# Bioreporters in microbial ecology

Johan HJ Leveau*‡ and Steven E Lindow†

Bioreporters are effective research tools for gaining an understanding of a microbe's perception of the world. Fitted with a fusion of an environmentally responsive promoter to a suitable reporter gene, a bacterial or fungal bioreporter is able to communicate its metabolic or transcriptional behavior in a habitat, and furnish us with information on the chemical, physical or biological properties of its immediate surroundings. This review details recent developments in the use of such bioreporters in microbial ecology. Emphasis is placed on reporter genes that allow detection in individual microbial cells, as they provide a high-resolution description of the habitat under investigation. In an outlook on the future of bioreporter technology, this review stresses the need to interpret the activity of a bioreporter within the context of its biology.

**Addresses**
University of California, Department of Plant and Microbial Biology, 111 Koshland Hall, Berkeley, California 94720, USA
*e-mail: leveau@uclink.berkeley.edu
†e-mail: icelab@socrates.berkeley.edu
‡JHJL is currently at the Netherlands Institute of Ecology (KNAW-NIOO), Centre for Terrestrial Ecology, Heteren, The Netherlands;
e-mail: LEVEAU@CTO.NIOO.KNAW.NL

**Current Opinion in Microbiology** 2002, **5**:259–265

1369-5274/02/$ – see front matter


***Published online 7 May 2002***

**Abbreviation**
**GFP** green fluorescent protein

## Introduction

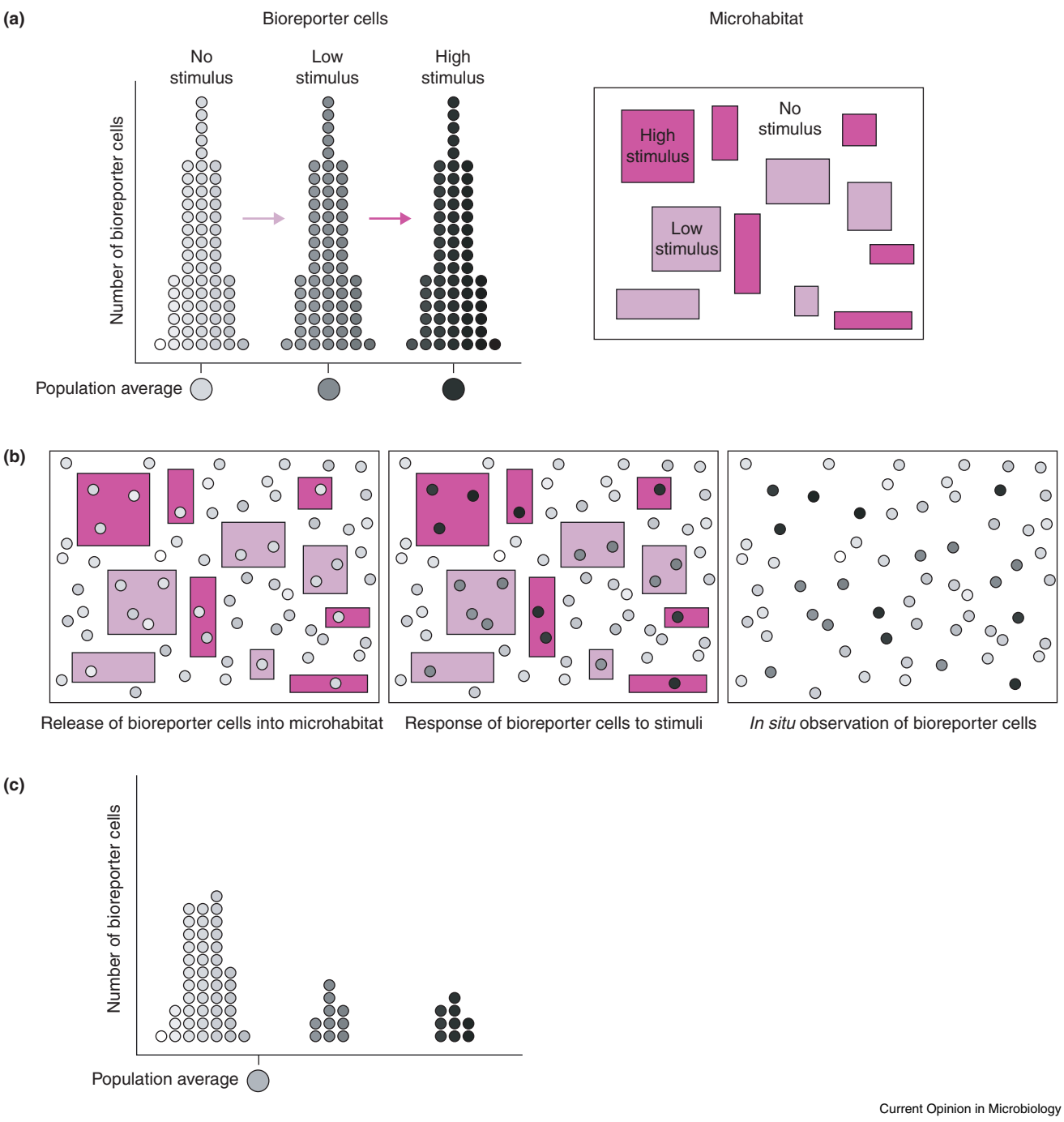
From the perspective of a bacterium 2 μm in length, the surface of a matchbook represents an area roughly the size of Rhode Island, or the Grand Duchy of Luxembourg. To the same bacterium, an Italian espresso compares in volume to Lake Baikal, the largest freshwater lake on Earth, while a hot air balloon takes on the proportions of Earth itself. This contrast in world view should make obvious the fact that we cannot afford to neglect the element of scale as we try to understand the behavior of bacteria and other microbes. There is a limited understanding of how microbes perceive their immediate environment, or changes to it, on a scale that is most relevant to them. How do physical, chemical and biological conditions vary along distances of 1, or 10, or 100 or more micrometers, and how do they affect a microbe's behavior? An exciting approach to address these questions involves the use of reporter gene technology. In simple terms, a reporter gene is the molecular equivalent of the sort of tagging device that wildlife biologists use to monitor animals in their natural habitat. If designed properly and with minimal impact on the organism, such a device can provide useful information concerning the physiology and activity of the organism under investigation, such as heart rate [1], body temperature [2] or muscle movement [3]. Similarly, reporter genes award the microbial ecologist with useful information on the activity of individual microbes. What's more, this information may help us gain an understanding of how microbes perceive their habitat in terms of chemical, physical and biological stimuli. In this review, we present a short synopsis of recent developments in the application of reporter genes in microbial ecology. We limit our discussion to the uses of reporter genes for habitat exploration [4] by which so-called bioreporter cells are released into a habitat with the task to report on their exposure to a specific stimulus. The focus will be mostly on reporter genes that allow the monitoring of individual microbial cells, as these have proven instrumental for describing habitats at the resolution of micrometer dimensions.

