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THE DETECTION OF BACTERIAL VIABILITY - A PATH FOR SENSING DEVICES

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Introduction

Microorganisms are extensively used for the benefit of socio-ecological system with a wide range of applications in medicine (e.g. development of many important pharmaceuticals), industry (e.g. food production) and environmental sciences (e.g. bioremediation, biocontrol of insects pests, biofertilisers). The advantage of using biological models is their self-replicating, self-repair, self-sustainable and biosignal-responsive abilities [1]. Since bacteria viability represents the cell ability to complete all the functions for its survival in a specific environment, the cell should have an undamaged cell membrane, metabolic activity and the capability to replicate and growth. Therefore, their enzymatic system acts as a key tool for many biological researches. In the last decades, biological system represented the basis on which new sensing applications were developed. Among all those approaches the lab on a chip have been defined as an extremely interesting approach. More recently, 3D printing and microfluidic systems could be an advantageous rapid, cost-effective sensing tool for pathogen detection [2]. The aim of this study was to point out a brief review of specific detection methods for bacterial viability.

Materials and methods

Numerous detection methods were described to highlight bacterial cells viability, among which traditional culture-based and alternative culture-independent methods. Traditional culture-based methods (colony count, liquid culture, plate count) involve several steps such as: a) a pre-enrichment / inoculation on non-specific medium; b) enrichment / inoculation in selective media to allow the growth of target bacterial strains; c) enumeration and biochemical diagnosis. Fluorescent dyes-based, cellular and metabolic properties-based as well as nucleic acid-based were considered alternative culture-independent methods [3].

Results and conclusions

Traditional culture-based methods proved to be the gold standard in terms of bacterial viability assessment. The method is cost-effective, although it can be tedious, labour-intensive and time-consuming, since requires overnight incubation and about a week to assess the viability of mesophilic heterotrophic bacteria.

Some alternative methods enable viability to be assessed by staining techniques using microscopy or flow cytometry with the help of fluorescent dyes, among which

propidium iodide, ethidium monoazide, propidium monoazide, tetrazolium salts, cyanine dyes, calcein, fluorescein diacetate, rhodamine dyes and resazurin. The cell viability can be detected by cell enzyme activity, cell energy and cell membrane integrity. The cell membrane integrity works as a biomarker for viability, thus it can be emphasized by intercalating dyes which can either impregnate intact and damaged cells, or dyes which can only penetrate damaged cells.

Nucleic acid-based assays are rapid, sensitive, compared with the conventional methods, but a drawback of the protocol is that do not differentiate DNA from live/death cells as well as it may overrate viable cell numbers due to amplification of DNA from dead cells and extracellular DNA within samples. To overcome this limitation, biological dyes have been widely used for bacterial viability detection, especially for foodborne pathogens.

Moreover, considering cellular and metabolic properties, bacterial viability was positively correlated with adenosine triphosphate (ATP) concentration. Thus, ATP as the energy of all living organisms, was utilized as a biomarker for microbial contamination in different environments. In addition, the presence of oxidoreductase and dehydrogenase enzymes in viable cells, could reduce tetrazolium salt and resazurin, indicating the respiratory activity of viable cells [3].

Overall, all living organisms have developed particular adapting mechanisms to contest the effect of pressure factors by spontaneous mutations and activation of various defense mechanisms. Although organisms respond differently to stressors according to their species, their defense mechanisms could be very similar from prokaryotes to eukaryotes. Biological models, especially the bacterial ones, are very useful tools to observe the toxic effect of different pollutants. Although numerous detection methods have been described for bacterial cell viability, the feasibility and the results interpretation, must be considered when applying the techniques.

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