Phage-based biosensors for pathogen detection

Researchers at TEAGASC and Queen's University Belfast are developing a bacteriophage-based biosensor for the detection of food-borne pathogens.

The threat to our socioeconomic balance and healthcare system from bacterial contamination of food has become a global burden. The World Health Organisation estimates that 600 million people in the world (approximately one in 10) fall ill following consumption of contaminated food every year, and of these, 420,000 die. With the changing of food preparation and food styles over recent years, where more processed and ready-to-eat foods are available, cooking processes have altered significantly, and thus the risk of consuming food products containing pathogenic bacteria has increased. Food-borne pathogens such as *Campylobacter*, *E.coli*, *Salmonella* and *Listeria monocytogenes* are responsible for numerous outbreaks of disease and the recall of food products worldwide. The gold standard for detection of food-borne bacteria is still conventional culture-based diagnostic protocols, due to their sensitivity and the benefit of yielding colonies that can be subjected to diagnostic tests. However, these methods are time consuming and labour intensive. The development of simple-to-use diagnostics for end product or process line testing is essential to ensure that the integrity of the food chain is maintained.

An introduction to bacteriophage biology

Bacteriophages have existed alongside bacteria for billions of years and have evolved systems that may be exploited for our benefit, such as the use of bacteriophages to detect food-borne pathogens. A bacteriophage (phage) is a virus that infects bacterial cells. Phages are the most abundant biological entity found naturally, with an estimated number of 10^{31} phages present in the biosphere (Casey *et al.*, 2017). In order to survive and replicate, phages hijack their host bacteria's metabolism. Phages are acutely host specific, meaning that they only infect particular bacteria through recognising certain receptors present on the host bacteria's cell surface. Phages can use a number of cell surface moieties as receptors, such as glycolipids, integral membrane proteins and flagellar proteins used by *Salmonella* phages, and wall teichoic acid (WTA), lipoteichoic acid (LTA), polysaccharides, S-layer proteins and PIP protein used by *Lactococcus* phages (**Figure 1**). The recognition of the bacterial host by the phage occurs through receptor-binding proteins, which are generally located in the tail of the phage. Once phages encounter their host bacteria, they attach to the surface of the cell via these receptors in their tail proteins and begin to degrade the cell wall. Following this, phages inject their genetic material through the tail and into the host cell. Depending on the phage life cycle, this intracellular phage will eventually result in the lysis of the host cell and release of newly formed phages into the environment.

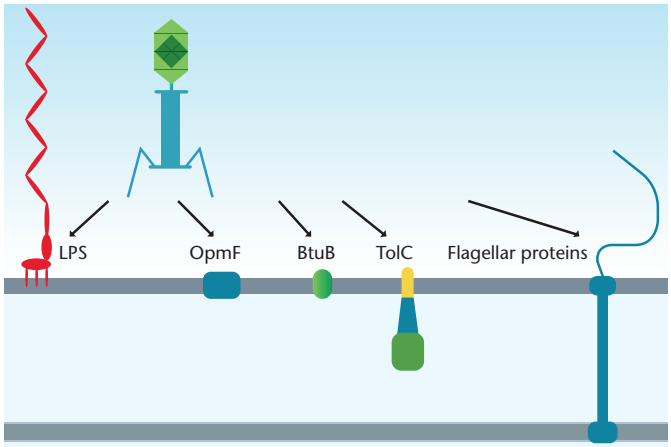

FIGURE 1: Potential cell surface moieties that phages can use as receptors.

