Engineered Microbial Co-culture Systems for Biosensing: Design and Communication Mechanisms

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Abstract. Microbial co-culture biosensors achieve the sensing, transmission and output of target signals through the functional division and synergistic effects of different microorganisms. Systematically review the functional Design, intercellular communication mechanism and sensing process of microbial co-culture biosensors; focus on the synergistic pattern of different strains in target recognition, signal transduction and reporter output; and summarize their application progress in environmental monitoring, biomedicine, food safety and bioprocess monitoring. Based on the above research, co-culture systems can achieve multi-functional synergy through inter-strain communication and metabolic cooperation; thus, the metabolic burden of a single microorganism performing multiple functions is reduced, and the functional flexibility and complex signal processing ability of the sensing system are enhanced. At the same time, other reasons for varying performance in co-culture biosensors include differences in microbial communities, substrate compatibility, metabolic competition, environmental conditions, and differences between microbial abundance and reporter activity. Some problems still exist in the present studies, such as a lack of system stability, difficulty in accurately regulating the proportion of microbial communities, complex population dynamics, and limited repeatability and accuracy of detection results. Future research will improve the accuracy of control over microbial community dynamics and intercellular interactions, as well as signal-output mechanisms, to enhance the sensitivity and quantitative detection capability of co-culture biosensors.

Keywords: Co-culture System, Biosensor, Communication Mechanism.

1. Introduction

A biosensor is a device that employs a sensing element based on life. It is a transducer that converts biological changes into another form that can be easily observed by humans, such as optical, thermal, electrical or electrochemical signals [1]. It will help people live a better life. Biosensing technology has good prospects and a wide range of applications. Environmental Monitoring, Healthcare and Food Safety are examples.

A Microbial Biosensor is a kind of biosensor. Living cells are used to detect biological signals, and then these signals are converted into simple visual signals. Strain engineering of some sensors has been used to quickly and accurately detect biochemical signals. All the required functions of target recognition, signal processing, signal transduction, signal generation and reporting have been combined in a single cell, thus creating problems. An increase in the number of gene functions for a strain will also raise the complexity of the system and metabolic demand. It is mainly because of the high engineering demands and the narrow environmental tolerance of the microorganisms involved. The above reasons limit the application range of whole-cell bioelectronic sensors and thus prevent their widespread use. Whole-cell biosensors still have defects in sensitivity, selectivity, response speed, stability and signal interpretation [2]. Microbial co-culture systems that contain two or more microbial populations offer a potential solution to have different microbial populations carry out different functions and communicate with each other; thus, the co-culture system can achieve more complex tasks through the cooperation of various strains.

This paper will introduce microbial co-culture-based biosensors. The general ideas of function and intercellular communication in co-culture biosensors are introduced, and their applications in environmental monitoring, biomedical sensing, food safety and bioprocess monitoring are listed. Finally, the main problems in the field and future prospects will be introduced.

2. Co-culture System

2.1. Function of Co-culture

Interaction in a co-culture system can achieve collective sensing and coordination that single microbial communities cannot. Many relatively complex activities take place in a single cell, such as numerous synthetic enzymes and catalytic reactions, and thus impose a considerable burden on that cell. Divide the different functions among the various strains to reduce the metabolic load on the cell. At the same time, it also avoids the interference caused by different cooperative paths, and the different ring cell environments of multiple strains meet the requirements of different functions better to improve work performance and efficiency [3]. Different microbial communities can communicate through diffusible signals, such as quorum-sensing molecules and metabolic products. Alnahhas et al. designed a two-strain E. coli consortium that can sense. Communication and Quorum Sensing (QS) sense the relative abundance of the two populations. By inhibiting the signal synthesis and fluorescent reporter gene expression of another strain with the QS signals produced by the majority type of strains in the system, the communication mode enabled the system to sense the relative abundance of the populations and determine which strain had a larger population. This newly designed system has the ability to determine which population is in the majority [4]. This indicates that the different microbial populations in the co-culture system can sense each other to determine the ratio of various strains in the system, and then produce a response based on this population ratio; this is not possible for a single strain. Therefore, with the development of technology, co-development technology will have an expanding range of applications.

