 0.09$ | $1.8 \pm 0.07$ |
| *p*-Xylene | $2.36 \pm 0.10$ | $1.8 \pm 0.03$ |
| Styrene | $1.54 \pm 0.07$ | $2.0 \pm 0.03$ |
| Toluene | $0.15 \pm 0.01$ | nsi |
| Ethylbenzene | $0.43 \pm 0.01$ | nsi |
| Acetone | $0.46 \pm 0.04$ | nsi |
| *p*-Cymene | $5.15 \pm 0.09$ | $1.2 \pm 0.03$ |

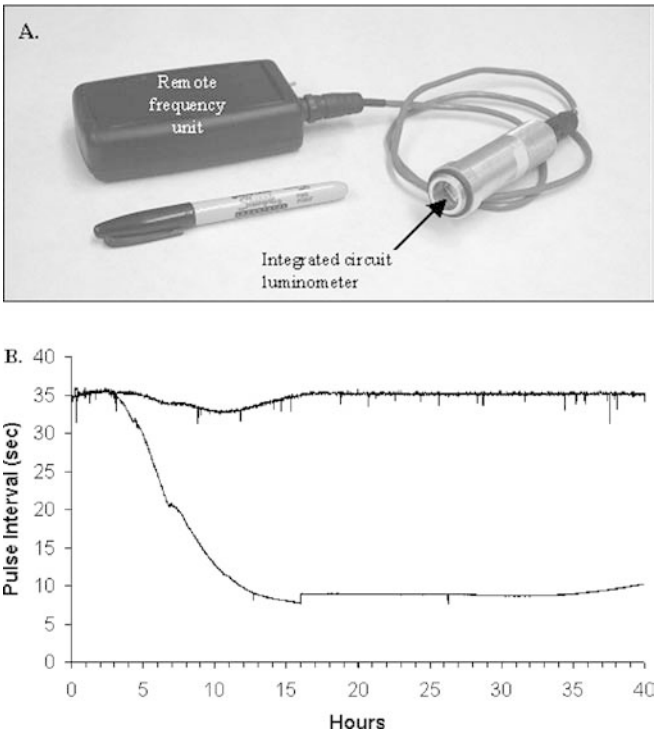


**Fig. 3** **A** The BBIC sensor probe and remote frequency unit. **B** Bioluminescence profile established with the remote BBIC detection system. Unexposed controls (*upper line*) produced pulse intervals of approximately 35 s. Bioluminescence induction from *P. putida* UT93 in response to *P. roqueforti* growth (*lower line*) yielded a pulse interval response significantly differing from the baseline within 3.5 h and maximizing at approximately 9 s (the pulse interval is inversely proportional to measured bioluminescence) ($n = 2$)

on YEPG + Kn initially and after 35 h indicated that cell numbers had decreased from approximately $1\times10^6$ to $1\times10^3$ CFUs, falling below the calculated threshold number of cells typically required for on-chip detection (the lower limit approaches approximately $5\times10^3$ fully induced cells [6]). Visual inspection of the agar matrix after 35 h revealed it to be highly desiccated, which was likely a main contributor towards this loss in bioreporter viability.

GC/MS headspace analysis of the BBIC chamber itself failed to indicate any presence of *p*-cymene. However, sampling occurred from the top of the chamber and likely did not realistically exemplify what was transpiring at the photoluminometer–fungal culture interface. To achieve a better profile of *P. roqueforti* metabolite synthesis, duplicate cultures were grown in reduced-headspace 300-ml stoppered flasks. The *p*-cymene concentrations in these cultures averaged 0.022 ppm over the first 2–6 h and then decreased to an average of 0.013 ppm from 8–48 h.

Similar experiments were done using a plate of *E. coli* K12 as a source of volatiles. No significant bioluminescence was produced by the UT93 bioreporter upon exposure to *E. coli* growth.