Bacteriophage-based biosensor for pathogen detection

Recent years have seen the development of biosensors as novel methods of pathogen detection, which are designed to overcome the many limitations of conventional detection platforms. Biosensors are selective, sensitive, cost-effective, rapid and, more recently, portable devices. The platforms that have leveraged phage-based probes have used primarily intact bacteriophage particles as the recognition element, and have successfully detected pathogens in various food matrices (**Figure 2**). However, significant disadvantages are associated with such biosensors,

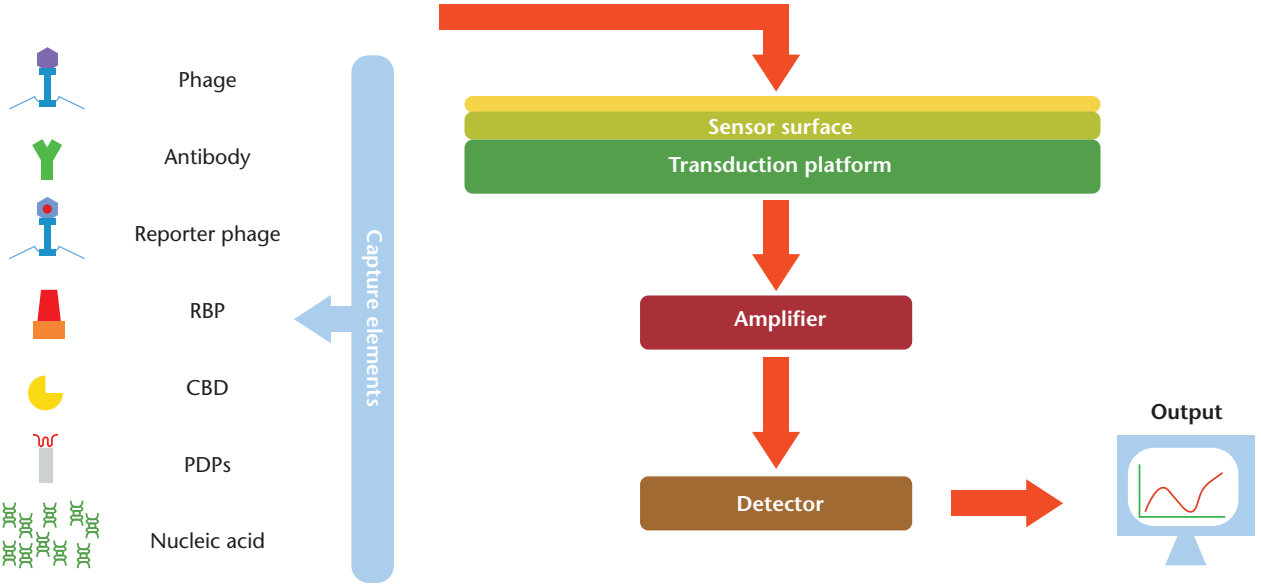

FIGURE 2: The components of a biosensor showing various biorecognition elements that may be attached to the sensor surface.

including the large size of the intact phage particles, making it difficult to integrate them into certain platforms, and the potential of infection and lysis of cells at certain time periods, which will result in a decrease of signal. To avoid these difficulties, research is now focusing on the use of phage-derived molecules as the biorecognition element in the biosensor.

Rapid diagnostic test for *L. monocytogenes*

Our research aims to develop a rapid diagnostic test for *Listeria monocytogenes*, a food poisoning bacterium that is particularly associated with ready-to-eat foods. *Listeria*-related economic losses now run into the billions per year worldwide, following many high-profile epidemics in recent years. Two independently successful technologies will be combined: the rapid portable nature of biosensors based on planar waveguide technology; and, the extraordinary specificity of bacteriophage-host interactions for *L. monocytogenes* detection. To date, novel phages against *L. monocytogenes* strains of the 4b and 4e serotypes have been isolated from mushroom compost. The genome sequences of two of these phages, phage vB_Lmo_188 and phage vB_Lmo_293, were elucidated and identified as belonging to a recently defined group of *Listeria* bacteriophages known as orthocluster IV. Their specificity for *L. monocytogenes* strains of serotypes 4b and 4e was determined to be likely to be due to a small cluster of putative tail fiber genes, which were thought to function in bacterial-host recognition. Through a series of mutational analysis experiments, the receptor-binding proteins in the phage tails were identified (Casey *et al.*, 2015). Recombinant production of these proteins is ongoing, with biosensor integration of these phage-derived affinity proteins planned as the next stage. By harnessing phage receptor-binding proteins, a rapid real-time test can be developed for the online monitoring of key serotypes of *L. monocytogenes* food matrices with improved sensitivity over current methods.

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