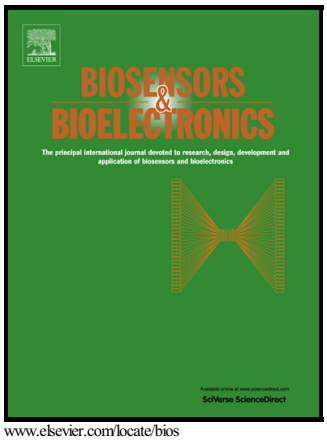

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Title

An unconventional approach to impedance microbiology: detection of culture media conductivity variations due to bacteriophage generated lyses of host bacteria

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Abstract

A novel and unconventional approach to impedance microbiology has been under investigation. In our approach, solution conductivity variations are generated from bacteriophage lyses of infected host cells and the consequent release of conductive endoplasmic material. To sensitively detect the lysis, low conductive growth media have been developed. A microchip has been fabricated to perform the analysis. The microchip is made of two bare gold electrodes and PDMS microchamber of 36 nL volume. *Escherichia coli* and selective phages T4 have been used as case study. Proof-of-principle experiments are here presented and discussed. The method was characterised in a wide range between 10^4 and 10^8 CFU/mL, where linear relation was found between conductivity variation and cell concentration in a $\log_{10}$ *vs.* $\log_{10}$ plot. The method is suited to integration with sample preparation based on phage-functionalised magnetic beads. It has a potential detection limit below 1 CFU/chamber and a total assay time of less than 1 hour.

Keywords

Impedance microbiology, bacteriophage, pathogen detection, microsystem, low conductivity broth, system integration.

1. Introduction

The detection of foodborne pathogens is a critical and costly practice of the agro-food industry that is necessary in quality control and quality assurance protocols. Since the introduction of Hazard Critical Control Points (EC Health & Consumer Protection General Directorate, 2005) a high testing rate has been generated (Alocilja and Radke, 2003) and the industry has developed a strong demand for cheap, sensitive and selective methods and technologies that can provide quick results on-site (Mortari and Lorenzelli, 2014). Furthermore, hygiene regulations for foodstuffs (EC, 2005) require selective and sensitive analysis of 10-25 gr samples (ISO, 2008) and the detection of colony forming units; which implies that microbiological methods must be able to differentiate between viable and dead cells.

Impedance Microbiology (IM) is a certified action method for the detection of viable cells (AOAC, 1995) that has been widely used by the industry. In the simplest implementation, 2 electrodes are exposed to the sample to measure solution conductivity. In particular, IM detects variations of solution conductivity due to bacterial catabolism of non-conductive substrates, such as sugars, into conductive catabolites, such as organic acids. It employs bare electrodes, that are robust and reusable, and conventional electrochemical cells of high volumes, in the order tens of mLs. Since IM relies on bacterial metabolic time frames, it is time consuming, often taking over one day to produce results. It is also characterised by low sensitivity, which is mainly due to broth culture salinity and its off-setting effect, but also due to the dilution that the produced conducting molecules experience in a high volume electrochemical cell (Gómez et al., 2002). Furthermore, selectivity is still an unsolved issue as IM must rely on selective broths. Overall, IM has not experienced major advancements since its first development. However, Gomez *et al*. have proved that concentrating cells in a micro-electrochemical cell can decrease detection time and improve the method sensitivity by decreasing the dilution of bacteria catabolites (Gómez-sjöberg et al., 2005).–Phages have recently been suggested as a promising recognition element (Goodridge and Griffiths, 2002). They are locked in an evolutionary arms race with bacteria and are highly specific to their target host. Their replication cycle begins with the specific recognition and binding of their tail fibre proteins to the bacterial host. After phage infection via the viral DNA injection, amplification inside the host cells takes place. Finally, cell wall-hydrolysing enzymes (endolysins) are produced to lyse the host cell wall and release progeny phages along with endoplasmic material (Young and Blasi, 1995). Fit phages generate cell lysis in 30 minutes. Phages are easy to isolate (Tlili et al., 2013), cheap to produce and present a long shelf life (Goodridge and Griffiths, 2002). They can also be directionally immobilised on planar surface (Gervais et al., 2007) and on beads (Mortari et al., 2014), maintaining their recognition and infection properties. Most importantly, bacteriophages (phages) require viable cells for their amplification and reproduction.