## Bioreporters and reporter genes

The principle of bioreporting is illustrated in Figure 1. Most bioreporters used in microbial ecology today are products of genetic engineering by which an environmentally or metabolically responsive promoter is fused to a suitable reporter gene and introduced into an appropriate microbial host either on a plasmid or as a stable chromosomal insertion. Changes in the abundance of the corresponding reporter protein are indicative of changes in the transcriptional activity of the promoter and, thus, of changes in the stimulus to which that promoter is responsive. Stimuli can be either chemical (for example, nutrients [5••,6•], metals [7] or antibiotics [8•]), physical (for example, ultraviolet light [9], temperature [10] or water potential [11]), or biological (for example, the signal molecule *n*-acylhomoserine lactone [12•,13], which bacteria use to monitor their population density [14•]). Metabolically responsive promoters are also commonly used, namely to monitor a microbe's growth rate [15,16] or its deficiency in an essential resource such as carbon [17••], oxygen [18], nitrogen [19] or phosphorus [20]. For a comprehensive overview of promoters that have been used in conjunction with reporter genes, we refer the reader to reviews by Hansen and Sørensen [21], Daunert *et al.* [22], and Köhler *et al.* [23].

The most commonly used reporter genes are those which code for green fluorescent protein (GFP), bacterial luciferase, firefly luciferase, β-galactosidase, β-glucuronidase, catechol 2,3-dioxygenase and the ice nucleation protein, InaZ. Each reporter protein has its advantages and disadvantages, and for a comparison of these and other reporter proteins, we refer the reader to recent reviews by Loper and Lindow [24], and Daunert *et al.* [22]. The one property we would like to single out in this review in *Current Opinion in Microbiology* is whether or not the abundance of a reporter protein can be detected and quantified in a single microbe. GFP owes much of its popularity to its easy

**Figure 1**




The utility of bioreporters for microbial exploration of a microhabitat. **(a)** We start with a population of bioreporter cells for which it has been established in culture that they exhibit elevated reporter gene activity in response to a known metabolical, physical, chemical or biological stimulus. For many bioreporters, reporter gene activity is proportional to the magnitude of the stimulus (no, low or high exposure, in this example). Bioreporter activity is expressed as the abundance of reporter protein averaged over the entire population, or on a cell-to-cell basis (for example, in a histogram). The bioreporter can be used to search a microhabitat for the stimulus to which it is responsive. As symbolized by the coloring, there may be variation within the habitat in terms of stimulus magnitude, but this variation is essentially unknown. **(b)** Following release of the bioreporter cells into the habitat, and after a defined period of time or at different time intervals, bioreporter cells can be examined for reporter protein directly in their natural surroundings, that is, if both the reporter protein and the habitat allow. Note that the coloring in the final panel has been removed to indicate that variation in stimulus exposure is not an observable feature, but instead the kind of information that we try to infer from the bioreporters' behavior. **(c)** Alternatively, or in addition, cells may be recovered from the habitat and analyzed for reporter activity. Depending on the reporter gene that is being used, this activity is expressed as an average over all the bioreporters recovered from the habitat, or as a distribution of reporter activity among individual bioreporter cells. Obviously, the latter is inherently more informative.