## Discussion

The bioluminescent bioreporter *P. putida* UT93 was constructed by fusing the 34-bp *cymB* promoter from *P. putida* F1 upstream of a promoterless *V. fischeri lux-CDABE* cassette to generate a sensor for the MVOC *p*-cymene. Detection limits ranged from 0.02 to 850 ppm, with a response time of less than 1.5 h. Representative concentrations of *p*-cymene in "clean" residential structures are estimated at 1 part-per-trillion [25], thus bioluminescent signaling in response solely to *p*-cymene would occur only during a biocontamination event, whereupon *p*-cymene emissions would become uncomfortable (~1.5 ppm) to nauseous (~1,000 ppm and beyond) [8]. These concentrations fittingly parallel the detection range of strain UT93, allowing for perceptible detection prior to extensive fungal infiltrations.

The primary application of strain UT93 is as an indirect monitor for fungal growth in enclosed spaces, for example, within wall cavities or heating, ventilating, and air conditioning (HVAC) systems. We therefore tested the ability of UT93 to function as a remote sensor in conjunction with an integrated-circuit luminometer. The bioreporter cells were encapsulated in an agar matrix and layered onto the polydimethylsiloxane-treated surface of the integrated circuit. Upon exposure to a single plate of *P. roqueforti*, a significant bioluminescent signal was produced within 3.5 h. The incorporation of a frequency-hopping spread-spectrum radio provided low-error telemetry of the BBIC measurement over a line-of-sight distance of up to 300 m. Headspace analysis of *P. roqueforti* volatile metabolites indicated low-level (0.012–0.025 ppm) synthesis of *p*-cymene 2–48 h post-transfer onto SYES media. These concentrations were at or below the experimentally derived minimum detection limit (0.02 ppm) of strain UT93. However, this limit was determined using a liquid light cable/photomultiplier tube set-up for bioluminescence measurement. The integrated circuit lowers these detection limits because of the near-direct interface of the bioreporter with the integrated-circuit luminometer, thereby bypassing the less efficient transfer of photons through a liquid light cable. Additionally, there are likely cumulative induction events occurring in response to MVOCs besides *p*-cymene. *P. roqueforti*, like other fungi, produces a diverse profile of volatile metabolites whose synthesis and concentration are highly dependent on environmental factors and media compositions [16]. Maintaining high bioreporter specificity under such conditions would be difficult and was not expected. However, the lack of significant bioluminescence in response to *E. coli* indicates that strain UT93 preserves specificity for fungal growth via sensory perception of fungal MVOCs (inclusive of *p*-cymene) under the conditions evaluated in this study.

Strain UT93 also responds to VOCs emitted from sources such as building materials and cleaning supplies that contribute to substandard indoor air quality. Many

potentially harmful VOCs have been detected in residential homes and office buildings [18]. We chose several of the most common (toluene, ethylbenzene, *m*- and *p*-xylene, acetone, and styrene) for determining potential interfering effects on UT93 bioreporter bioluminescence induction. At 0.05 ppm, *m*- and *p*-xylene and styrene each were capable of inducing strain UT93 within 1.5–2 h, but corresponding bioluminescence remained more than two-fold lower than that for *p*-cymene at similar concentration. VOC concentrations in enclosed structures would typically be found on an individual basis only in part-per-trillion concentrations, and would be "diluted out" by MVOCs during a biocontamination event [18]. Therefore, under normal circumstances, peripheral VOCs would rarely interfere with UT93 signaling. Nevertheless, if the probability for elevated VOC concentrations exists, the UT93 bioreporter could be used in conjunction with other bioreporters capable of monitoring desired VOCs to establish a multi-sensing platform for MVOC detection with VOC controls (i.e., *P. putida* TVA8 for BTEX compounds [4], *P. fluorescens* HK44 for naphthalene [20], *E. coli* DPD3063 for phenol derivatives [10], etc.). Indeed, the integrated circuit is fabricated with a quadrated luminometer designed to hold and individually sense multiple bioreporters, making it ideally suited for such monitoring regimens. Although further work is necessary to implement multiplexed bioreporter arrays, preliminary data presented here fully indicate that fundamental bioreporter/BBIC hybrid sensors can function as simple and effective monitors for MVOC signatures.

