Prevention of bacterial foodborne disease using nanobiotechnology

Abstract: Foodborne disease is an important source of expense, morbidity, and mortality for society. Detection and control constitute significant components of the overall management of foodborne bacterial pathogens, and this review focuses on the use of nanosized biological entities and molecules to achieve these goals. There is an emphasis on the use of organisms called bacteriophages (phages: viruses that infect bacteria), which are increasingly being used in pathogen detection and biocontrol applications. Detection of pathogens in foods by conventional techniques is time-consuming and expensive, although it can also be sensitive and accurate. Nanobiotechnology is being used to decrease detection times and cost through the development of biosensors, exploiting specific cell-recognition properties of antibodies and phage proteins. Although sensitivity per test can be excellent (eg, the detection of one cell), the very small volumes tested mean that sensitivity per sample is less compelling. An ideal detection method needs to be inexpensive, sensitive, and accurate, but no approach yet achieves all three. For nanobiotechnology to displace existing methods (culture-based, antibody-based rapid methods, or those that detect amplified nucleic acid) it will need to focus on improving sensitivity. Although manufactured nonbiological nanoparticles have been used to kill bacterial cells, nanosized organisms called phages are increasingly finding favor in food safety applications. Phages are amenable to protein and nucleic acid labeling, and can be very specific, and the typical large “burst size” resulting from phage amplification can be harnessed to produce a rapid increase in signal to facilitate detection. There are now several commercially available phages for pathogen control, and many reports in the literature demonstrate efficacy against a number of foodborne pathogens on diverse foods. As a method for control of pathogens, nanobiotechnology is therefore flourishing.

Keywords: bacteriophage, pathogen control, detection, food safety

Introduction

The impact of foodborne disease is a significant economic and clinical issue, despite recent advances in food safety. In clinical terms, the number of cases per year in the US has been estimated at 9.4 million, with 55,961 hospitalizations and 1,351 deaths. The annual consequent cost of foodborne illness is $51 billion to $77.7 billion, depending on the model used. Reducing the occurrence of foodborne illness through the use of rapid, cost-effective detection procedures and new ways to control pathogens is the focus of much current research.

Nanobiotechnology can be defined as the intersection of nanotechnology and biotechnology. This concise review article considers nanobiotechnological detection of, and interventions to control, bacterial foodborne pathogens. We have chosen to focus...
on nanostructures that either incorporate biological elements or are entirely biological in origin, and particularly on applications of bacteriophages (phages). We have excluded nonbiological materials such as silver nanoparticles that are used to kill pathogens and have been reviewed elsewhere. We also acknowledge that many examples and applications have been omitted from this review because of space limitations, and readers are referred to the many excellent recent reviews that are available.

Detection of bacterial pathogens

In an ideal world it would be desirable to detect even a single pathogen present on/in a food sample. Current “traditional” culture-based methods tend to use an enrichment step in which low numbers of target cells are encouraged to multiply to detectable numbers. Enrichment also ensures that any bacteria detected are alive. This approach allows a theoretical detection limit of 0.04 cell/g (ie, one cell in 25 g, a normal analytical weight). However, enrichment typically adds 1–2 days to the total analysis time. When enrichment is not used, it is usual for a food sample to be diluted one in ten prior to analysis, thus also reducing the cell concentration available for detection tenfold (eg, ten cells/g of food become one cell/mL in the diluent).

It is imperative to have methods that are able to detect low concentrations of pathogens, as it can sometimes take only a few cells in a food to cause disease (Table 1). Another factor to be considered is the accuracy of testing methods, as judged by the proportion of false positive and false negative results. For a method to be adopted widely it is usual for it to receive official validation. An example of this is a “performance-tested method” by AOAC Research Institute (http://goo.gl/413U6y). Receiving such validation endorses all the performance parameters of the test.

For nanobiotechnology to offer improvements over traditional methods it will need to allow the detection of pathogens faster, less expensively, and/or more accurately than existing methods. Ideally, it should be sufficiently sensitive to eliminate the need for enrichment, though probably requiring enhanced sample preparation methods. This need has been addressed in some papers. For example, pathogens have been detected at 20–50 colony-forming units (CFU)/mL within 2 hours without enrichment by prior concentration of bacteria in food rinses using immunomagnetic separation (IMS).