detection in individual cells by epifluorescence and confocal laser scanning microscopy [25–27], and its amenability to quantitative single-cell analysis using image cytometry [5••,7] or flow cytometry [27,28]. β-galactosidase activity can also be measured in individual cells, using chromogenic [29] and fluorigenic substrates [30] or by immunofluorescence [17••,31]. Luciferase has been detected in individual bacteria [32], but low resolution has hampered its use for analysis in single-cell bioreporters. The ability to measure reporter protein in individual cells enhances the information that can be obtained from a bioreporter, as is illustrated in Figure 1. First, it allows *in situ* observation by microscopy that may provide a spatial context within which bioreporter activity can be understood. Second, it creates the opportunity to assess variation within the bioreporter community and, thus, within the habitat. In the following sections, we discuss each of these in greater detail.

## *In situ* observation of bioreporters

Judging from a sizable number of recent journal covers, one would get the impression that *in situ* observation is a favorite pastime of microbial ecologists. Certainly, a reporter protein such as GFP has proven to be a powerful tool to relate the activity of a microbe to its actual location. At least two reports have clearly documented how relevant micrometer distances are for a microbe's perception of its surroundings. In a study by Møller *et al.* [33], *Pseudomonas putida* bioreporters in a toluene-fed flow-chamber biofilm reported the presence of benzoate only when they were located less than several micrometers away from colonies of an *Acinetobacter* sp. that were presumed to be leaking benzoate as an intermediate of toluene metabolism. Furthermore, induction appeared to spread with the direction of the flow in narrow bands approximately 100 μm wide, suggesting that no perpendicular diffusion of benzoate occurred over these distances to nearby bioreporter cells. In a more recent study, Jaspers *et al.* [34••] showed substantial differences in the availability of alkanes to bioreporter cells along millimeter distances from a fixed alkane source. Other research groups have linked the activity of single reporter cells to microscopic differences in habitat topography. On bean leaves, for example, both sugar consumption [5••,6•] and plasmid transfer [35] seemed to be taking place preferentially near stomata, at the junctions of epidermal cells, or in the cracks between veins. Rapid growth of a bioreporting *P. putida* inoculant on barley roots was limited spatially to sloughing sheath cells and temporally to the first day after inoculation [16].

There are several issues with the interpretation of *in situ* observations. In many instances, it is probably fair to ask how representative such observations are within the larger context of the habitat under investigation. Another problem often overlooked with *in situ* observation of reporter activity is its bias towards those bioreporter cells that are actively reporting. Non-reporting cells may easily escape detection, resulting in an overestimation of the number of reporting cells. Also, it may be just as interesting to know why certain bacteria are not reporting, and whether it can be explained by their whereabouts. This bias towards actively reporting cells can be avoided by marking the bioreporter cells with an independently expressed fluorescent protein such as DsRed [36], or by making the bioreporter cells visible using fluorescent *in situ* hybridization [33,37]. A third problem inherent to *in situ* observation is the difficulty in adequately determining the abundance of reporter protein. Actual quantification of reporter content in individual bioreporter cells *in situ* has, to the best of our knowledge, not been done, although Møller *et al.* [33] were able to estimate the proportion of bioreporting cells at different depths within a biofilm. β-galactosidase activity cannot be easily measured *in situ*, owing to the need to permeabilize the bioreporter cells and/or provide them *in situ* with substrate. GFP does not share this requirement, but inconsistencies in habitat structure may cause problems with microscope focusing, uneven excitation of the cells, or interference from background fluorescence.

## *Ex situ* analysis of reporter protein abundance in bioreporter cells

Many of the problems associated with *in situ* observation of bioreporter cells can be overcome by analyzing bioreporter cells after they are recovered from the habitat. Unfortunately, not all habitats permit such a recovery, and a major drawback of this approach is that any correlation between the location and activity of a bioreporter is lost in the process. However, *ex situ* interrogation is free from habitat interference and amenable to any kind of manipulation that is required for measurement of reporter protein abundance. Furthermore, because many more cells can be interrogated simultaneously, bioreporter data become more statistically representative of the population as a whole.

