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Reporter bacteriophage assays as a means to detect foodborne pathogenic bacteria

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Abstract

Bacterial disease due to the consumption of contaminated food is a global problem that has necessitated the need for modern rapid bacterial detection techniques. There has been much recent interest in the use of reporter bacteriophages as a tool to aid in the detection of foodborne, and clinical bacterial pathogens. The reporter bacteriophage concept provides a sensitive method for bacterial detection and sensitivity to antimicrobial agents. This review presents the current status of reporter bacteriophage technology. The bacterial and eucaryotic luciferases, the ice nucleation protein, and the *E. coli* β-galactosidase reporter genes are discussed, along with many examples that demonstrate the usefulness of reporter bacteriophage as tools to detect foodborne bacterial contamination.

Keywords: Foodborne bacterial pathogens; Contamination; Rapid; Detection; Reporter bacteriophages; Luciferase; Ice nucleation protein; β-galactosidase

1. Introduction

Several recent, large foodborne disease outbreaks have necessitated increased vigilance in the area of food safety (Ahmed & Cowden 1997; Health Canada, 1999; Health Canada Population and Public Health Branch, 2000; Watanabe et al., 1999). These outbreaks have highlighted the need for simpler, reliable, labor saving methods, capable of detecting specific pathogenic microorganisms present in low numbers in food, within a relatively short time-frame. Present methods are either (1) too long, (2) too laborious, (3) too costly, (4) lack specificity, or (5) lack sensitivity. For example, cultural, genetic, and immunological based detection methods are often labor intensive, costly, or require intensive training of laboratory personnel.

Alternatively, reporter bacteriophages represent an unique and sensitive alternative to conventional methods for detection of bacteria within food. In this technology, DNA carrying a reporter gene is introduced into a target bacterium via a bacteriophage. Once the reporter gene has been introduced to the bacterium, it is expressed, thereby allowing bacterial cells to be rapidly identified (Fig. 1). Since bacteriophages need host cells to replicate, the phages will remain "dark" (i.e. the reporter gene will not be expressed until the phage DNA has been injected into the host). Therefore, expression of the reporter gene is indicative of the presence of the infected organism. So far, the reporter genes employed in this system are the prokaryotic, and eukaryotic luciferase (*lux* and *luc*) genes, the bacterial ice nucleation (*inaW*) gene, and the *E. coli* β-galactosidase (*lacZ*) gene (Goodridge & Griffiths, 2002; Ulitzur & Kuhn, 1987; Wolber & Green, 1990). To date, reporter bacteriophages have been developed that can detect *E. coli*, Mycobacteria, *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes* (Chen & Griffiths 1996; Jacobs et al., 1993; Loessner, Rudolf, & Scherer 1997; Pagotto, Brovko, & Griffiths 1996; Ulitzur & Kuhn 1987).

Several reviews have described some of the work accomplished with lux^+ bacteriophage mediated detection of bacteria (Baker, Griffiths, & Collins-Thompson, 1992; Billard & DuBow, 1998; Kricka, 1988; Stewart &

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Williams, 1992). Nevertheless, the majority of research conducted on the use of reporter bacteriophages to detect pathogenic bacteria has not been comprehensively reviewed. Therefore, the purpose here is to review the current research surrounding reporter bacteriophage technology, and the reporter systems employed in such detection systems.

2. Luciferase reporter systems

The majority of transducing bacteriophage detection assays have employed the bioluminescence (*lux* and *luc*) genes as the reporter system (Table 1). Bioluminescence can be defined as the emission of visible light in living organisms from enzyme-catalyzed reactions (Campbell 1989; Meighen, 1993). Bioluminescent organisms are widely distributed in nature, comprising a diverse set of species. Among light emitting species are dinoflagellates, fungi, fish, insects, shrimp, squid, and bacteria (Harveyi, 1952).

There are several components necessary for the bioluminescent reaction to occur. These include an oxidant (usually O_2) a protein catalyst (luciferase), a substrate (luciferin), a cofactor (FMN or ATP), a cation, and an ancillary protein (Stewart, Smith, & Denyer, 1989).