Assessment of methods cited in literature

Pathogen detection methods using nanobiotechnology that are cited in the literature need to be examined from the perspective of what they bring to a crowded rapid methods diagnostics market. For example, one study reports the detection of three pathogens at 400–800 cells/mL. For the suggested application in testing milk, this assay is too insensitive to be used without enrichment, so the associated improved test speed is largely negated.

Currently, there are rapid pathogen detection kits available that use antibodies to capture pathogens, and many of these produce a qualitative visual signal (eg, lateral flow devices) or a colored enzymatic reaction-derived product that can be measured by spectrophotometry (eg, enzyme-linked immunosorbent assay). In general, low-sensitivity methods are applicable only to detecting pathogens in enrichments. There are also many methods available that are based on the detection of amplified segments of nucleic acid (polymerase chain reaction [PCR]), which can be sensitive, rapid, and specific.

Quantum dots, nanobeads, and nanorods

There has been considerable research into the use of nanosized quantum dots (QDs) to detect foodborne pathogens. These semiconductor nanocrystals have been used as fluorophores for cellular imaging, as they possess superior properties to conventional fluorophores. QDs have been coupled with specific antibodies to facilitate detection of organisms, including the parasites Cryptosporidium parvum and Giardia lamblia and the bacteria Mycobacterium bovis, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella, and Shigella. Indeed, a modified cellphone has been used as a detection system for E. coli. Toxins, including shiga-like toxin, cholera toxin, and ricin, have been detected using a QD protocol.

Table 1 Examples of low concentrations of foodborne pathogens in foods associated with disease outbreaks

| Pathogen          | Food                  | Concentration | Reference |
|-------------------|-----------------------|---------------|-----------|
| Campylobacter     | Raw beef liver        | 3.6 MPN/g     | 4         |
| Escherichia coli  | Raw beef liver        | 0.04–0.18 CFU/g | 4         |
| O157:H7           | Frozen burger patties| 1.45 MPN/g    | 4         |
| Listeria monocytogenes | Hard cheese     | Approximately 20 CFU/g | 5         |
| Salmonella        | Frankfurters          | <0.03 MPN/g   | 6         |
|                   | Paprika-flavoured chips | 0.04–0.45 CFU/g | 7         |
|                   | Flour                 | 0.003–0.02 CFU/g | 8         |
|                   | Tahini products       | <0.03–0.46 MPN/g | 9         |

Note: Both MPN and CFU are loosely equivalent to the number of cells present.

Abbreviations: CFU, colony forming units; MPN, most probable number.
Antibody- and nucleic acid-based biosensors

Biosensors make use of antibodies or phage components (see Phage-based nanobiotechnological detection methods) that have specific recognition elements for pathogenic microbes. When the microbe is captured by the recognition element, a measurable response occurs. In these systems the antibody is attached to a suitable matrix in close proximity to a transducer, which can be optical, electrochemical, piezoelectric, or thermometric and which produces a measurable signal. Although biosensors may offer the prospect of near to real-time detection, they currently lack the sensitivity to be used without an initial enrichment (see Narsaiah et al). Silicon nanorods onto which antibodies were attached have also been used to detect *Salmonella*, although fluorescence microscopy, a low throughput detection method, was used to detect the labeled cells. The limit of detection was not reported.

Phage-based nanobiotechnological detection methods

Phages are naturally occurring nanosized killers of bacteria. The smallest group are icosohedra (diameter 27 nm), but they can be up to 2,400 nm long in other groups. Phages are environmentally common and are the most numerous biological entities on the planet. Their high specificity and lethal effect, and the relative ease of engineering their genomes and structures lend them to nanobiotechnological applications for food safety.

Rapid detection techniques have been developed using the bactericidal and specificity properties of free phage particles. Impedance–conductance methods have been combined with phages for the rapid detection of *E. coli* O157:H7 and *Salmonella*. Selective enrichments are performed and electrical properties of the media monitored for changes resulting from metabolism of substrates by target bacteria. The added phages retard the growth of the target bacterium, resulting in a characteristic change in electrical profile of the medium, so enabling rapid identification. These methods may be prone to false negative results and require careful tuning of conditions.