For the interpretation of *ex situ* bioreporter data, it makes a notable difference whether reporter activity is expressed as a population average or for individual cells. An average value is perfectly adequate to describe microbial behavior when all or most bioreporters in the habitat under study are expected to behave in the same way or encounter uniform conditions (such as *Salmonella typhimurium* within the vacuolar microenvironment of a macrophage [38], or *Yersinia pestis* in the phagolysosome [39]). If, however, the habitat is less homogeneous, an average value becomes inevitably less informative and possibly misleading. This is illustrated in Figure 1c, which shows that the existence of two small subpopulations of actively reporting bioreporter cells cannot be inferred from the low average reporter activity. An interesting situation occurs when heterogeneity becomes so extreme that only a very small fraction of the cells is positively reporting. In case of a GFP-based bioreporter, this would necessitate the interrogation of many more individual cells, which may not always be practical. For such situations, the InaZ reporter protein presents an ideal alternative. Although its activity cannot be expressed on a single-cell basis, InaZ exhibits an

**Figure 2**

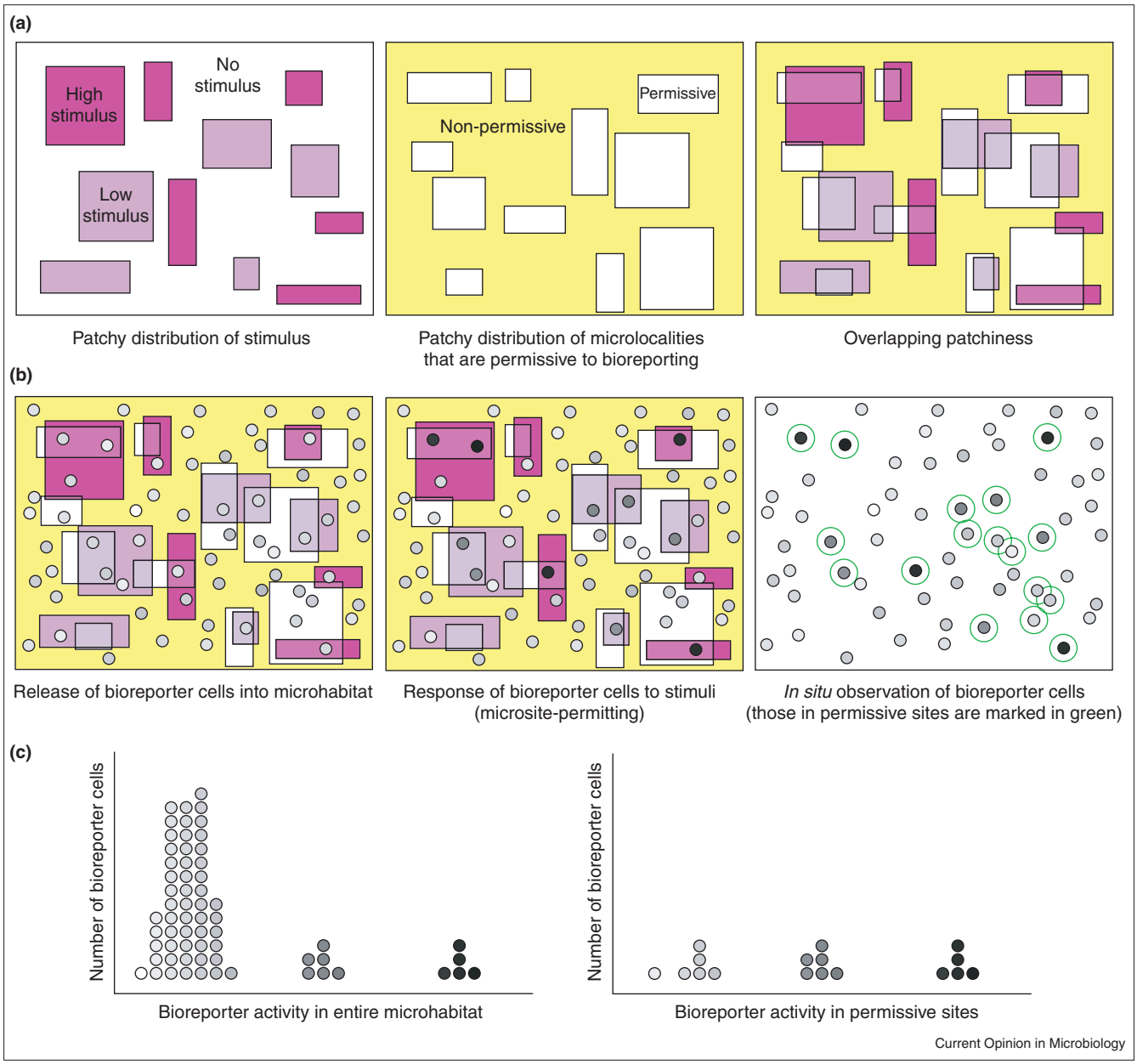



Illustration of the need for contextual interpretation of bioreporter data. **(a)** The microhabitat under investigation may be variable not only in stimulus magnitude, but also in the availability of microsites that are permissive to bioreporting. Outside these sites, the cells are exposed to conditions that prevent them from producing reporter protein even when they are exposed to the stimulus. Examples of such conditions are lack of nutrients or exposure to toxicants, which may render cells metabolically less active or non-active, but also, for example, the suppression of reporter gene expression owing to catabolite repression. **(b)** Upon release and establishment of the bioreporter in the habitat, reporter activity becomes a combined function of both stimulus exposure and site permissiveness. **(c)** Analysis of all bioreporters may correctly identify cells that were exposed to the stimulus, but their numbers are an underestimate, given that there might have been an unknown number of apparently non-reporting cells that had been exposed to the stimulus but were located in non-permissive sites. Cells that reside in permissive sites may be identified by different criteria, for example, in the case of metabolic status on cell size, ribosome content or energy levels. Comparative analysis of only bioreporter cells from permissive sites presents an accurate estimate for the variability in stimulus presentation in such sites.

exponential relationship between abundance and ice nucleation activity [24], so that it remains detectable even at very low numbers of reporting cells [6•]. This property has been exploited in several bioreporter applications using *inaZ* reporter fusions [40–44].

A recent study by Joyner and Lindow [7] demonstrates the advantage of a single-cell reporter like GFP for the recognition of heterogeneous activity within a population of bioreporters. The authors described a strain of *Pseudomonas syringae* that carries a fusion of the iron-regulated *pvd*