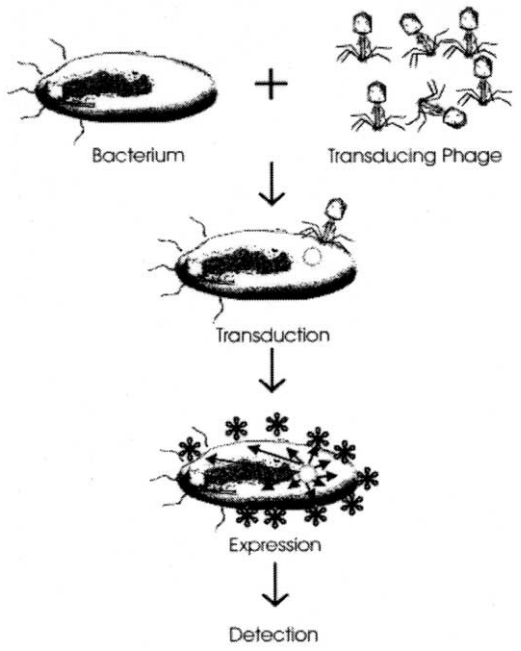

Fig. 1. Reporter phages as a tool for detection of foodborne pathogenic bacteria. A recombinant transducing bacteriophage, carrying a reporter gene inserted downstream ofa phage promoter is used to infect a host bacterium. This infection results in expression of the reporter gene, which can then be detected using various techniques.

2.1. Principles of bacterial bioluminescence

Luminescent bacteria are the most ubiquitous of the luminescent organisms found in marine, freshwater, and terrestrial habitats. The primary habitat of luminescent bacteria is the ocean, where they live in symbiotic, saprophytic, and parasitic relationships; they can also dwell in a free living mode (Meighen, 1994). The many species of luminescent bacteria that have been studied include the marine bacteria *Vibrio harveyi, Vibrio fischeri, Photobacterium phosphoreum* and *Photobacterium leiognathi*, as well as the terrestrial bacterium *Photorhabdus luminescens* (Campbell, 1989).

The principles of bacterial bioluminescence have been extensively reviewed (Meighen, 1991, 1993, 1994; Szittner & Meighen, 1990). In bacteria, the luminescent reaction can be represented as follows:

$$FMNH_2 + RCHO + O_2 \rightarrow FMN + H_2O + RCOOH + h\upsilon\ (490nm)$$

This light emitting reaction involves an intracellular luciferase-catalysed oxidation of the reduced form of flavin mononucleotide ($FMNH_2$) and a long chain aliphatic aldehyde (RCHO), such as dodecanal, by molecular oxygen (Stewart, Denyer, & Lewington, 1991; Stewart & Williams, 1992).

Bacterial luciferase is a heterodimeric enzyme of 77 kDa, composed of α and β subunits with molecular masses of 40 and 37 kDa, respectively (Stewart & Williams, 1992). While the natural aldehyde for the bioluminescence reaction appears to be tetradecanal, shorter chain aldehydes, such as decanal and dodecanal, are routinely used in in vitro assays for luciferase activity (Meighen, 1994).

Table 1
Bacterial species detected by recombinant reporter bacteriophages

Bacterial species	Reporter gene	Reference
E. coli O157:H7	*lux*	Waddell & Poppe, 2000
E. coli	*lux*	Duzhii & Zavilgelskii, 1994
E. coli	*lacZ*	Goodridge & Griffiths, 2002
E. coli	*lux*	Ulitzur & Kuhn, 1987
Salmonella spp.	*lux*	Chen & Griffiths, 1996
S. Typhimurium	*lux*	Turpin et al. 1993
Salmonella spp.	*inaW*	Wolber & Green 1990
M. bovis BCG	*lux*	Pearson et al. 1996
M. smegmatis	*lux*	Sarkis et al. 1995
M. tuberculosis	*lux*	Jacobs et al. 1993
Staphylococcus aureus	*lux*	Pagotto et al. 1996
L. monocytogenes	*lux*	Loessner et al. 1996
Enteric bacteria	*lux*	Kodikara et al. 1991

2.2. Lux *gene organization*

The genes of the *lux* operon from three bacterial genera, *Photobacterium, Vibrio*, and *Xenorhabdus*, have been cloned and sequenced (Stewart & Williams, 1992). In these genera the *lux* operon consists of the *luxA* and *luxB* genes, which encode the luciferase α and β subunits, as well as genes that code for a reductase (*luxC*), a transferase (*luxD*), and a synthetase (*luxE*). The latter three genes are required for the conversion of fatty acids into the long chain aldehyde required for bioluminescence (Wall & Meighen, 1986).