In a novel alternative technique, the sample containing target bacteria was first inoculated with a low concentration of phages for a short time (2 hours) to enable at least one phage replication cycle (Figure 1). Bacterial “helper cells” known to be susceptible to the phage were then added to the sample to amplify phage numbers during another short 1-hour to 2-hour incubation. The ratio of intact to damaged helper cells was measured by Live/Dead staining, enabling the calculation of the original number of cells present in the sample. The limit of detection of these QD methods is reported to be $10^{-10}$–$10^{-14}$ organisms/mL, depending on the detection method used, although a limit of five to ten organisms/mL has also been claimed. If an enrichment is used, the detection limit can be reduced to ten organisms/mL, but this also increases the test time.

Carboxylated magnetic nanobeads (diameter 180 nm) coated with antibodies have been used to detect *Salmonella typhimurium*, *E. coli* O157:H7, and *L. monocytogenes* in foods by multiplex PCR, but the detection limit, approaching $10^4$ CFU/mL, means that enrichment of the food would be needed for practical application. Silicon nanorods onto which antibodies were attached have also been used to detect *Salmonella*, although fluorescence microscopy, a low throughput detection method, was used to detect the labeled cells. The limit of detection was not reported.
sample. The authors were able to detect 100 CFU/mL of *Pseudomonas aeruginosa* using this method.

The detection of progeny phages following infection of bacteria during enrichment is also an approach for rapid bacterial detection. Such assays have been used in vitro for the detection of *Pseudomonas* and *Salmonella*, with sensitivities of 40 CFU/mL and 600 CFU/mL, respectively, in 4 hours. A commercial assay for the detection of *Mycobacterium avium* subspecies *paratuberculosis* in milk is under development using this technique and is currently able to determine presence/absence within 48 hours (which is rapid for this slow-growing microorganism). Amplification assays have also been combined with mass spectrometry where phage proteins are the target for bacterial identification and detection. This approach has been demonstrated for the detection of *E. coli* and *Salmonella*, and for *S. aureus*, though improvements in the sensitivity would be required for commercial use.

A number of methods have been proposed for the detection of bacteria by phages that involve modification of the phage DNA or capsid (head) protein. The phage genome is relatively amenable to genetic engineering, and this has allowed the insertion of a number of reporter genes, facilitating rapid detection of bacteria. In these systems (summarized in Table 2), the engineered gene is expressed soon after bacterial infection, which enables both specific and rapid detection compared with conventional techniques. The sensitivity of these methods is good (eg, 1 CFU/mL with 6 hours’ enrichment), but a potential limitation is the need for phages with the correct specificity. Rather than detecting the expression of a gene in the target bacterium, an alternative is direct fluorescent DNA staining or phage capsid labeling. These techniques are flexible, in that the fluorescent signal can be detected by flow cytometry, multwell plate readers, or direct microscopy. Goodridge et al described the use of IMS combined with fluorescent staining of a phage to detect 2.2 CFU/g *E. coli* O157:H7 in ground beef following a 6-hour enrichment and 10–100 CFU/mL *E. coli* O157:H7 in raw milk after 10 hours’ enrichment. The capsid of *E. coli* phage T7 has been labeled with QDs by conjugation with biotin, and the approach was able to detect as few as 20 CFU/mL *E. coli* in a water sample within 1 hour, compared with 24 hours with conventional techniques.

### Immobilized phage-based devices

The immobilization of phages onto surfaces for pathogen detection has several advantages over using free particles, particularly by allowing the possibility of incorporating phages into high-throughput or hand-held devices. Phages have been used in a magnetoelectric biosensor to detect *Salmonella* on the surfaces of watermelons, tomatoes, and egg shells and in milk. The phages are coated onto the biosensor chip, and when *Salmonella* cells bind to the phages the mass of the biosensor increases. The change in mass and resultant decrease in resonance were measured when a surface-scanning coil detector was passed within close proximity. This technique raises the possibility of immobilizing sensors in food packaging that could then be checked for the presence of a pathogen by passing a detector across the product. The current limitation is the relative lack of sensitivity (limit of detection 150 CFU/mm²) compared with conventional methods.

Surface plasmon resonance (SPR) is a method that has been harnessed to detect pathogens using gold immobilized phages. SPR is an optical technique that involves measuring small changes in the refractive index at the boundary of two media when illuminated by polarized light. Like magnetoelectric sensors, this technique promises rapid, label-free detection of pathogens, but more work is required to improve sensitivity. Phage-based SPR biosensors have been used in vitro for the detection of *E. coli* K12, *E. coli* O157:H7, and *S. aureus*, with detection limits of ~1,000 CFU/mL in 20 minutes.