promoter to the gene for GFP and fluoresces when $Fe^{3+}$ becomes scarce. Previous analyses of bioreporters with the *pvd* promoter fused to the *inaZ* reporter gene [45] had revealed that the average *P. syringae* cell on bean plant surfaces had access to low but non-limiting concentrations of $Fe^{3+}$. From the distribution of GFP content in individual bioreporter cells, however, it was obvious that this average was, in fact, a combination of a few very fluorescent cells and many dim cells, suggesting that, within the population of plant colonists, there existed considerable differences in the availability of iron. Such observations of heterogeneity have been made in other habitats and for other environmental stimuli as well. For example, *Erwinia herbicola* cells experience highly different levels of sucrose and fructose on bean plant leaves [5••,6•]. Hansen *et al.* [8•] took a bioreporter approach to search soil microcosms for the presence of oxytetracycline produced *in situ* by a strain of *Streptomyces rimosus*, and showed that only a subset of the GFP-based *Escherichia coli* reporter cells was exposed to the antibiotic. Likewise, *Pseudomonas* bioreporter cells varied considerably in their reporting of carbon limitation [17••] and metabolic activity [16] in soil and rhizosphere habitats, respectively.

## Obvious and obscure sources of heterogeneity in bioreporter activity

Apparently, it is not uncommon to observe heterogeneity in the activity of bioreporters recovered from habitats. This leaves us with a compelling question. Does this heterogeneity truthfully reflect spatial and/or temporal heterogeneity in the stimulus that we set out to measure? Surely, there is no reason to assume that variability exists only for that one stimulus, and not for all the other stimuli that the bioreporter is likely to experience. What if variation in the latter would affect bioreporter performance? A scenario in which a habitat is patchy with respect to microlocalities that do and do not permit the bioreporter to function properly is illustrated in Figure 2. Such sites must exist, at least in habitats for which it has been observed that a substantial fraction of bioreporter cells is metabolically challenged and, as such, may not be able to report. As Figure 2 clearly shows, different estimates of habitat variation are obtained when activity of bioreporters is or is not related to their metabolic status. Such contextual evaluation of bioreporter data will have to become an important component of future bioreporter applications. This holds true not only for applications that are aimed to describe microbial perception of a habitat, but also for those that use reporter genes for the demonstration of habitat-specific gene expression. A few precedents for such multiparameter approaches to relate a reporter activity of a cell to other activities in which it might be engaged have already been set. We [5••] used a combination of fluorescent *in situ* hybridization and inducible GFP expression to show that the presence of sugars on bean leaf surfaces was a key determinant for foliar growth of *E. herbicola*. Normander *et al.* [35] showed that plasmid exchange among bacteria on plant surfaces, as visualized by a GFP-based reporter system, was not limited by the metabolic activity of donor and recipient populations, based on incorporation rates of leucine *in situ*. Koch *et al.* [17••] used a double reporter for carbon and nitrogen limitation in soil, and showed that the amendment of straw changed the soil habitat from carbon- to nitrogen-limited.

Aspects of bioreporter biology other than the ones above should also be considered. For example, it is a misconception that reporter abundance is a function only of promoter activity. We have recently described a mathematical model that helps understand how parameters such as growth rate and protein stability also affect the reporter content of individual bacteria [46•]. The predictions of the model are remarkably intuitive. For example, a decrease in growth rate or increase in reporter stability may cause an increase in reporter protein abundance without changes in promoter activity. Integration of this type of modeling approach will undoubtedly prove essential for the interpretation of bioreporter data from complex environments.