The order of the genes that code for luciferase (*luxAB*) and the fatty acid reductase (*luxCDE*) is the same in all operons, with *luxC* and *luxD* upstream of the luciferase genes and *luxE* downstream of the luciferase genes. Although the luciferase and fatty acid reductase genes are the only *lux* genes common to all luminescent bacteria, other *lux* genes (*luxF*, *luxG*, *luxH*, *luxI*, *luxR*) as well as unlinked *lux* gene loci have been identified in specific luminescent strains (Eberhard, 1972; Engebrecht & Silverman, 1984; Mancini, Boylan, Soly, Graham, & Meighen, 1988; Stewart & Williams 1992; Swartzman, Kapoor, Graham, & Meighen, 1990).

Regulatory genes controlling the expression of bacterial luminescence from *V. fischeri* and *V. harveyi* have been cloned and sequenced, and induction of luminescence has been characterized for the *V. fischeri* lux system (Meighen, 1993). Two regulatory genes, designated as *luxI* and *luxR*, are located upstream of the *luxCDABEG* genes in the *V. fischeri lux* operon (Meighen, 1993). LuxI is a 22 kDa regulatory protein required for synthesis of an autoinducer that interacts with luxR, a 29 kDa protein, to activate the expression of the *lux* operon (Swartzman, Miyamoto, Cao, Sun, & Meighen, 1993). In *V. fischeri*, the genes of the lux system are organized into two operons; the left operon contains *luxR*, while the right operon contains *luxICDABEG* (Engebrecht & Silverman 1984). In *V. harveyi*, a regulatory gene (*luxR*) also controls expression of the *lux* genes (Meighen, 1993). Yet, it has no sequence homology with the *V. fischeri luxR* gene (Swartzman et al. 1993).

2.3. *Principles of eucaryotic bioluminescence*

The single polypeptide *luc* gene, encoding a 62 kDa luciferase from the North American firefly *Photinus pyralis*, has been cloned and expressed in *E. coli* (De Wet, Wood, Helsinki, & Deluca, 1985). The luciferase of *P. pyralis* emits in the yellow-green range, with peak emission at 560 nm, while other eukaryotic organisms, including different firefly species and click beetles, produce light ranging from green (547 nm) to red (604 nm).

The production of light in the luc-based bioluminescence system occurs when adenosine triphosphate (ATP), reacts with the heterocyclic carboxylic acid, luciferin, in the presence of molecular oxygen (O_2). The reaction is catalyzed by the firefly enzyme luciferase, according to the following equations:

$$\text{luciferase} + \text{luciferin} + \text{ATP---}Mg^{2+} \rightarrow \text{lucerase-luciferyl-AMP} + \text{Ppi} \quad (1)$$

$$\text{luciferase-luciferyl-AMP} + O_2 \rightarrow \text{luciferase+oxyluciferin+AMP} + CO_2 + \text{light} \quad (2)$$

These reactions produce a highly efficient flash of light, with an estimated quantum yield of 0.88, which is more efficient than the light generated by bacterial luciferase (Bronstein, Fortin, Stanley, Stewart, & Kricka, 1994). The fact that luc based bioluminescence systems produce a flash of light as opposed to a stable light signal is a disadvantage in bacterial detection systems. The flash kinetics of the luc reaction, caused by the interaction of luciferase and oxyluciferin, have been addressed to some degree by the use of chemicals such as coenzyme A, reported to prolong the half-life of light to 10 min. LucLite™, is a series of reagents developed to specifically improve the light output of luc based reactions to over 5 h, with the production of a glow type light signal (Roelant, Burns, & Scheirer, 1996). Additionally, it has been shown that a single serine to threonine substitution at position 198 of the luc polypeptide chain results in an enzyme with a 150 fold increase in half-time for light emission decay (Thompson et al. 1997).

3. Ice nucleation proteins

Water can be supercooled below 0 °C, and still remain in liquid form. The introduction of an ice nucleus into supercooled water at a low enough temperature, will result in a rapid chain reaction of freezing, and ice formation. Ice nuclei are employed in such applications as snow-making, and the possibility exists for ice nuclei to be used in the production of ice cream and other frozen foods, in immunoassays, and as a replacement for silver iodide in cloud seeding (Warren, 1987).

