Use of the MicroFoss system for the detection and enumeration of *Escherichia coli* in oysters

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Summary

E. coli **is used as an indicator of depuration and an index of pathogens in oysters. A low limit of detection is required, and current methods have proven problematic. Using the semi-automated MicroFoss system, very low levels (~1 cfu) of *E. coli* were detected in less than 9 hours, with high accuracy (*r* = - 0.97). The MicroFoss system was found to be sensitive, accurate and efficient for the enumeration of *E. coli* in oyster flesh.**

Introduction

In Australia, as elsewhere, oysters have been identified in several outbreaks of bacterial or viral disease as the vehicle of transmission [5]. To minimise the likelihood of carriage of human pathogens, oysters produced commercially must undergo a process of *depuration*, during which they disgorge their gut contents [7].

While bacteriophage may be a more appropriate index of human viral pathogens, *E. coli* remains as the standard indicator of adequacy of depuration, especially as an index of bacterial pathogens [2, 3]. After depuration, oysters should contain less than 2.3 *E. coli*/g. To achieve this level of sensitivity in detection, undiluted oyster flesh must be analysed either by the direct plate method, using multiple plates, or using the most probable number (MPN) method. Aspects of both of these methods are problematic.

An alternative system, potentially offering automation and acceleration of analysis as well as the required sensitivity, is the MicroFoss (Figure 1) [1]. The method relies on optical detection of biochemical activity by a general population or specific organism (Figure 2). To date, the system has been applied to determination of several microbial analytes [1, 4, 6, 8, 9], among them *E. coli* [1], in a range of foods including ground beef [8], chicken carcasses [8] and ground pork [9]. Oysters have not been examined using this technology.

The aim of this study was to investigate the use of the MicroFoss system for the enumeration of *E. coli* in oysters.

Materials and Methods

Media for conventional tests, including Lauryl Tryptose broth, EC broth, EMB agar and Tryptone water, were supplied by Oxoid Australia (Melbourne). The MicroFoss system and *E. coli* vials were supplied by Foss Pacific (Sydney). Sydney Rock Oysters *(Saccostrea commercialis)* were obtained from a commercial farmer. Five strains of *E. coli*, obtained as field isolates from sewage, were used as a cocktail for artificial inoculation.

The most probable number (MPN) and direct plate methods were performed according to Australian Standard method AS1766.2.3 [10] and AS1766.2.12 [11] respectively. A protocol for analysis of *E. coli* in oysters using the MicroFoss was developed using variables including the volume of oyster flesh used and the volume of diluent (0.1% peptone water) used. The incubation temperature (42°C) and initial operational settings of the MicroFoss instrument were recommended by Foss Pacific, based on analyses for *E. coli* in other flesh foods. Data points for a calibration curve were derived from comparative analysis of oyster samples using the MicroFoss and MPN methods. A depuration experiment, in duplicate, was monitored using MPN, direct plate, and MicroFoss methods. Non-*E. coli* isolates from vials were identified using the ID32GN system (bioMérieux, Sydney).

Results

The most consistent curves, also yielding the greatest change in light transmission, were obtained when 3ml of stomached oyster flesh and 2ml of peptone water were added to the *E. coli* vial (Figure 3, blue, green). When *E. coli* was present, the increase in light transmission was typically substantial (Figure 3, blue and green).

Figure 1. The MicroFoss MF128 system.

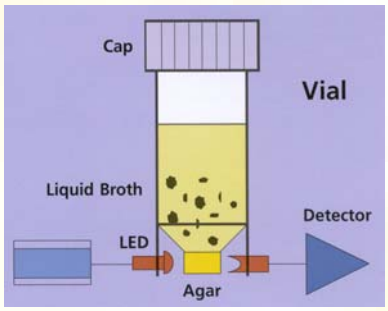

Figure 2. Principle of MicroFoss detection. Light passes from an LED through an agar plug to a detector. Metabolites formed during growth in the liquid medium change the indicator in the plug, in turn altering the intensity of light at the detector. At a given time, related to the initial microbial population, the change is recognised by the system as significant and a detection is recorded. Detection times are correlated with counts from standard methods, and a calibration curve is established.