## Conclusions

Without question, bioreporters have become popular and useful tools in microbial ecology. They provide us with a line of communication to a world of micrometer dimensions. As we continue to make use of their services and try to understand what it is that they are trying to convey, we need to stay aware that bioreporters have a biology, and that their bioreporting skills should be understood within the ramifications of that biology. Sports journalist Red Smith once said that a "reporter has one of the toughest jobs in the world — getting as near to the truth as possible is a terribly tough job." [47]. We wish to argue that, in microbial ecology, this responsibility lies not with the bioreporter but with the microbial ecologist, whose greatest challenge it is to translate bioreporter data into a meaningful account of the microbe's biology and its perception of the world.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Giacometti M, Janovsky M, Fluch G, Arnold W, Schober F: **A technique to implant heart-rate transmitters in red deer.** *Wildl Soc Bull* 2001, **29**:586-593.

2. Brain C, Mitchell D: **Body temperature changes in free-ranging baboons (*Papio hamadryas ursinus*) in the Namib desert, Namibia.** *Int J Primatol* 1999, **20**:585-598.

3. Thorstad EB, Okland F, Koed A, McKinley RS: **Radio-transmitted electromyogram signals as indicators of swimming speed in lake trout and brown trout.** *J Fish Biol* 2000, **57**:547-561.

4. Lindow SE: **The use of reporter genes in the study of microbial ecology.** *Mol Ecol* 1995, **4**:555-566.

5. •• Leveau JHJ, Lindow SE: **Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere.** *Proc Natl Acad Sci USA* 2001, **98**:3446-3453.
This study reveals spatial and temporal variation in the availability of fructose to bacterial colonizers of bean leaf surfaces. GFP content of the *Erwinia*-based bioreporter was quantified by image cytometry, which provides an accurate measure for comparison of bioreporter behavior at different times

during plant colonization. Furthermore, the authors employed fluorescent *in situ* hybridization to correlate sugar consumption by the bioreporter cells with their growth status.

6. Miller WG, Brandl MT, Quiñones B, Lindow SE: **Biological sensor for**
• **sucrose availability: relative sensitivities of various reporter genes.** *Appl Environ Microbiol* 2001, **67**:1308-1317.
The authors compared the limits of detection of reporter proteins GFP, β-galactosidase and InaZ. They showed that the *inaZ* reporter gene is the most sensitive, as it allowed detection of the lowest amounts of sucrose by a sucrose responsive promoter in an *E. herbicola* reporter strain.

7. Joyner DC, Lindow SE: **Heterogeneity of iron bioavailability on plants assessed with a whole-cell GFP-based bacterial biosensor.** *Microbiology* 2000, **146**:2435-2445.

8. Hansen LH, Ferrari B, Sørensen AH, Veal D, Sørensen SJ: **Detection**
• **of oxytetracycline production by *Streptomyces rimosus* in soil microcosms by combining whole-cell biosensors and flow cytometry.** *Appl Environ Microbiol* 2001, **67**:239-244.
This paper describes an application of the resolving power of flow cytometry for the analysis of GFP-based bioreporters from a complex habitat such as soil, as it allows the detection of a few fluorescent cells among a majority of non-fluorescent reporter bacteria.

9. Kim JJ, Sundin GW: **Regulation of the *rulAB* mutagenic DNA repair operon of *Pseudomonas syringae* by UV-B (290 to 320 nanometers) radiation and analysis of *rulAB*-mediated mutability *in vitro* and *in planta*.** *J Bacteriol* 2000, **182**:6137-6144.

10. Ullrich MS, Schergaut M, Boch J, Ullrich B: **Temperature-responsive genetic loci in the plant pathogen *Pseudomanas syringae* pv. *glycinea*.** *Microbiology* 2000, **146**:2457-2468.

11. Axtell CA, Beattie GA: **Use of a *proU-gfp* transcriptional fusion to quantify water stress on the leaf surface.** Abstract 41 of the 10th International Congress on Molecular Plant–Microbe Interactions: 2001 July 10–14; Madison, Wisconsin.

12. Andersen J, Heydorn A, Hentzer M, Eberl L, Geisenberger O,
• Christensen B, Molin S, Givskov M: ***gfp*-based *N*-acyl homoserine-lactone sensor systems for detection of bacterial communication.** *J Bacteriol* 2001, **67**:575-585.
Description of a novel type of bioreporter for eavesdropping on bacterial communication. The study monitors communication through *N*-acylhomoserine lactone between individual cells within swarming colonies of *Serratia* species.