The ability of organisms to cause heterogeneous nucleation of ice in supercooled water, has been well documented (Watanabe, Southworth, Warren, & Wolber, 1990; Wolber, Deininger, Southworth, Vandekerckhove, van Montagu, & Warren, 1986; Wolber & Warren, 1989). Several of these organisms possess the extraordinary ability to nucleate at temperatures warmer than −10 °C. (Warren & Wolber, 1987). Bacteria from the genera *Pseudomonas*, *Erwinia*, and *Xanthomo-*

nas possess the ability to nucleate at relatively warm temperatures, a capability shared by several eukaryotic organisms, including some insects and at least one angiosperm (Duman & Horwath, 1983; Krog, Zachariassen, Larsen, & Smidsrod, 1979).

Bacteria have been the most studied of the various types of biological ice nucleators. The identification and characterization of the ice nucleating bacteria have been the subjects of several reviews (Franks, 1987; Lindow, 1982, 1983). Since then, much work has centered around the characterization of bacterial ice nucleators. The nucleotide sequences of two open reading frames, each encoding a single ice nucleation (*ina*) gene from *Pseudomonas*, have been deduced (Green & Warren, 1985). The genetic sequences of the *inaZ* gene from *Pseudomonas syringae*, and the *inaW* gene from *Pseudomonas fluorescens* are very similar. Additionally, Orser, Staskawicz, Panopoulos, Dahlbeck, and Lindow (1985) used Southern hybridizations to show that another *ina* gene from *P. syringae* has homology to the ina-conferring region from *E. herbicola*, and these researchers hypothesize that, in *Erwinia*, a single gene is probably responsible for conferring ice nucleation activity.

The ice nucleation genes were originally identified by the isolation of ina$^-$ mutants, followed by screening cloned sequences for complementations of the mutations. Using this method, Orser et al. (1985) discovered that it was unnecessary to return the cloned genes to their original host species in order to detect their phenotype, because the genes were able to impart ice nucleation activity to the heterologous host *E. coli*. A 4.5 kb fragment of DNA from *P. syringae*, and a 5.7 kb fragment from *E. herbicola* both conferred the *ina*$^+$ phenotype on *E. coli*, and were also sufficient to complement all ina$^-$ mutations in the original species. A 5.7 kb fragment from a strain of *Pseudomonas fluorescens* has also been identified (Corotto, Wolber, & Warren 1986).

4. Beta-galactosidase

Beta-galactosidase is a glycosidase that catalyzes the hydrolysis of β-galactoside sugars such as lactose (Wallenfels & Weil, 1972). Beta-galactosidases have been found in numerous microorganisms, animals and plants (Wallenfels & Weil, 1972). The importance of lactose fermentation tests as a diagnostic tool for the Enterobactericeae led to extensive investigation of β-galactosidases in Gram negative bacteria. Beta-galactosidases have been reported in strains of the Enterobacteriaceae, Pseudomonodaceae, Parvobacteriaceae, and Neisseriaceae (Corbett & Catlin, 1968).

The *lac Z* encoded β-galactosidase from *E. coli* has been the most studied. This enzyme is tetrameric, with a subunit size of 116,000 daltons and a total molecular weight of 465,000 daltons (Schenborn & Groskreutz, 1999).

The properties of the enzyme vary depending on the substrate employed in the hydrolysis reaction. The most common substrates for assaying β-galactosidase are the chromogenic galactosides. The introduction of o-nitrophenyl β-D-galactoside (ONPG) as a sensitive substrate (Lederberg, 1950) brought general acceptance to the β-galactosidase assay. Chlorophenol red β-D-galactopyranoside is another colourimetric based substrate that is 10 times more sensitive than ONPG (Eustice, Feldman, Colberg-Poley, Buckery, & Neubauer 1991). The substrate 5-bromo-4-chloro-3-indoyl β-D-galactoside (X-Gal) is often used in in situ histochemical analyses. Enzymatic hydrolysis of X-Gal followed by oxidation produces a blue precipitate (Lim & Chae, 1989). Fluorescent based substrates such as β-methylumbelliferyl galactoside (MuGal) and fluorescein digalactoside (FDG) are very sensitive and allow detection of enzyme activity in single cells (Krasnow, Cumberledge, Manning, Herzenberg, & Nolan 1991). Chemiluminescent substrates offer the highest sensitivity and the largest dynamic range of the three classes of substrates (Jain & Magrath, 1991), and β-galactosidase assays that employ chemiluminescent substrates are similar in sensitivity to the bioluminescent luciferase assay (Schenborn & Groskreutz, 1999).