Laube et al described a phage-based detection method for *Salmonella* where a phage was coated onto paramagnetic beads. The beads were used to capture and concentrate *Salmonella* from milk samples, with or without enrichment, and the bacteria were detected using peroxidase conjugated to *Salmonella*-specific antibodies. Limits of detection were 19 CFU/mL with no enrichment (assay time 2.5 hours) and 1.4 CFU/mL with brief enrichment (assay time 6 hours), which are comparable with existing methods taking 24 hours.

### Table 2: Reporter genes used in phage detection of foodborne pathogens

| Reporter gene | Target | Reference |
|---------------|--------|-----------|
| lux (luciferase) | *Listeria* spp. | 36 |
| lux | *Escherichia coli* O157:H7 | 37 |
| lux | *Salmonella enterica* | 38 |
| lux | *Staphylococcus aureus* | 39 |
| lux/luxIR (quorum sensing) | *E. coli* O157:H7 | 35 |
| inoW (ice nucleation) | *Salmonella* | 40 |
| celB (β-glycosidase) | *Listeria* spp. | 41 |
| locZ (β-galactosidase) | *E. coli* O157:H7 | 42 |
| gfp (green fluorescent protein) | *E. coli* | 43 |
| ccp (cytochrome c peroxidase) | *E. coli* | 44 |

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One limitation of immobilized phage biosensors is the potential for false negative results arising from the failure to detect injured or viable but not culturable cells. Fernandes et al.\textsuperscript{55} offer a possible solution to this with the use of a lytic phage bound to a magnetoresistive biochip and antibody-coated nanoparticles for detection. Lytic phages are usually not favored for biosensors, as they destroy the cell before it can be detected, but here the authors used physiological conditions enabling cells to survive long enough to be detected. They were able to detect injured cells using the biosensor.

One of the main challenges in the development of nano-biosensors using intact phage particles is to immobilize them at a sufficiently high density. An approach to circumvent this is to use phage-encoded proteins that have high affinity for the bacterial cell wall, either receptor proteins from phage tail fibers or the cell-binding domains (CBDs) from endolysins (for Gram-positive bacteria only). The receptor-binding protein from a Campylobacter phage has been immobilized and used with SPR to detect as few as 100 CFU/mL.\textsuperscript{56} Technology based on this principle has been developed by Hyglos GmbH (Bernried am Starnberger See, Germany) and licensed to bioMérieux (Craponne, France) for use in its VIDAS\textsuperscript{®} detection systems for E. coli O157:H7, Salmonella, and Listeria.

Walcher et al.\textsuperscript{57} immobilized the CBD from a Listeria phage endolysin onto paramagnetic beads and combined this with real-time PCR to develop a rapid method for detecting Listeria. They were able to recover 10–100 CFU/mL Listeria from raw milk spiked with each of five isolates tested. The same group also successfully immobilized a Listeria endolysin CBD on a gold screen-printed electrode for detection by electrochemical impedance spectroscopy, but sensitivity is currently ten- to 100-fold poorer.\textsuperscript{58}

**Control in food production and distribution**

**Phage-based control**

Intuitively, it is clear that testing of food alone cannot be relied upon to assure food safety, and there is a need to inactivate (control) any pathogens that are present before distribution to the consumer. Phage-based pathogen control offers an attractive proposition for control purposes, as phages are naturally present in food, and there is accumulating evidence that they are both safe and effective.

For meat it would be optimal to intervene at the pre-harvest stage, as bacteria are spread to carcasses via direct or indirect cross-contamination from feces on the hide in ruminant animal processing,\textsuperscript{59} from feathers during mechanical plucking of poultry,\textsuperscript{60} and from the digestive tract of seafood during processing.\textsuperscript{61} Supporting this premise, modeling of pre-slaughter intervention strategies, such as the use of phages to reduce shedding of E. coli O157:H7 in cattle feces, had the largest positive impact on carcass contamination.\textsuperscript{62}