2.2. Intercellular Communication and Signal Processing

Microorganisms are able to communicate in nature. It is required to have good stability and dynamic properties in the co-culture system. A co-culture system can have different microorganisms perform different functions by communicating between them. Li and others have designed an e-COSENS (electroactive co-culture sensing) system. This is a plug-and-play bioelectronic sensor system composed of two parts: a "sender" bacterium that has been genetically modified to respond to specific analytes and produce the electron mediator 1,4-dihydroxy-2-naphthoic acid (DHNA), and a "receiver" bacterium that detects and utilises DHNA to generate an electrical signal via a DHNA-mediated extracellular electron transfer (EET) pathway. DHNA is a link between the two microbial communities that serves as an electron mediator [5]. This co-culture system divides the labour and assigns different functions to the various cell populations to reduce the difficulty of building single-function strains. The sender in this system is responsible for analysing the analyte and producing DHNA; the receiver is responsible for converting the DHNA signal into an electrical output. A single microorganism is no longer needed to perform recognition of the analyte, signal processing and electrical output. Instead, this system uses DHNA to transmit information between two populations with different functions and has reduced the complex engineering strategy of strain design; modular co-cultures can be employed to build multiple functional sensing platforms.

2.3. Design Considerations

Construction of this co-culture system requires a large amount of effort and is therefore a significant problem; however, such a system has good prospects for addressing complex detection problems. Good co-culture design needs to coordinate the composition of cell types to ensure the metabolism of the strain's cells and the functions of the co-culture system. Chen and others have shown this idea in an experiment with an E. coli-E. coli co-culture for indigo biosynthesis. The two parts of the synthetic pathway in this experiment are: the provision of tryptophan and the conversion of tryptophan into indigo. The ratio of the two strains is a required Design parameter, and different ratios of the two strains will result in a significant difference in indigo production. Under glucose conditions, the optimal ratio is 4:1 and the yield is 7.7 mg/L; under glycerol conditions, the optimal

ratio is 9:1 and the yield is 15.9 mg/L [6]. Therefore, the ratio of strains and substrate compatibility need to be considered in the design of the co-culture system.

When designing factors for biological co-culture, another factor that needs to be considered is distinguishing between strain abundance and reporter activity. Avery J.C. Noonan et al. use CRAGE (chassis-independent recombinase-assisted genome engineering) technology to express RFP into the chromosome of the biosensor strain and use GFP to serve as an inducible biosensor. OD600 can reflect the total number of cells. During the deceleration phase of the microbial growth curve, a nearly linear increase relation intensity between RFP fluorescence and OD600 value can be observed; this allows the OD600 value of a specific strain to be represented by the fluorescence signal, thus RFP was used to show the quantity of the sensor bacteria. GFP fluorescence increased when the biosensor detected the target signal, representing sensing activity. The change in population abundance from changes in biosensor activity can be determined by those two signals. Also, if the proportion of sensor strains is too low, the RFP signal will be close to the background, and about 25% can be considered a practical lower limit [7]. Therefore, designing a co-culture system needs to consider the proportion of strains; both too low and too high ratios of the strains can affect the sensitivity of the detection.

Although microbial co-culture technology has already demonstrated its great potential, there is still significant room for advancement in the technology.