13. Wu H, Song ZJ, Hentzer M, Andersen JB, Heydorn A, Mathee K, Moser C, Eberl L, Molin S, Hoiby N *et al.*: **Detection of *N*-acylhomoserine lactones in lung tissues of mice infected with *Pseudomonas aeruginosa*.** *Microbiology* 2000, **146**:2481-2493.

14. Withers H, Swift S, Williams P: **Quorum sensing as an integral**
• **component of gene regulatory networks in Gram-negative bacteria.** *Curr Opin Microbiol* 2001, **4**:186-193.
This is an up-to-date review on cell-to-cell communication by Gram-negative bacteria through small diffusible molecules such as *N*-acylhomoserine lactones. The review explores the phenomenon of quorum sensing within the context of bacterial adaptation and bacterial infections of humans and animals.

15. Sternberg C, Christensen BB, Johansen T, Nielsen AT, Andersen JB, Givskov M, Molin S: **Distribution of bacterial growth activity in flow-chamber biofilms.** *Appl Environ Microbiol* 1999, **65**:4108-4117.

16. Ramos C, Molbak L, Molin S: **Bacterial activity in the rhizosphere analyzed at the single-cell level by monitoring ribosome contents and synthesis rates.** *Appl Environ Microbiol* 2000, **66**:801-809.

17. Koch B, Worm J, Jensen LE, Højberg O, Nybroe O: **Carbon limitation**
•• **induces $\sigma^S$-dependent gene expression in *Pseudomonas fluorescens* in soil.** *Appl Environ Microbiol* 2001, **67**:3363-3370.
Using a $P_{fic}$–*lacZ* fusion in *Pseudomonas fluorescens* DF57, the authors reveal how the soil habitat is experienced by individual bacteria in terms of carbon limitation. This is one of the first studies to employ a double reporter, which simultaneously monitors both carbon and nitrogen availability. The authors describe the use of immunofluorescence to detect concurrently by flow cytometry individual bioreporter cells (using an antibody to outer membrane lipopolysaccharides of DF57) and their β-galactosidase content (using an antibody to β-galactosidase).

18. Højberg O, Schnider U, Winteler HV, Sørensen J, Haas D: **Oxygen-sensing reporter strain of *Pseudomonas fluorescens* for monitoring the distribution of low-oxygen habitats in soil.** *Appl Environ Microbiol* 1999, **65**:4085-4093.

19. Jensen LE, Nybroe O: **Nitrogen availability to *Pseudomonas fluorescens* DF57 is limited during decomposition of barley straw in bulk soil and in the barley rhizosphere.** *Appl Environ Microbiol* 1999, **65**:4320-4328.

20. Kragelund L, Hosbond C, Nybroe O: **Distribution of metabolic activity and phosphate starvation response of *lux*-tagged *Pseudomonas fluorescens* reporter bacteria in the barley rhizosphere.** *Appl Environ Microbiol* 1997, **63**:4920-4928.

21. Hansen LH, Sørensen SJ: **The use of whole-cell biosensors to detect and quantify compounds or conditions affecting biological systems.** *Microb Ecol* 2001, **42**:483-494.

22. Daunert S, Barrett G, Feliciano JS, Shetty RS, Shrestha S, Smith-Spencer W: **Genetically engineered whole-cell sensing systems: coupling biological recognition with reporter genes.** *Chem Rev* 2000, **100**:2705-2738.

23. Köhler S, Belkin S, Schmid RD: **Reporter gene bioassays in environmental analysis.** *Fresenius J Anal Chem* 2000, **366**:769-779.

24. Loper JE, Lindow SE: **Reporter gene systems useful in evaluating *in situ* gene expression by soil- and plant-associated bacteria.** In *Manual of Environmental Microbiology*, edn 2. Edited by Hurst CJ. Washington, DC: ASM Press; 2001:627-637.

25. Phillips GJ: **Green fluorescent protein — a bright idea for the study of bacterial protein localization.** *FEMS Microbiol Lett* 2001, **204**:9-18.

26. Tombolini R, Unge A, Davey ME, De Bruijn FJ, Jansson JK: **Flow cytometric and microscopic analysis of GFP-tagged *Pseudomonas fluorescens* bacteria.** *FEMS Microbiol Ecol* 1997, **22**:17-28.

27. Cormack BP, Valdivia RH, Falkow S: **FACS-optimized mutants of the green fluorescent protein (GFP).** *Gene* 1996, **173**:33-38.

28. Valdivia RH, Ramakrishnan L: **Applications of gene fusions to green fluorescent protein and flow cytometry to the study of bacterial gene expression in host cells.** *Methods Enzymol* 2000, **326**:47-73.

29. Poulsen LK, Dalton HM, Angles ML, Marshall KC, Molin S, Goodman AE: **Simultaneous determination of gene expression and bacterial identity in single cells in defined mixtures of pure cultures.** *Appl Environ Microbiol* 1997, **63**:3698-3702.