Preharvest interventions using phage biocontrol have been trialed for a number of food animals, the most notable being applications for poultry. Bardina et al.\textsuperscript{63} were able to reduce concentrations of Salmonella in chicken ceca by 4.4 log\textsubscript{10} CFU/g in 2 days and up to 2 log\textsubscript{10} CFU/g, compared with controls, at 8 days (end of experiment) following oral administration of phages. In a similar trial, Wong et al.\textsuperscript{64} achieved a 2.9 log\textsubscript{10} CFU/mL reduction in bacterial concentrations after 6 hours, with Salmonella becoming undetectable in the cecum at 24 hours. A novel approach taken by Waseh et al.\textsuperscript{65} was to purify the receptor (tail-spike) protein from a phage and feed it to chickens, attaining a 1–2 log\textsubscript{10} CFU/mL reduction in Salmonella concentration in chicken ceca.

Campylobacter is a significant food safety issue for the poultry industry,\textsuperscript{66} and there have been several promising recent trials using phages to control Campylobacter in chickens. A study performed in three commercial broiler houses by Kittler et al.\textsuperscript{67} showed that one of three experimental groups exhibited a significant reduction in Campylobacter concentration of >3.2 log\textsubscript{10} CFU/g cecal contents at slaughter. However, there was no difference in the other two groups. Carvalho et al.\textsuperscript{68} achieved a 2 log\textsubscript{10} CFU/g reduction in fecal Campylobacter concentration using oral gavage to deliver phages, but this was increased by 0.5 log\textsubscript{10} when the phages were delivered in feed.

Shiga toxigenic E. coli O157:H7 is a potentially fatal foodborne pathogen, and its primary reservoirs are cattle and other ruminants.\textsuperscript{69} Phage-based preharvest interventions for ruminants have been studied extensively with mixed results, and several studies report small or insignificant effects. Nevertheless, a single oral administration of phages reduced E. coli O157:H7 in the gastrointestinal tract of sheep by up to 4 log\textsubscript{10} and a multidose phage protocol reduced fecal shedding of E. coli O157:H7 in cattle by 1 log\textsubscript{10} CFU/g for up to 72 days.\textsuperscript{70}

OmniLytics, Inc. (Sandy, UT, USA) (http://www.omnilytics.com) has taken a different approach and produced a phage-based hide wash for cattle, Finalyse\textsuperscript{TM} (marketed by Elanco, Greenfield, IN, USA), to control E. coli O157:H7. It appears to be widely used in the US, although independent verification of its efficacy is lacking. A study of the application of phages to cattle hides\textsuperscript{72} demonstrated that up to 1.5 log\textsubscript{10} CFU/cm\textsuperscript{2} reduction in E. coli O157:H7 could be
achieved on detached hides 1 hour post-treatment. Clearly, there appears to be promise in the use of phages for hide decontamination, but techniques to control the pathogen within the gastrointestinal tract will require some further development.

For economic and public health reasons, control of *Vibrio* spp. is important in aquaculture. Phage biocontrol of *V. vulnificus* in live shucked oysters achieved up to 5 log_{10} CFU reductions in pooled tissue using a nine-phage cocktail.\(^3\) Subsequently, the same cocktail was evaluated in vitro for the control of *V. vulnificus* either alone or with oyster extract.\(^4\) Over 24 hours, the phage cocktail reduced the concentration of *V. vulnificus* from 6 log_{10} to 3 CFU/mL at 4°C, and the addition of oyster extract had a small additive effect. Phages may therefore have potential as a biodepuration treatment for *V. vulnificus* in oysters and other shellfish.

The easiest and most common application of phages for food safety purposes is to add them directly to foods during processing, and some phage products are now available commercially. Listex™ (P100), produced and marketed by Micreos BV (Wageningen, the Netherlands) (http://www.micreos.com), targets *L. monocytogenes*, and its efficacy has been demonstrated for several foods (Table 3). Intralytix Inc. (Baltimore, MD, USA) (http://www.intralytix.com) markets a range of similar phage products, including ListShield™, EcoShield™ (formerly ECP-100), and SalmoFresh™.

Beyond commercial products, phages have proven to be effective for pathogen biocontrol in a wide range of foods, including meat, dairy, fruit, and produce, and against most of the significant foodborne pathogens (Table 3). Other ways of harnessing phages to control pathogens include using inactivated particles, immobilizing particles on packaging, or immobilizing phage-encoded lytic proteins (endolysins). Hudson et al.\(^6\) produced inactive (nonreplicating) phage particles that retained the ability to kill *E. coli* on meat. The use of inactive phage particles may have advantages for perceived safety or regulatory classification purposes.

