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- Review -

Applications of Microbial Whole-Cell Biosensors in Detection of Specific Environmental Pollutants

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Microbial whole-cell biosensors can be excellent analytical tools for monitoring environmental pollutants. They are constructed by fusing reporter genes (e.g., *lux, gfp* or *lacZ*) to inducible regulatory genes which are responsive to the relevant pollutants, such as aromatic hydrocarbons and heavy metals. A large spectrum of microbial biosensors has been developed using recombinant DNA technology and applied in fields as diverse as environmental monitoring, medicine, food processing, agriculture, and defense. Furthermore, their sensitivity and target range could be improved by modification of regulatory genes. Recently, microbial biosensor cells have been immobilized on chips, optic fibers, and other platforms of high-throughput cell arrays. This paper reviews recent advances and future trends of genetically modified microbial biosensors used for monitoring of specific environmental pollutants.

Key words : Whole-cell biosensor, recombinant DNA technology, aromatic hydrocarbons, heavy metals

Introduction

Microbial whole-cell biosensors have been developed to detect target pollutants. Being considered as first monitoring systems, they can provide enough information for routine testing and screening of samples in various fields [15]. For environmental monitoring, microbial biosensors can provide fast and specific data on contaminated sites; not only the concentration of pollutants but also their biological effects, toxicity and bioavailability. They also offer other advantages such as portability, working on-site, and ability of measuring pollutants in complex matrices with minimal sample preparation.

Microorganisms as sensing elements in microbial biosensors have several advantages over other sensing elements such as enzymes, antibodies, or sub-cellular components: ability to detect wide range of chemicals, amenability to genetic modification, and adaptation to broad environmental reaction conditions [20]. In the earlier microbial biosensors, the respiratory and metabolic functions of the microorganisms have been exploited to detect such substrates or inhibitors of these processes. For example, toxicity responses of naturally luminescent *Vibrio fischeri* (the commercial microbial biosensor, Microtox® test) are detected by inhibition of light production. Recently, the recombinant DNA technologies have been used to tail the microorganisms for a given purpose by artificial fusing of natural regulatory genes (coding a transcriptional regulator plus promoter/operator) with a promoter-less reporter gene [36,48]. Transcriptional regulators activated by the target chemicals interact with the promoter triggering production of the measurable signal from the reporter gene (Fig. 1). Several reviews have discussed various aspects of the use of such constructs for environmental applications [2,8,14,17]. Furthermore, their biosensor capabilities (e.g., target range and sensitivity) could be improved by modifying regulatory genes. Our group [30,37] as well as Galvão and de Lorenzo [12] demonstrated increased sensitivities and specificities of microbial biosensors by engineering of effector-binding sites in regulatory proteins. More recently, microbial biosensor cells have been incorporated into chips, optic fibers, and other platforms to construct high-throughput biosensors [19,23,42]. This paper reviews recent progress in microbial whole-cell biosensors and their future trends with a focus on the development and application of genetically engineered biosensors used for monitoring of specific environmental pollutants.

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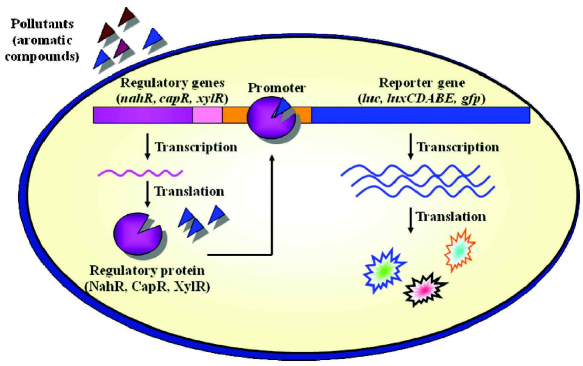

Fig. 1. The signal transmission of a specific microbial biosensor. Here a specific chemical (e.g., aromatic compounds) induces a tightly regulated promoter by binding to the regulatory protein (e.g., NahR, CapR, XylR). The response is usually a measurable increase in reporter protein production.

Configurations

Microbial whole-cell biosensors are constructed by fusing regulatory genes to a promoter-less reporter gene. The regulatory genes, in the presence of the relevant pollutants or environmental signals, activate the promoter which turns on the expression of reporter gene downstream (Fig. 1). Thus, the regulatory genes determine the specificity and sensitivity of microbial biosensors. Two categories of biosensors are classified in regards to target property: 'chemical responsive' biosensors showing the identity of a single or a few structurally related specific chemicals and 'stress responsive' biosensors showing the presence of a group of toxic chemicals or adverse physiological conditions that cause stress responses such as DNA or protein damage. Stress responsive biosensors will not be described in this communication, but comprehensive reviews have been published [39,48]. The development of chemical responsive biosensors exploits the high specificity of regulatory protein interactions with chemicals. Examples are depicted in microbial biosensors used for detection of aromatic hydrocarbon [29,40,49], heavy metals [9,11,13], pesticides/herbicides [10,47], and various organophosphorous nerve agents [21].