3. Applications

3.1. Environmental

Microbial co-cultures have a large potential for testing environmental quality. Yudina et al. developed a co-culture system that can detect biological oxygen demand (BOD). The concentration of this substance can determine whether the water body has been polluted. A three-member co-culture consisting of Pichia angusta, Arxula adeninivorans, and Debaryomyces hansenii shows the widest substrate specificity, including oxidizing monosaccharides, disaccharides, alcohols, carboxylic acids, and amino acids, compared with a single-strain D. hansenii biosensor. When this three-member co-culture was applied to actual complex environments, such as river water, bog water, and municipal wastewater, it demonstrated excellent detection sensitivity [8]. This metabolic complementarity within a designed co-culture system improves the range of environmental biosensors and supports more comprehensive water-quality detection.

3.2. Biomedical

Microbial co-culture biosensors have also been explored for biomedical applications, particularly the detection of disease states. Huang et al. developed three different bacterial system to used to detect Heme and Lactate. The first one uses an IFFL (incoherent type-1 feedforward loop strain to create a signal. The second one regulates the CHL (acyl-homoserine lactone 3OHC14:1-HSL) signal directly. The third depends on the outside to offer the signal. IFFL shows better performance on detecting Heme and Lactate. The IFFL Network System includes three strains; the first two are used to detect the biomarkers, and the third strain is used to show the signal at a low level by detecting Heme and lactate in patients' feces. Diseases of the symptomatic enteropathy, colitis and rectal cancer may occur. A co-culture system was tested using humanised mouse fecal samples, and it was found that both Heme and Lactate could be detected in this complex biological matrix [9]. Zhou and others have constructed a liver injury-on-a-chip microfluidic co-culture system that combines hepatocytes and stellate cells to simulate signalling under alcohol-induced liver injury. TGF-β (Transforming Growth Factor-beta) will be released by injured hepatocytes, and this can stimulate stellate cells to release more TGF-β. Aptamer-modified electrodes are used to detect TGF-β, and a two-cell communication model in the liver is constructed [10]. Thus, the microfluidic co-culture can simulate dependent intercellular signals and detect molecular responses; therefore, it is proposed that a co-culture system can be used to model tissue injury and disease. Nurdiani and others developed an ethanol biosensor in this system using Saccharomyces cerevisiae and Bacillus megaterium. The system has extended

the measurable range of ethanol concentration to 0.02–6.0% and has a fast response time of 11 s [11]. Microbial consortia can enhance the performance of biosensors by combining multiple functions of microorganisms and offer more advantages than a single strain.

4. Challenges and Optimization

Co-culture technology has already represented huge advantages and potential in the field of biosensing; however, maintaining stable relative abundance among different microorganisms remains a significant challenge, as the growth and metabolic rates of different strains, as well as the proportion of strains, can have a significant impact on sensing performance and product formation. Population dynamics can affect the fluorescence intensity of the reporter, which means changes in the sensing signal don't always reflect changes in analyte detection. So it's also really important to monitor the population dynamics of specific strains [6,7]. In addition, the e-COSENS system shows that reliable communication is required and needs to be established between strains. Future directions could use genetic circuits to regulate population ratios, metabolic pathways, and communication signals.[5]. So, the optimization should not only focus on strain sensitivity but also needs to pay more attention to the interactions, metabolism, and communication between different strains in co-culture to increase system stability.

5. Conclusion

This review discusses the functions, intercellular communication mechanisms, design considerations, and applications of microbial co-culture systems. Co-culture systems assign different sensing tasks to strains, achieving collective sensing of microbial communities by communication and metabolic interactions and coordination between the strains. Different microorganisms can coordinate their functions and convert biological information into detectable signals; this gives a method to overcome the limitations of single-microorganism biosensors. The main advantage of microbial co-culture systems is using the interactions and functional differences between them to build a more flexible sensing system. Other reasons for the variations in performance of co-culture systems include changes in the ratio of microbial populations, substrate incompatibility, and differences in population abundance from reported activity. Changes in population, metabolic competition and the environment will all affect the performance of the sensing system. In the future, research will be conducted to enhance the stability of microbial co-culture systems, optimise the ratio and interaction of strains, and improve the sensitivity and accuracy of detection. Many applications of microbial co-culture biosensors are available.

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