**Acknowledgements** Research support was provided by the NASA Advanced Environmental Monitoring and Control Program and the Department of Energy, Office of Science, Laboratory Technology Research Program. Portions of this study were done at the Oak Ridge National Laboratory, managed by UT-Battelle, LLC for the US DOE under contract # DE-AC05-00OR22725.

## References

1. Ahern DG, Crow SA, Simmons RB, Price DL, Noble JA, Mishra SK, Pierson DL (1996) Fungal colonization of fiberglass insulation in the air distribution system of a multistory office building: VOC production and a possible relationship to sick building syndrome. J Ind Microbiol 16:280–285
2. American Conference of Governmental Industrial Hygienists (1986) Bioaerosols: airborne viable microorganisms in office environments—sampling protocol and analytical procedures. Appl Ind Hyg April: R-22
3. Applegate B, Kelley C, Lackey L, McPherson J, Kehrmeyer S, Menn F-M, Bienkowski P, Sayler G (1997) *Pseudomonas putida* B2: a *tod-lux* bioluminescent reporter for toluene and trichloroethylene co-metabolism. J Ind Microbiol Biotech 18:4–9
4. Applegate BM, Kehrmeyer SR, Sayler GS (1998) A chromosomally based *tod-luxCDABE* whole-cell reporter for benzene, toluene, ethylbenzene, and xylene (BTEX) sensing. Appl Environ Microbiol 64:2730–2735
5. Begerow J, Jermann E, Keles T, Ranft U, Dunemann L (1995) Passive sampling for volatile organic compounds (VOCs) in air at environmentally relevant concentration levels. Fresenius J Anal Chem 351:549–554
6. Bolton EK, Sayler GS, Nivens DE, Rochelle JM, Ripp S, Simpson ML (2002) Integrated CMOS photodetectors and signal processing for very low-level chemical sensing with the bioluminescent bioreporter integrated circuit. Sens Actuators B 85:179–185
7. Borjesson T, Stollman U, Schnurer J (1990) Volatile metabolites and other indicators of *Pencillium aurantiogriseum* growth on different substrates. Appl Environ Microbiol 56:3705–3710
8. Cometto-Muniz JE, Cain WS, Abraham MH, Kumarsingh R (1998) Sensory properties of selected terpenes—thresholds for odor, nasal pungency, nasal localization, and eye irritation. Ann NY Acad Sci 855:648–651
9. Cometto-Muniz JE, Cain WS, Abraham MH, Kumarsingh R (1998) Trigeminal and olfactory chemosensory impact of selected terpenes. Pharmacol Biochem Behav 60:765–770
10. Davidov Y, Rozen R, Smulski DR, Van Dyk TK, Vollmer AC, Elsemore DA, LaRossa RA, Belkin S (2000) Improved bacterial SOS promoter::*lux* fusions for genotoxicity detection. Mutat Res 466:97–107
11. de Lorenzo V, Herrero M, Sanchez JM, Timmis KN (1998) Mini-transposons in microbial ecology and environmental biotechnology. FEMS Microbiol Ecol 27:211–224
12. Dewey S, Sagunski H, Palmgren U, Wildeboer B (1995) Microbial volatile organic compounds: a new approach in assessing health risks by indoor mould? Zbl Hyg Umweltmed 197:504–515
13. Drewello R, Weissmann R (1997) Microbially influenced corrosion of glass. Appl Microbiol Biotech 47:337–346
14. Eaton RW (1996) *p*-cumate catabolic pathway in *Pseudomonas putida* F1: cloning and characterization of DNA carrying the *cmt* operon. J Bacteriol 178:1351–1362
15. Eaton RW (1997) *p*-cymene catabolic pathway in *Pseudomonas putida* F1: cloning and characterization of DNA encoding conversion of *p*-cymene to *p*-cumate. J Bacteriol 179:3171–3180
16. Fiedler K, Schutz E, Geh S (2001) Detection of microbial volatile organic compounds (MVOCs) produced by moulds on various materials. Int J Hyg Environ Health 204:111–121
17. Hodgson AT, Rudd AF, Beal D, Chandra S (2000) Volatile organic compound concentrations and emission rates in new manufactured and site-built houses. Indoor Air 10:178–192
18. Jones AP (1999) Indoor air quality and health. Atmos Environ 33:4535–4564
19. Kim YM, Harrad S, Harrison RM (2001) Concentrations and sources of VOCs in urban domestic and public microenvironments. Environ Sci Technol 35:997–1004
20. King JMH, DiGrazia PM, Applegate B, Burlage R, Sanseverino J, Dunbar P, Larimer F, Sayler GS (1990) Rapid, sensitive bioluminescence reporter technology for naphthalene exposure and biodegradation. Science 249:778–781
21. Lam HR, Ladefoged O, Ostergaard G, Lund SP, Simonsen L (1996) Four week's inhalation exposure of rats to *p*-cymene affects regional and synaptosomal neurochemistry. Pharmacol Toxicol 79:225–230
22. Larsen TO, Frisvad JC (1995) Characterization of volatile metabolites from 47 *Penicillium* taxa. Mycol Res 99:1153–1166
23. Larsen TO, Frisvad JC (1995) Chemosystematics of *Penicillium* based on profiles of volatile metabolites. Mycol Res 99:1167–1174
24. Meighen EA (1994) Genetics of bacterial bioluminescence. Annu Rev Genet 28:117–139
25. Otson R, Fellin P, Tran Q (1994) VOCs in representative Canadian residences. Atmos Environ 28:3563–3569
26. Pasanen A-L, Lappalainen S, Pasanen P (1996) Volatile organic metabolites associated with some toxic fungi and their mycotoxins. Analyst 121:1949–1953
27. Pasanen A-L, Korpi A, Kasanen J-P, Pasanen P (1998) Critical aspects on the significance of microbial volatile metabolites as indoor air pollutants. Environ Int 24:703–712
28. Pasanen P, Kalliokoski P, Pasanen A (1996) The effectiveness of some disinfectants and detergents against microbial activity. Building Environment 32:281–287