Phages have been successfully immobilized onto a cellulose substrate\(^8\) and could control *Listeria* and *E. coli* O157:H7 on raw and ready-to-eat meat under different storage temperatures and food packaging conditions. A reduction in *Listeria* concentration of up to 4 log_{10} CFU/g and in *E. coli* O157:H7 of up to 1.2 log_{10} CFU/g was achieved. It has been reported that a variety of phages can be immobilized on silica particles\(^9\) and incorporated into electrospun fibres,\(^10\) both of which are potential applications in food packaging.

### Table 3 Examples of phage biocontrol of foodborne pathogens

| Food         | Phage(s) | Log_{10} reduction (CFU/mL or g) | Reference |
|--------------|----------|---------------------------------|-----------|
| *Listeria monocytogenes* |          |                                 |           |
| Melon slices | Listex P100 | 1.5 (per slice) | 75        |
| Pear slices  | Listex P100 | 1.0 (per slice) | 75        |
| Sausage      | Listex P100 | 2.5                         | 76        |
| Catfish      | Listex P100 | 1.4–2.3                    | 77        |
| Salmon       | Listex P100 | 1.8–3.3                   | 78        |
| Queso fresco cheese | Listex P100 | 3.5 (per cm²) | 79        |
| Cheese       | Listex P100 | 3.5 (per cm²) | 80        |
| Hot dogs     | Listex P100 | 3.0                         | 81        |
| Ham          | Listex P100 | 1.0                         | 82        |
| Chicken roll | FWLLmL1     | 2.5 (per cm²) | 83        |
| *Escherichia coli* O157:H7 |          |                                 |           |
| Beef (raw)   | FAHecl     | 2.6 (per 4 cm²) | 84        |
| Beef (raw)   | FAHecl     | 3.8                         | 85        |
| Beef (cooked)| FAHecl     | 4.6                         | 85        |
| Beef (raw)   | EcoShield™ | 2.6                         | 86        |
| Lettuce      | EcoShield™ | 1.3                         | 86        |
| Spinach      | 8 phages   | 3.5                         | 87        |
| Lettuce      | 8 phages   | 3.8                         | 87        |
| Cantaloupe   | ECP-100    | 3.1                         | 88        |
| Lettuce      | ECP-100    | 1.9                         | 88        |
| Broccoli     | ECP-100    | 2.3                         | 89        |
| Tomatoes     | ECP-100    | 2.1                         | 89        |
| Spinach      | ECP-100    | 3.2                         | 89        |
| Beef (ground)| ECP-100    | 1.3                         | 89        |
| *Salmonella enterica* |          |                                 |           |
| Chicken (skin)| Wksl3     | 2.5                         | 90        |
| Pork (skin)  | 4 phages   | 4.3                         | 91        |
| Mung beans   | 6 phages   | 3.3                         | 92        |
| Chicken      | 72 phages  | 3.0                         | 93        |
| Beef (raw)   | P7         | 5.9                         | 94        |
| Beef (cooked)| P7         | 4.8                         | 94        |
| Chicken (skin)| P22 and 29C | 2.0                      | 95        |
| Cheese       | SJ2        | 3.0                         | 96        |
| *Campylobacter jejuni* |          |                                 |           |
| Beef (raw)   | Cj6        | 2.2                         | 94        |
| Beef (cooked)| Cj6        | 3.7                         | 94        |
| Chicken (skin)| NCTC12673 | 2.0                        | 95        |
| Chicken (skin)| e2        | 1.3                         | 97        |
| *Bacillus cereus* |          |                                 |           |
| Mashed potato | FWLBc1 and 2 | 6.0                     | 98        |
| *Staphylococcus aureus* |          |                                 |           |
| Cheese (soft)| IPLA35 and 88 | 3.8                    | 99        |
| Cheese (hard)| IPLA35 and 88 | 4.6                    | 99        |

Note: EcoShield™ (Intralytix Inc, Baltimore, MD, USA).

Abbreviation: CFU, colony forming units.

