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FOOD-BORNE PATHOGENS
Use of bioluminescent *Salmonella* for assessing the efficiency of constructed phage-based biosorbent †,‡

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A bacteriophage-based biosorbent for *Salmonella enteritidis* was constructed, and bacterial bioluminescence was used for assessment of the efficiency of cell capture. A strain of *S. enteritidis* with bioluminescent phenotype was constructed by transformation with plasmid pT7 carrying the entire *lux* operon from *Photorhabdus luminescens*. The relation between relative light output (RLU) and colony-forming units (CFU/ml) of the bioluminescent strain was established. The bacteriophage specific to *S. enteritidis* was biotinylated, and the biotinylation procedure was optimized based on the maximum retention of phage infectivity. The biotinylated phages were then coated onto streptavidin-labeled magnetic beads, and were used to capture the bioluminescent *S. enteritidis* cells. Our preliminary results showed that the number of cells captured by constructed biosorbent was five times higher than that of the control, magnetic beads coated with nonbiotinylated phage, indicating the capture is specific. *Journal of Industrial Microbiology & Biotechnology* (2001) **27,** 126–128.

Keywords: bioluminescence; *Salmonella enteritidis*; bacteriophage; magnetic beads; biosorbent

Introduction

Contamination of food products with bacterial pathogens, such as *Salmonella*, *Campylobacter*, *Escherichia coli* O157, and *Listeria* is a major concern for the food industry. Conventional detection methods, which take 3 to 5 days to detect and confirm pathogens in foods, are not satisfactory for monitoring the microbial quality of foods. Consequently, cheaper, simpler, and more rapid detection methods are needed. Although many rapid procedures have been proposed for *Salmonella* detection, the use of immunomagnetic separation (IMS) has been suggested as a method of reducing total analysis time and improving sensitivity of detection [2,5]. The specificity of bacteriophage–host cell interaction makes it possible to use the bacteriophage instead of specific antibodies as a biosorbent. A phage-based biosorbent consisting of a *Salmonella*-specific phage passively immobilized on a polystyrene membrane has been used to separate *Salmonella* from food materials by Bennett *et al* [1]. However, the efficiency of cell capture was poor. In this study, a bacteriophage-based biosorbent for *S. enteritidis* was constructed by coating magnetic beads with bacteriophage *via* streptavidin–biotin interaction, and the efficiency of the biosorbent was assessed by *in vivo* bioluminescence.

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Materials and methods

Bacterial strain, phage, and plasmid

The strain of *S. enteritidis* and its phage SJ2 was isolated from chicken eggs in our laboratory. A bioluminescent strain of *S. enteritidis* was constructed by introducing plasmid pT7 from Dr E. Meighen [4] carrying the entire *lux* operon from *Photorhabdus luminescens* according to techniques described previously [3]. Bioluminescent colonies were selected on LB agar plates supplemented with 100 μg/ml of ampicillin and confirmed in a BIQ Image Quantifier (Cambridge Imaging, Cambridge, UK). The plasmid pT7-*lux* in *S. enteritidis* was further confirmed by restriction enzyme analysis.

Calibration of bioluminescent signal and viable cell numbers

The correlation between relative light units (RLU) and colony-forming units (CFU) of the bioluminescent strain was established in LB broth and LB broth with beads using a 96-well solid-white microtiter plate at room temperature. Fivefold serial dilutions of the culture were prepared in LB broth with 200 μl per well. The magnetic beads (Dynabeads® M-280 Streptavidin, Dynal Inc., Lake Success, NY, USA) were added at the concentration of 20 μl/ml broth. RLU in each well was measured by a microplate luminometer (1258 plate reader, Bio-Orbit, Mandel Scientific Company Ltd., Guelph, Canada). Samples from each well were plated on LB-Amp plates, and CFU/well was determined.

Construction of the bacteriophage-based biosorbent

The bacteriophage-based biosorbent was prepared as follows: bacteriophage was prepared and purified according to the method

described by Sambrook *et al* [6]. The purified bacteriophage was resuspended in 1 ml of 0.85% saline and then biotinylated with sulfosuccinimidobiotin (Sulfo-NHS-Biotin, Pierce, Rockford, IL, USA) at room temperature for 30 min, followed by dialysis against 0.85% saline at room temperature for 24 h. The phage infectivity was examined by the soft agar overlay technique [6] following biotinylation and dialysis, respectively. The biotinylated phage was then coated onto streptavidin-labeled magnetic beads by rocking 100-μl beads in phage solution overnight at room temperature. The constructed phage-based biosorbent was then collected, resuspended to 100 μl in saline, and used to capture the target cells of *S. enteritidis*. The bacteriophage without biotinylation was subjected to the same procedure as the biotinylated phage. Magnetic beads coated with the unbiotinylated phage were employed as a control for the following bioluminescent assay.