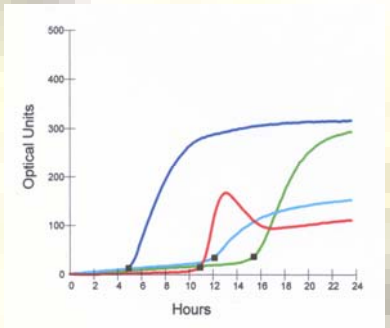

Figure 3. Curves obtained during analysis of oysters: contaminated with high (blue) and low (green) levels of *E. coli*, *Enterobacter* (light blue) or a mixed flora including *E.coli* (red).

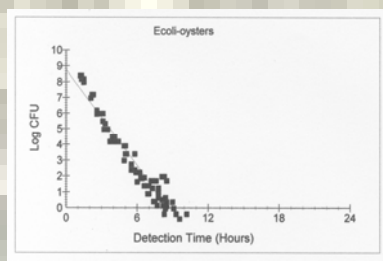

Figure 4. Calibration curve for analysis of *E. coli* in oyster flesh.

Results (continued)

The final protocol for detection used incubation at 42°C, a detection threshold of 12, a skip factor of 1, a shuteye period of 2 h, a cutoff time of 8 h, a caution time of 10 h and a test duration of 12 h. Although there was some variability in the time to detection (DT), statistically a single cfu of *E. coli* was detected in approximately 8.8 h. Using the established protocol, 76 data points, across a 9-log cycle, were used to construct a calibration curve by comparison with matched data from MPN analysis (Figure 4). The curve is described by the equation:

$$\log \text{cfu/ml} = 8.777 - 1.020 \times DT \quad (r = -0.97)$$

Monitoring *E.coli* in oysters during the depuration process was achieved with greater sensitivity and efficiency using the MicroFoss than with the MPN or direct plate method (data not shown). *E. coli* was detected occasionally at low levels in uninoculated control oysters and depuration samples when testing proved negative by the MPN or direct plate methods. Such samples were confirmed positive through plating and identification from the vials.

For some samples, an initial sharp rise in light transmission was curtailed, and transmission began to decrease then plateau (Figure 3, red). Upon plating, these samples yielded a mixed flora. For other samples, a smaller increase in transmission was observed with a late detection time (Figure 3, light blue). In both cases, non-*E.coli* isolates were identified as members of the genus *Enterobacter*.

Discussion

This poster describes the detection and enumeration of *E. coli* in oysters, representing the first research performed on the MicroFoss system in Australia and the first analysis of oysters on the platform.

The correlation coefficient of -0.97 compares very favourably with those published for any other MicroFoss protocols in which other foods, and a range of microbial analytes, including *E. coli*, were enumerated [8, 9].

The method is considered highly effective for routine analysis of oysters during depuration, as part of the quality assurance process. Using the established calibration curve, oyster homogenate simply has to be added to a vial, and the position of the vial entered into the instrument. A result below the required threshold of 2.3 cfu/g will be obtained within 12 h.

While *Enterobacter* was isolated from some vials for which a detection was registered, this was not considered a major problem for routine analysis of *E. coli* in oysters. When *E. coli* was present, a typical sharp rise in transmission was observed, and the detection time correlated well with the MPN result. During routine testing, observation would reveal any atypical curves that could then be investigated further.

The atypical curves when *Enterobacter* was present can be explained in terms of the metabolism in this genus. While *E. coli* exhibits a mixed acid fermentation, producing significant quantities of various acids from lactose, *Enterobacter* produces more neutral end products, resulting in less change in the bromocresol purple indicator in the medium in the vial. In conclusion, the MicroFoss system offers an efficient and accurate approach to the enumeration of *E. coli* in oysters.

Acknowledgements

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References

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