29. Ray R, Little B, Wagner P, Hart K (1997) Environmental scanning electron microscopy investigations of biodeterioration. Scanning 19:98–103
30. Ripp S, Nivens DE, Ahn Y, Werner C, Jarrel J, Easter JP, Cox CD, Burlage RS, Sayler GS (2000) Controlled field release of a bioluminescent genetically engineered microorganism for bioremediation process monitoring and control. Environ Sci Technol 34:846–853
31. Seifert B, Abraham HJ (1982) Indoor air concentrations of benzene and some other aromatic hydrocarbons. Ecotoxicol Environ Saf 6:190–192
32. Simpson ML, Sayler GS, Ripp S, Nivens DE, Applegate BM, Paulus MJ, Jellison GE (1998) Bioluminescent bioreporter integrated circuits form novel whole-cell biosensors. Trends Biotech 16:332–338
33. Simpson ML, Sayler GS, Patterson G, Nivens DE, Bolton E, Rochelle J, Arnott C, Applegate B, Ripp S, Guillorn MA (2001) An integrated CMOS microluminometer for low-level luminescence sensing in the bioluminescent bioreporter integrated circuit. Sens Actuators B 72:135–141
34. Smet E, Van Langenhove H, De Bo I (1999) The emission of volatile compounds during the aerobic and the combined anaerobic/aerobic composting of biowaste. Atmos Environ 33:1295–1303
35. Termonia A, Termonia M (1999) Characterisation and on-site monitoring of odorous organic compounds in the environment of a landfill site. Int J Environ Anal Chem 73:43–57
36. Webb OF, Bienkowski PR, Matrubutham U, Evans FA, Heitzer A, Sayler GS (1997) Kinetics and response of a *Pseudomonas fluorescens* HK44 biosensor. Biotech Bioeng 54:491–502
37. Wessen B, Schoeps K-O (1996) Microbial volatile organic compounds-what substances can be found in sick buildings? Analyst 121:1203–1205