5. Reporter bacteriophage assays

Reporter bacteriophage assays based upon the above mentioned reporter genes have been developed to detect several species of bacteria including *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and *Mycobacterium* spp. (Chen & Griffiths, 1996; Duzhii & Zavilgelskii, 1994; Goodridge & Griffiths, 2000; Kodikara, Crew, & Stewart, 1991; Loessner, Rees, Stewart, & Scherer, 1996; Pearson, Jurgensen, Sarkis, Hatfull, & Jacobs, 1996; Sarkis, Jacobs, & Hatfull, 1995; Turpin, Maycroft, Bedford, Rowlands, & Wellington, 1993; Ulitzur & Kuhn, 1987). Table 1 summarizes the bacterial species detected by recombinant reporter bacteriophages.

Ulitzur and Kuhn (1987) first showed that bacteriophages carrying a reporter gene could be used to detect microorganisms. These researchers engineered bacteriophage λ Charon 30, with the *lux* genes from *Vibrio fischeri*. The *lux* genes were placed into the bacteriophage chromosome by ligating the λ Charon 30 DNA, to DNA from a plasmid that contained the entire *lux* operon of *V. fisheri*. The ligated DNA was packaged into the bacteriophage, and luminescent phage were identified. Using the *lux*$^+$ phage, it was possible to detect between 10 and 100 *E. coli* cells/ml of milk or urine, within 1 h (Ulitzur & Kuhn, 1987). Since then,

reporter bacteriophage assays have been developed that are capable of detecting several pathogenic foodborne microorganisms.

Waddell and Poppe (2000) used transposon mutagenesis to construct a luciferase transducing bacteriophage that was capable of detecting *E. coli* O157:H7. The phage used in this study, ΦV10, is a temperate phage that is used to phage type *E. coli* O157:H7. A mini-Tn 10 transposon containing the *luxA* and *luxB* genes was constructed and used to deliver a 3.6 kb insertion to the phage chromosome which conferred n-decanal-dependent bioluminescence on bacterial cells upon subsequent infection with lux^+ ΦV10. The lux^+ phage detected a wide range of *E. coli* O157:H7 phage types, and in broth culture, bioluminescence was observed and easily measured after 1 h.

Chen and Griffiths (1996) have used bacteriophage P22 as part of a cocktail of bacteriophages to detect *Salmonella* spp. Bacteriophage P22 and two uncharacterized lysogenic bacteriophage were engineered to carry the *lux* genes of *V. fischeri*, by homologous recombination between the phages and a plasmid carrying the *V. fisheri lux* genes. The lux^+ phage assay was capable of detecting *Salmonella* isolates from groups B, C, and D. Seventy-nine bacterial cultures (57 *Salmonella* spp. and 22 non-*Salmonella* strains representing 14 different genera) were tested using this assay. All 57 of the *Salmonella* isolates were detected, while none of the non-*Salmonella* samples gave a positive result.

With a 6 h preincubation, 10 CFU/ml of *Salmonella* in the original broth culture were detectable. The detection system also allowed *Salmonella* to be detected in whole eggs by the direct addition of the recombinant bacteriophages into the eggs. After 24 h of incubation, as few as 10 CFU/egg could be detected, thereby offering the ability to not only detect bacterial contamination in whole eggs, but also to determine its location (Chen & Griffiths, 1996). Luminescence could be detected using a variety of instruments, including a photon-counting charge-coupled device (CCD) camera, a luminometer, or X-ray film.

The ability of the *ina* genes to confer ice nucleation activity on bacteria previously unable to produce ice nuclei, is the basis of a commercially available assay known as the Bacterial Ice Nucleation Diagnostic (BIND) *Salmonella* assay. This assay works by allowing *Salmonella* cells to be infected with a bacteriophage carrying an *ina* gene. Bacteriophage infected *Salmonella* cells will produce ice nucleation proteins, that will cause the cells to freeze when the temperature is lowered to −9.3 °C or below. The recombinant bacteriophage was created by introducing the *inaW* gene from *P. fluorescens* into the *Salmonella* bacteriophage P22 chromosome, with the use of standard recombinant DNA techniques. The resulting reporter bacteriophage allows for expression of the *inaW* gene upon infection of a susceptible host, thereby causing ice to form, which is easily detected with the use of a fluorescent freezing-indicator dye (Wolber & Green, 1990). The BIND assay was tested on samples of culture medium and raw egg, to which *Salmonella* had been added, to give a range of bacterial concentrations. *Salmonella* were detected in all of the samples with high sensitivity ($\leqslant$10 bacteria per ml). Additionally, results with culture medium samples showed that phage P22 transduced the *inaW* gene with high efficiency, and the viscosity or complexity of the raw egg samples did not affect the assay. Also, P22 was capable of detecting 10 *Salmonella* Dublin cells/ml, in the presence of 10^7 non-target bacteria/ml (Wolber & Green, 1990), suggesting that a selective enrichment step may not be necessary for the assay to work.