30. Rowland B, Purkayastha A, Monserrat C, Casart Y, Takiff H, McDonough KA: **Fluorescence-based detection of *lacZ* reporter gene expression in intact and viable bacteria including *Mycobacterium* species.** *FEMS Microbiol Lett* 1999, **179**:317-325.

31. Kang YW, Saile E, Schell MA, Denny TP: **Quantitative immunofluorescence of regulated *eps* gene expression in single cells of *Ralstonia solanacearum*.** *Appl Environ Microbiol* 1999, **65**:2356-2362.

32. Sternberg C, Eberl L, Poulsen LK, Molin S: **Detection of bioluminsecence from individual bacterial cells: a comparison of two different low-light imaging systems.** *J Biolumin Chemilumin* 1997, **12**:7-13.

33. Møller S, Sternberg C, Andersen JB, Christensen BB, Ramos JL, Givskov M, Molin S: ***In situ* gene expression in mixed-culture biofilms: evidence of metabolic interactions between community members.** *Appl Environ Microbiol* 1998, **64**:721-732.

34. Jaspers MCM, Meier C, Zehnder AJB, Harms H, van der Meer JR:
•• **Measuring mass transfer processes of octane with the help of an *alkS-alkB*::*gfp*-tagged *Escherichia coli*.** *Environ Microbiol* 2001, **3**:512-524.
This paper reports an elegant study that reveals the significance of mass transfer at micrometer scales. Using a sophisticated experimental setup, the researchers were able to compare the exposure of *gfp*-based *E. coli* bioreporter cells to alkanes as a function of time and space. The results indicate a critical role for air spaces in the accelerated transport of hydrophobic pollutants.

35. Normander B, Christensen BB, Molin S, Kroer N: **Effect of bacterial distribution and activity on conjugal gene transfer on the phylloplane of the bush bean (*Phaseolus vulgaris*).** *Appl Environ Microbiol* 1998, **64**:1902-1909.

36. Jakobs S, Subramaniam V, Schoenle A, Jovin TM, Hell SW: **EGFP and DsRed expressing cultures of *Escherichia coli* imaged by confocal, two-photon and fluorescence lifetime microscopy.** *FEBS Lett* 2000, **479**:131-135.

37. Brandl MT, Quiñones B, Lindow SE: **Heterogeneous transcription of an indoleacetic acid biosynthetic gene in *Erwinia herbicola* on plant surfaces.** *Proc Natl Acad Sci USA* 2001, **98**:3454-3459.

38. Heithoff DM, Conner CP, Hentschel U, Govantes F, Hanna PC, Mahan MJ: **Coordinate intracellular expression of *Salmonella* genes induced during infection.** *J Bacteriol* 1999, **181**:799-807.

39. Pollack C, Straley SC, Klempner MS: **Probing the phagolysosomal environment of human macrophages with a $Ca^{2+}$-responsive operon fusion in *Yersinia pestis*.** *Nature* 1986, **322**:834-836.

40. Jaeger CH III, Lindow SE, Miller W, Clark E, Firestone MK: **Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan.** *Appl Environ Microbiol* 1999, **65**:2685-2690.

41. Li XZ, Starratt AN, Cuppels DA: **Identification of tomato leaf factors that activate toxin gene expression in *Pseudomonas syringae* pv. *tomato* DC3000.** *Phytopathology* 1998, **88**:1094-1100.

42. Loper JE, Henkels MD: **Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere.** *Appl Environ Microbiol* 1999, **65**:5357-5363.

43. Pierson EA, Wood DW, Cannon JA, Blachere FM, Pierson LS: **Interpopulation signaling via *N*-acyl-homoserine lactones among bacteria in the wheat rhizosphere.** *Mol Plant Microb Interact* 1998, **11**:1078-1084.

44. Zhang ZG, Pierson LS: **A second quorum-sensing system regulates cell surface properties but not phenazine antibiotic production in *Pseudomonas aureofaciens*.** *Appl Environ Microbiol* 2001, **67**:4305-4315.

45. Loper JE, Lindow SE: **A biological sensor for iron available to bacteria in their habitats on plant-surfaces.** *Appl Environ Microbiol* 1994, **60**:1934-1941.

46. • Leveau JHJ, Lindow SE: **Predictive and interpretive simulation of green fluorescent protein expression in reporter bacteria.** *J Bacteriol* 2001, **183**:6752-6762.
The authors of this paper formulated a numerical model for the accumulation of GFP in bacterial reporter cells, which takes into account promoter activity, GFP maturation and its susceptibility to proteolytic degradation, and bacterial growth rate. The model proved highly predictive for the behavior of bioreporters in culture, which suggests that it may also facilitate and advance the interpretation of bioreporter data obtained from complex habitats.

47. Smith WW: *The Red Smith Reader*. New York: Random House; 1982.