Microbial biosensors report biological interaction with target chemicals by a reporter gene. The interactions reported by reporter genes can be detected in color or bioluminescence change simply by the naked eye or converted by transducers into a measurable response such as absorption of light, current, or potential through optical or electrochemical means for being further amplified, processed and analyzed. The reporter gene usually codes an enzyme that catalyzes an easily monitored reaction: for example, *lacZ* codes for the enzyme β-galactosidase, *lux* or *luc* genes for the bacterial or firefly luciferase, and the *gfp* gene for green fluorescent protein (GFP). The intensity of the color or bioluminescence is proportional to the level of enzyme activity present in the reaction. In addition to the reporters described, other enzymes such as alkaline phosphatase [29], horseradish peroxidase [50], and the *crtA* gene product [11,51] have been employed in biosensor constructions. The bioluminescence reporter gene (*lux* or *luc*) is the predominant gene used for the construction of biosensors for environmental monitoring. The *Luc* has higher sensitivity than bacterial luciferase (*lux*) although it requires the addition of the substrate and ATP for generation of bioluminescence. Bacterial luciferase (*Lux*) is heat-labile and difficult to use in mammalian cells. GFP is a reporter produced fluorescence activity after simple irradiation at the excitation wavelength without the addition of an exogenous substrate or ATP or lysed cells [40]. However, the application of GFP to bacterial biosensors is not recommended since it has a high background signal and takes time to form the fully active fluorophore of GFP.

Aromatic hydrocarbons

Aromatic hydrocarbons are volatile toxicants, and thus may be lost during transportation and the various steps of the analytic process, possibly leading to false negatives. In such cases, on-site monitoring by microbial biosensors could be a useful alternative to chemical analysis [38]. Diaz and Prieto [6] scrutinized the various bacterial regulator-promoter pairs capable of biodegrading aromatic pollutants, and gained insight into the molecular mechanisms through which regulatory proteins sense a given signal and activate transcription from their cognate promoters. Several microbial biosensors have been successfully developed to monitor aromatic hydrocarbons [18,29-32,40,49-50] and biodegradation of BTEX (benzene, toluene, ethylbenzene, xylene) compounds-impacted soils [4,7,33]. Paitan et al. [29] constructed whole cell electrochemical biosensors by using the *lacZ* and *phoA* genes to detect different aromatic compounds. These biosensors could be potentially used for on-line and *in situ* monitoring. Bioavailability of BTEX compounds [40,50] and naphthalene [49] have been monitored by using GFP- or horseradish peroxidase-based and filter-immobilized microbial biosensors. The detection limit was 10-50 μM for BTEX [50]. Our own group also developed

microbial biosensors for detection of BTEX, naphthalene, and phenolics [18,30-32,37-38]. We demonstrated on-site detection of phenolic compounds in soil and wastewater by a color change with the naked eye [38]. For this biosensor, a plasmid was constructed by fusing the *β-gal* gene with the *capR* promoter [32], which is activated in the presence of phenolics. The estimated concentration of phenolic compounds (detection range at 0.1 μM-10 mM) was similar to the values obtained by chemical analysis. The use of intact whole cells might be responsible for the excellent response at much higher concentrations of phenol (at 10 mM). Furthermore, mutagenesis of the effector binding sites of regulators may increase their sensitivities and specificities to toxic aromatic compounds [12,30,37].

Heavy metals

Several microbial biosensors have been constructed to detect heavy metals by fusing metal responding regulatory genes (e.g., *merR, arsR*) with various reporter genes: biosensors for mercury [16,35], arsenite [11,41,45], cadmium [3], chromate [34], and nickel [44]. Cadmium biosensors also responded to lead and antimony. Biran et al. [3] reported the novel use of a cadmium biosensor for *in situ* and on-line monitoring of water, sea water and soil samples. They used a cadmium-responsive promoter fused to a promoterless *lacZ* gene, and monitored the results with an electrochemical assay of β-galactosidase activity. The system was able to detect cadmium at concentrations as low as 25 nM in water and 5 μM in untreated soil samples under anaerobic conditions. Stocker et al. [41] constructed colorimetric qualitative paper strips using β-galactosidase as the reporter which were found to produce a visible blue color at arsenite concentrations above 8 μg/l. Fujimoto et al. [11] constructed an arsenite biosensor using the *crtA* gene as the reporter and photosynthetic bacteria as the host strain. The color change could be detected with the naked eye at 5 μg/l arsenite in liquid culture. The time required for color development (12~24 hr) was too long for this system to be of practical on-site use [11], but future developments may broaden its applicability. Another example that has received enormous attention is the contamination of groundwater with inorganic arsenic species in Vietnam. The application of luminescent arsenic biosensor bacteria [45] to samples from nearly 200 groundwater wells from the Red River and Mekong River deltas in Vietnam resulted in more than 90% accurate measurements, suggesting that it is a quite reliable assessment. This is a successful example of using genetically modified biosensors outside of laboratories.