Initial attempts to construct lux^+ bacteriophages to detect *Listeria monocytogenes* were unsuccessful, due to the narrow host ranges of the known *listeria* phages. The isolation of a phage with a wide host range allowed the construction of a lux^+ reporter phage assay for *L. monoctyogenes*. The phage, A511, is a genus specific, virulent myovirus, capable of infecting 95% of *L. monocytogenes* serovar 1/2 and 4 cells. Loessner and coworkers (1996) constructed a recombinant bacteriophage with a *luxAB* gene, which encodes a fused *Vibrio harveyi* luxAB protein. In the recombinant phage (A511::*luxAB*), the *lux* gene was inserted immediately downstream of the major capsid protein gene, by homologous recombination between phage A511 DNA and a plasmid carrying *luxAB* flanked by A511 DNA. The recombinant phage A511::*luxAB* was tested for its ability to detect *L. monocytogenes* in pure culture, and a variety of foods. To determine lower detection limits for the A511::*luxAB* assay, low numbers of six different strains of three *Listeria* species were directly challenged with A511::*luxAB* without a prior enrichment step. As few as 500 cells were detected, and a positive result was defined as those samples that resulted in luminescent signals that were twofold or more greater than the background signal (Loessner et al., 1996). The presence of less than one CFU/g salad could be identified within 24 hours. Additionally, the lux^+ assay could detect *L. monocytogenes* cells from naturally contaminated foods, including meat, poultry, and various cheeses (Loessner et al., 1997).

Several most probable number (MPN) methodologies have been developed for use in conjunction with reporter bacteriophages. Turpin et al. (1993) developed a reporter phage MPN method which allows for enumeration of *Salmonella* Typhimurium in environmental materials within 24 h, based upon light production transduced to the *Salmonella* cells by a lux^+ bacteriophage. Positive samples were defined as those that produced light 100–1000 times that of background. This method has been developed for use with soil, water, and

sewage sludge, but should be easily modifiable to test other environmental samples and food (Turpin et al., 1993).

Loessner and coworkers (1997) describe a lux MPN method for *Listeria* detection based on Turpin's technique. In this work, *Listeria*-free samples of four different foods (minced meat, shrimp, Ricotta cheese, and lettuce) were thawed and spiked with low numbers of *L. monocytogenes* Scott A. The food samples were enriched for 20 and 40 h, serially diluted, mixed with phage A511::*luxAB*, and incubated at 20 °C for 2 hours. Luminescence was measured in a photon-counting, single-tube luminometer. Tubes that initially contained at least one viable *Listeria* cell, could be easily detected by the assay. The lux MPN method enabled the rapid enumeration of *Listeria* cells in a variety of foods. The method proved to be simple, yielded reliable results, and is suitable for automation in a microtitre plate format. It also appears that this technique would be useful for all applications in which direct plating for determination of cell count is not feasible (Loessner et al. 1997), such as in cases where a strong competing microflora can overwhelm the selective agents and mask *Listeria* colonies (Curtis & Lee, 1995).

Goodridge and Griffiths (2000) created a $lacZ^+$ T4 phage that was capable of detecting *E. coli*. The $lacZ^+$ assay could detect as few as 10^2 CFU/ml in broth culture when a chemiluminescent substrate (Galacto-Star®) was used. The $lacZ^+$ phage was used to develop a $lacZ^+$ phage MPN assay. This assay was capable of detecting as few as 10^1 CFU/ml in broth culture within 8 h when the fluorescent substrate Mu-Gal was used.