Solanki et al.\(^2\) immobilized phage endolysins on both silica and starch nanoparticles, and reductions of 4.0 log_{10} CFU/g and 1.2 log_{10} CFU/g *Listeria*, respectively, were recorded on iceberg lettuce. These nanoparticles may also be able to be incorporated onto the surfaces of equipment and into the infrastructure of food processing premises, in order to minimize cross-contamination.
Nonphage-based nanobiotechnological controls

Nanotechnology has begun to be explored as a means of targeted delivery of antimicrobial agents into food or as a means of protecting those agents from adverse environmental conditions prior to their application. A wide range of encapsulation delivery systems exists, but new developments in nanotechnology promise improvements over those that are currently available. Targeted delivery of bacterial materials can be achieved by several means, including liposomes, archaosomes, nanochelates, casein micelles, globular food proteins that can reassemble into nanofibrils at elevated temperatures and low pH values, and polymers, natural or synthetic, including nanospheres, nanocapsules, polymersomes, and micelles.

One example of a biological material used in nanotechnology is chitosan, which is nontoxic and biodegradable. It is used because of its inherent antimicrobial nature, which increases at low pH (with increased protonation). In addition, chitosan nanoparticles (CNPs) can be used to deliver other biologically derived antibacterial agents such as carvacrol, a component of some essential oils, to produce a synergistic antimicrobial effect. CNPs allow for a burst of release of the antimicrobial compound (eg, oregano essential oil), followed by a sustained release over time.

The antibacterial effects of oleoyl–chitosan nanoparticles (OCNPs) against *E. coli* and *S. aureus* have been explored. Variations in molecular weight and degree of substitution of the chitosan were shown to inhibit the two organisms differently, but inhibition was optimal at pH 6. Antibacterial activity against the same two organisms has also been reported for nisin-loaded CNPs. In this case, peak nisin release occurred at day 1, but then a residual concentration was maintained for up to 100 days, and it was claimed that the combination of nisin and the inherent antibacterial effect of chitosan was synergistic when compared with the effect of either alone. However, insufficient data were provided to rule out the possibility that the effect was additive rather than synergistic. The activity of chitosan, OCNPs, and nisin loaded CNPs involves the disruption of cell membranes.

Encapsulation of more than one bioactive component within a nonmaterial to allow simultaneous targeted and controlled release of both has been researched. For example, the incorporation of organic acid and essential oil in a chitosan matrix inhibited the growth of bacteria, including *Enterobacteriaceae*, on vacuum-packed cured meat products.

Chitosan can be used as a packaging film, but it has a number of drawbacks, including poor water resistance, gas barrier, and mechanical properties. However, when used in combination with nanoclay to form a bionanocomposite film, the properties of the resulting structure are improved. The addition of rosemary essential oil into this film resulted in antimicrobial activity against *L. monocytogenes* and spoilage organisms.

There is increasing interest in the use of biologically based polymers for food packaging purposes, with nanoscale fillers, including layered silicate nanoclays, being developed. Such nanocomposite films can contain inorganic material such as silver nanoparticles or biologically derived antimicrobials like essential oils, enzymes, and bacteriocins.

It may also be possible to induce antibody responses in the food animal against pathogenic bacteria. This has been achieved in black seabream by the oral administration of a DNA nanoparticle vaccine to induce an immune response against *Vibrio parahaemolyticus*.

Conclusion

The biggest challenge for the detection of pathogens is for new faster, more cost-effective methods to meet the sensitivity of conventional approaches. One way to achieve this might be to concentrate bacteria from food samples, ideally removing the need to use lengthy enrichment procedures. There may be aspects of nanobiotechnology that can help: eg, nanosized beads for IMS, which could be used to recover pathogens from complex samples prior to detection. Applications to detect bacteria that have some allowable level in foods, such as *S. aureus*, *Bacillus* spp., *E. coli*, and some clostridia, could be achieved more readily. Another challenge is to ensure that dead bacteria are not detected while injured bacteria are.

The use of advanced nanocomposite packaging films may help to combat foodborne disease if they can alert producers and consumers to the presence of pathogens or to deliver a sustained and targeted antimicrobial effect. As a control agent, phages show promise, as reflected in the appearance of commercially available products and a growing literature base. Drivers for adoption of bionanotechnologies for food safety include an increased awareness of the need for effective pathogen control by “green” technologies that do not negatively impact product quality.

Beyond food safety, applications in these technologies in human health may provide useful options in the future: eg, in the treatment of human infections caused by antibiotic-resistant bacteria.
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