Limitations and challenges

Several limitations of microbial biosensors could be due at least in part to the inherent limitations of microbial biosensors. Compared with instrumental analysis, microbial biosensors show lower sensitivity [46], poor specificity [3], and delay responses due to the time required for reporter gene expression. Continuous researches to resolve such limitations of microbial biosensors have been investigated by exploiting more sensitive promoters [26], refining host strain [1], designing strains to produce modifying regulatory proteins and enzymes [12,27,30,37], surface expressed or periplasmic binding proteins [21,24], and controlling bacterial physiology [22]. Indeed, the surface expressed proteins can directly react with substrates without the entry of substrates into cells, making microbial biosensors faster and highly sensitive [21,24-25]. Thus, it is a challenging and promising task to screen the natural variety for microbial biosensor developments and to extend the natural limits in terms of target range and sensitivity.

Another limitation of microbial biosensors is difficulties in maintaining the survival and activity of the cells in complex environments, due to lack of nutrients, inhibitory compounds and so on. It has been known that microorganisms show different survival and activity under various physiological conditions. Thus, their survival and activity of cells under harsh environment will be controlled by selecting proper microorganism and controlling cell's physiology [20].

Final limitation might be public perception of genetically modified microbial biosensors being released into nature. In reality the risk of an adverse consequence is very low; most biosensor cells are immobilized on matrices or suspended in a compartment, and environmental samples are applied to the biosensor systems. In addition, the benefits can far outweigh the possible risks. The arsenic biosensor used for large screening campaigns is an excellent example, as this simple, sensitive, and cheap sensor kit helped prevent arsenite-associated diseases in Asia [45]. Standardization and legislation in most countries for microbial biosensors could diminish some misconceptions about microbial biosensors.

Future trends

Future trends in biosensor research are likely to focus on miniaturized, high-throughput, wireless/mobile, and automated devices. This growing tendency might require a

group of emerging techniques (e.g., nanotechnology, genetic engineering, microelectronics, and etc.) and substantial development and optimization of refined instruments [43]. Examples of future trends could be found in several microbial array chips [23,42], single-cell biosensors [43]. Advanced new platforms, as shown recently in the development of modified silk derived carbon fiber mat [5] and chitosan matrix [28], might be developed for immobilization of electrochemical microbial biosensor. The development of multi-functional biosensor arrays composed of highly miniaturized signal tranducer elements enables the real-time parallel monitoring of multiple species and accesses to microenvironment which can not be easily accessed by chemical analysis [15]. The use of miniaturized and high-throughput biosensors has benefits not only to the environment but also to economy by reducing time, sample/ waste volume, and other reagents required.

Conclusions

The whole-cell microbial biosensors described herein have been used for *in situ* monitoring various kinds of environmental pollutants. However, there are several disadvantages that must be resolved: the need for sustainable cell viability and activity even under harsh environmental conditions, decreased response times, increased sensitivity, and improved selectivity. These critical advances might allow the growing biosensor industry to begin meeting the sizeable market demand for cheap, sensitive, selective and fast biosensors. At the same time, we need to reassure the public that it is safe to use genetically modified microbial biosensors. Recently, whole-cell microbial biosensor cells have been incorporated onto chips, fiber optics and other platforms of high-throughput arrays. Thus the future of microbial whole-cell biosensors will be advanced as more miniaturized, automated and ubiquitous devices for high-throughput monitoring of a large number of environmental variables.

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초록 : 특이 환경오염물질 검출을 위한 미생물 세포 바이오센서의 활용

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미생물 세포 바이오센서는 환경오염물질의 모니터링을 위한 좋은 분석도구가 될 수 있다. 이는 리포터유전자들(예로, *lux, gfp* or *lacZ*)을 방향족 화합물이나 중금속과 같은 오염물질에 반응하는 유도 조절유전자와 결합하여 만든다. 이러한 유전자 재조합기술을 이용하여 많은 종류의 미생물 바이오센서가 개발되었으며 환경, 의학, 식품, 농업, 및 방위 등 다양한 분야에서 활용되고 있다. 또한 바이오센서의 민감도와 검출범위는 조절유전자의 변형을 통해 증가시킬 수 있다. 최근에는 미생물 바이오센서 세포를 고효율 검색용 세포 에레이의 칩, 광섬유 등에 고착하여 활용하고 있다. 본 논문은 특이 오염물질의 검출을 위한 유전자 재조합으로 만든 미생물 세포 바이오센서의 현황과 미래에 대해 고찰한다.