As an alternative to the assay of bacterial pathogens, microbial contamination can also be identified via the detection of indicator bacteria. By definition, these are microorganisms present in significant numbers within a food which, while not pathogenic, can be related through increasing count to the increasing probability of pathogen contamination. Kodikara and researchers (1991) have endeavoured to explore the use of lux^+ recombinant bacteriophage for detecting enteric indicator bacteria. In this work, lux^+ bacteriophage were employed to detect enteric indicator bacteria without recovery or enrichment within 50 min, provided that the bacteria were present at levels greater than 10^4 CFU/g. Following a 4 h enrichment, as few as 10 CFU/g could be detected. One hundred and forty-one samples with a range of enteric counts from less than 10^1 to 10^8 CFU/ml were obtained from abattoirs, meat processing factory surfaces, and carcasses. Lux^+ bacteriophage, possessing specificity for the Enterobacteriaceae, were added to each sample, and from these data, a plot of bioluminescence vs mean enteric count was generated. In addition, the bioluminescent response of individual enteric isolates from food processing environments infected with the lux^+ phages showed that only 5.8% of the isolates were incapable of producing a bioluminescent signal. Since the assay is simple and short, the evaluation of indicator strains in less than 1 h can be accomplished. However, while this is a simple and effective approach to detecting enteric indicator bacteria, at present, it constitutes only a prototype assay, since a different enteric flora might require additional bacteriophage constructs with different host ranges (Kodikara et al., 1991).

The emergence of multidrug resistant strains has necessitated the development of methods to rapidly ascertain patterns of drug susceptibility. Mycobacterial infections due to multidrug resistant strains of *Mycobacterium tuberculosis* pose a significant challenge to public health. Since *M. tuberculosis* has a doubling time of 20–24 h, current methodology does not allow determination of antibiotic susceptibility for 2–18 weeks (M.M.W.R. 1992), leaving both patients and health care workers at risk. Reporter phages may help to reduce the time required for establishing antibiotic sensitivity of *M. tuberculosis* from weeks to days, which would accelerate screening for new antituberculosis drugs (Jacobs et al., 1993). In the presence of an antibiotic to which the host cell is sensitive, the infecting phage cannot complete its lytic cycle, and the amount of light emitted is therefore reduced, or abolished (Ulitzur & Kuhn, 1987).

The majority of reporter bacteriophage testing to determine antibiotic susceptibility has centered around the Mycobacteria. Jacobs et al. (1993) proposed the use of a recombinant reporter mycobacteriophage carrying a firefly luciferase reporter gene as a tool for the rapid determination of *M. tuberculosis* drug susceptibilities. In this research, mutants of *M. bovis* BCG were selected that were resistant to rifampicin, streptomycin, or isoniazid. When wild type *M. bovis* BCG and the mutants were grown for 24 h in the presence of the antibiotics and the lux^+ phage L5::*FFlux*, the wild type strain did not produce light, whereas, light was produced by the drug resistant mutants. Bacteriophage L5::*FFlux* was also tested on clinically derived *M. tuberculosis* strains, both singly and multiply drug resistant. The results established that L5:*FFlux* is very efficient at revealing the patterns of drug susceptibility or resistance of *M. tuberculosis* strains (Jacobs et al. 1993), and other researchers have shown that L5:*FFlux* can also determine the antibiotic susceptibility profile of other Mycobacteria (Sarkis et al., 1995; Zwadyk, Meyers, Jurgensen, Little, Jacobs, & Cole, 1995).

6. Conclusion

Reporter bacteriophage technology offers a rapid, reliable, and sensitive method for the detection of specific food borne pathogens. Despite this, the technology has not been widely accepted. One reason for this may

be the fact that it is difficult to genetically modify bacteriophages, that have bacterial hosts that have not been genetically characterized. Much of the early work in reporter bacteriophage technology centered around the well studied bacteria *E. coli* and *Salmonella* spp., and their equally well characterized bacteriophages lambda and P22. Hopefully, as the genetic sequences of more bacteria are determined, the technology will become popular. Another potential problem is the fact that an individual bacteriophage may not possess the host range required to detect all isolates of a bacterial species. Therefore, it will be necessary to modify the host ranges of the bacteriophages used, or employ a cocktail of bacteriophages in order to develop a reporter assay that will be able to detect the desired target bacteria. The many different bacteriophages, coupled with the use of various reporter genes, presents food microbiologists with an opportunity to revolutionize the detection of food borne bacteria. The potential for a revolution in microbial testing can be discerned with the near on-line detection of food borne microorganisms. By defining the microorganisms present in significant numbers within a food, it will be possible, using reporter bacteriophages, to relate increasing bacterial counts to the increased probability of pathogen contamination.

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