ELSEVIER

Making bio-sense of toxicity: new developments in whole-cell biosensors

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Bacterial whole-cell biosensors are very useful for toxicity measurements of various samples. Semi-specific biosensors, containing fusions of stress-regulated promoters and reporter genes, have several advantages over the traditional, general biosensors that are based on constitutively expressed reporter genes. Furthermore, semi-specific biosensors are constantly being refined to lower their sensitivity and, in combination, are able to detect a wide range of toxic agents. However, the requirement for a positive response of these biosensors to toxicants can result in false-negative responses. The application of *in situ* inoculation and single-cell detection, combined with the introduction of new reporter genes and refined detection equipment, could lead to the extensive use of semi-specific, stress-responsive biosensors for toxicity estimations in the future.

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Introduction

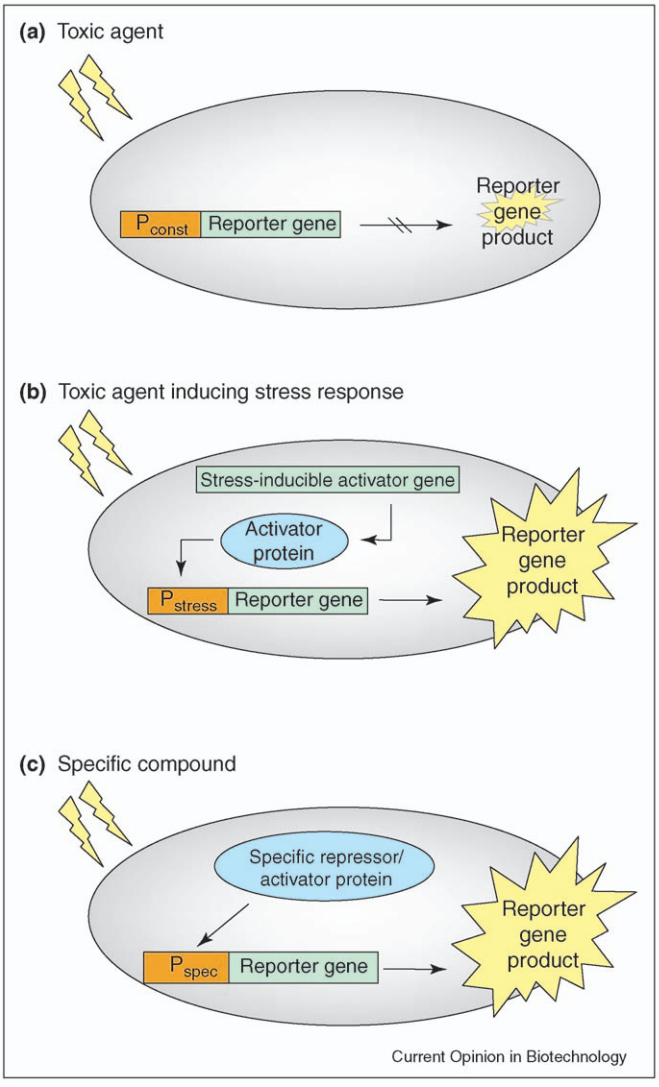
Whole-cell bacterial biosensors are widely used in evaluations of microbial habitats. They provide measurements of the bioavailable fraction of various compounds and report on the conditions to which bacteria are exposed [1,2]. Furthermore, biosensors have proven very useful in toxicity screenings of environmental samples, leading to the commercialization of several biosensor assays [3,4] that have supplemented or even replaced traditional methods for toxicity measurements which rely mainly on extraction and chromatography. The ease of use and low expense of biosensor assays represent some of the advantages compared with traditional methods.

Bacterial whole-cell biosensors produce measurable gene products encoded by reporter genes, which are either present naturally in the bacterial strain or introduced by genetic manipulation. The most frequently used reporter genes include the *lacZ* gene from *Escherichia coli*, the *lux* genes from, for example, *Vibrio fischeri* or the *gfp* gene from *Aequorea victoria*. Thorough descriptions of these and other reporter genes are given elsewhere [1,5•]. If the reporter gene is placed downstream of a constitutively expressed promoter, the biosensor reports a decrease in metabolic activity through a decline in the intensity of the signal produced. These biosensors are described here as general biosensors (Figure 1a). In some biosensors, the reporter gene is fused to a stress-responsive promoter, resulting in reporter gene expression when the biosensor strain is exposed to conditions triggering a stress response; for instance, DNA damage (SOS response) or protein damage (heat shock response). These are referred to as semi-specific biosensors (Figure 1b), as they respond only to classes of compounds or conditions that stress the cell in a certain way. The specific biosensors (Figure 1c) respond to the presence of a certain compound or condition. They usually contain a fusion of a regulated promoter to a reporter gene and, in some cases, also a regulatory protein responsible for the activation or repression of the promoter. This class of biosensors will not be described in this communication, but comprehensive overviews have recently been published [6,7]. In the following, we will focus on current and future trends in the use of general and semi-specific classes of biosensor in the detection of toxicity.

General biosensors

Using the inhibition of light production by naturally luminescent bacteria was first proposed as a simple and rapid method for monitoring the toxicity of aquatic samples over 27 years ago [3]. The test was commercialized soon after (Microtox® test) and has since been used in numerous studies, gaining wide acceptance in eco-toxicology [8]. The assay is based on the photometric detection of the change in light output from luminescent *V. fischeri* cells. Light emission depends on the presence of functional metabolism, including sufficient high-energy cofactors; hence, toxic compounds that compromise the bacterial metabolic state will cause a decrease in light emission proportional to the sample concentration (Figure 1a). One of the great advantages of non-specific general biosensors is that they can be used to measure mixed toxicants [9]. They can also detect unpredictable additive effects between chemicals in complex mixtures [10] and in environmental samples. Unfortunately, any decrease in metabolic activity will also result in decreased luminescence by these non-specific biosensor cells [11], resulting in false-positive results. Furthermore, sodium,

Figure 1



Schematic of the three different types of biosensors. **(a)** Response of a non-specific general toxicity biosensor such as Microtox®. The reporter gene is fused to a constitutive promoter (P_{const}) and toxicity is measured as a decrease in reporter protein activity. **(b)** A semi-specific biosensor responding to a toxic agent inducing a stress response. Toxicity is measured as an increase in reporter protein production from stress-induced promoters (P_{stress}), such as heat shock or SOS promoters. **(c)** The response of a specific biosensor. Here a specific chemical signal induces a tightly regulated promoter (P_{spec}), which responds specifically to the compound in question. The response is usually a measurable increase in reporter protein production.

potassium, calcium and magnesium ions have all been shown to influence light emission by *V. fischeri*. The use of non-specific general biosensors is therefore limited to short time exposures in a testing medium carefully designed and adjusted to provide optimal growth conditions for this marine bacterium. Over the years, several alternative tests using more robust natural luminescent bacteria (e.g. *Photobacterium leiognathi* [12]) or recombinant bacteria (e.g. the groundwater bacterium *Janthinobacterium lividum* [13] and pseudomonads isolated from contaminated wastewater [14]) have been developed. Despite the above-mentioned problems, however, owing to their simplicity the non-specific general biosensors remain the most widely used whole-cell biosensors today.

Semi-specific biosensors

Moving up a level in specificity is the group of semi-specific, stress-responsive biosensors. Bacteria respond to compounds or conditions that stress the cell by activating processes that protect the cell against the invoked stress. This has been exploited in the construction of biosensors for the detection of conditions or compounds eliciting a stress response (Figure 1b). As different stimuli can induce the same stress response in the cell, biosensors