Bioluminescent assay

The bioluminescent culture of *S. enteritidis* was grown in LB broth supplemented with 100 μg/ml of ampicillin to OD_{600}=1.0. The cells were collected by centrifugation (14,000 rpm, 2 min), washed once with LB broth, and resuspended in LB broth. *S. enteritidis* cells (1 ml) were mixed with 20 μl of biosorbent and rocked at room temperature for 20 min in a 1.5 ml-Eppendorf tube. The captured *S. enteritidis* cells on beads were collected by a magnetic particle separator (Boehringer Mannheim Canada, Laval, Quebec, Canada). The beads were washed once with 1 ml of LB broth, and then resuspended in 1 ml of LB broth. The capture efficiency of *S. enteritidis* by the biosorbent was assessed by comparing the RLU of the captured cells to the RLU of original *S. enteritidis* cells. Magnetic beads coated with the unbiotinylated phage, were employed as a control. The experiment was repeated three times.

Results and discussion

Correlation between bioluminescence and viable cell numbers

A strong correlation (log RLU=0.9797log CFU -2.3583, R^2=0.9982) (Figure 1) was observed between the luminescent signal (RLU) and colony-forming units (CFU), suggesting the reliability of bioluminescence for assessing the number of cells present in a sample. This linear relationship was also observed when 20 μl/ml of beads were added to the sample; however, the RLU was reduced by 50%, indicating that the beads can interfere with light output from bioluminescent cells (data not shown). By measuring the RLU and then referring to the calibration curves, the viable cell number can be estimated easily and in real time.

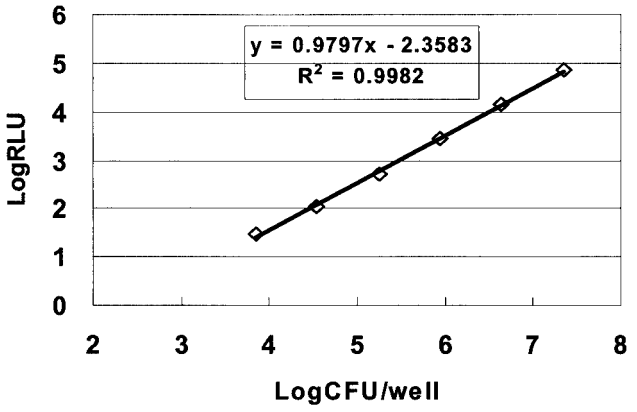

Figure 1 Correlation of luminescent signal (log RLU) and viable counts (log CFU) of bioluminescent *S. enteritidis* strain in a 96-well microplate at room temperature. Fivefold serial dilutions of the culture were prepared in triplicate in LB broth with 200 μl per well. The total RLU in each well was measured by a microplate luminometer. Samples from each well were plated, and CFU/well was determined.

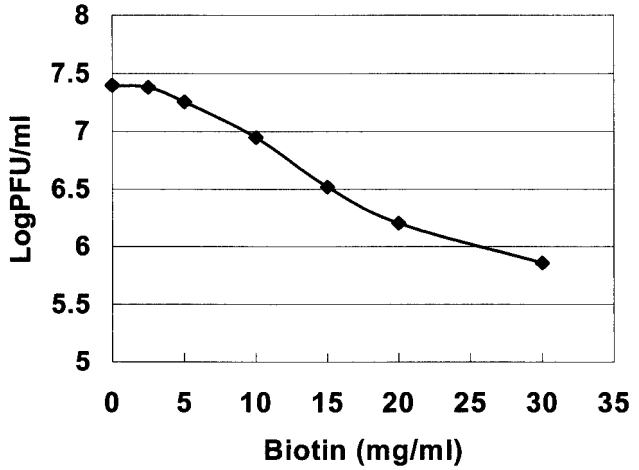

Figure 2 Effect of the biotin concentration on the infectivity of bacteriophages. Bacteriophage in 0.85% saline was biotinylated using different concentrations of biotin at room temperature for 30 min. The phage number, expressed as plaque-forming units (PFU)/ml, was determined by the soft agar overlay technique [6]. Each value in the figure represents the mean of three replicates.

Biotinylation of bacteriophage

Biotinylation of bacteriophage is the first step to construct the phage-based biosorbent. In this experiment, sulfosuccinimidobiotin, a biotin derivative that reacts with the phage surface protein through primary amines was used. Ideally, the phage head protein will be biotinylated and leave the phage tail free to attach to the host bacterial cells. Therefore, the biotin concentration is critical to optimize binding to the head while retaining maximum phage

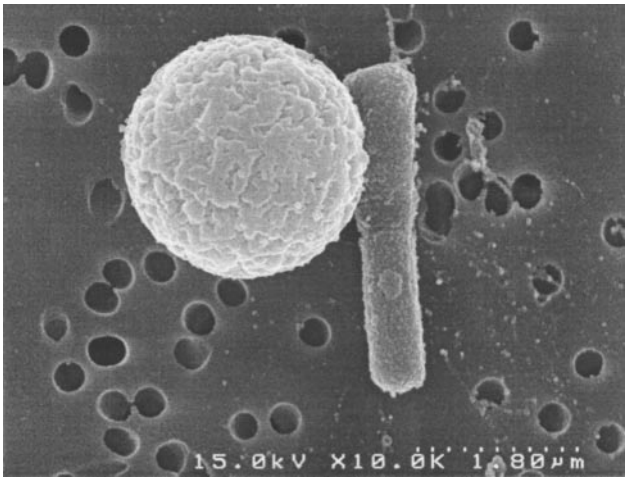

Figure 3 Scanning electron micrograph of an *S. enteritidis* cell captured by the constructed biosorbent.

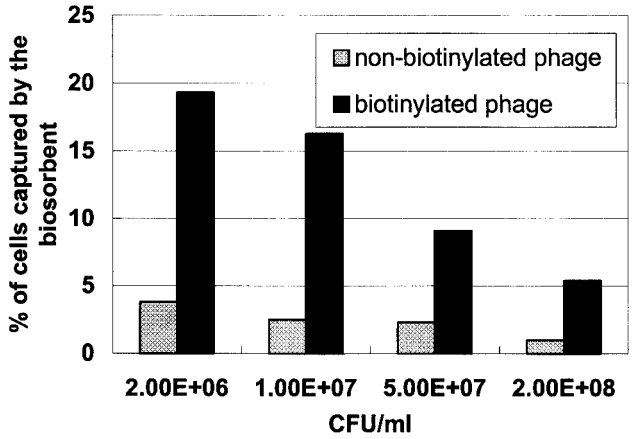

Figure 4 Percentage of *S. enteritidis* cells captured by the constructed biosorbent. Twenty microliters of the constructed biosorbent was used to capture the cells in 1 ml of LB medium. The percentage of capture was evaluated by comparing the RLU of the captured cells with that of the original of *S. enteritidis* cells. Magnetic beads coated with the unbiotinylated phage were used as a control. Each value in the figure represents the mean of three replicates.

activity. Figure 2 shows the effect of biotin concentration on phage activity. When the biotin concentration was low (<2.5 mg/ml), phage activity was not affected by the biotin used. The phage activity decreased 1.6 log cycle as the biotin concentration increased to 30 mg/ml. Therefore, 2.5 mg/ml biotin was chosen to biotinylate the bacteriophage.

Evaluation of the constructed biosorbent

The phage-based biosorbent was constructed by coating the biotinylated phage to streptavidin-labeled magnetic beads *via* biotin–streptavidin interaction following dialysis to remove the unreacted free biotin. The biosorbent was then employed to capture the *S. enteritidis* cells in LB broth (Figure 3). The captured cells were evaluated by *in vivo* bioluminescence as shown in Figure 4. The percentage of cells captured by the biosorbent increased from 5.4% to 19.3% as the *S. enteritidis* cell number decreased from 2×10^8 to 2×10^6 CFU/ml, suggesting that the ratio of beads to *S. enteritidis* cells was a major factor influencing the capture efficiency of the biosorbent. Furthermore, our constructed biosorbent showed a significant improvement to the systems described by Bennett *et al.*, [1] which required a culture of 10^7 CFU/ml to ensure enough cells (10^5 CFU/ml) were captured to generate a detectable PCR product (that represents a capture efficiency of <1%). With the aim of detecting low numbers of *S. enteritidis* cells in food samples, further experiments are being conducted to optimize parameters for coating the beads, bead sizes, and the ratio of beads to cells, to increase the efficiency of cell capturing by the biosorbent and decrease nonspecific binding.

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