International Food Research Journal 31(5): 1282 - 1297 (October 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Ornithine-decarboxylase activity assay during enrichment: Early identification of *Escherichia coli* O157:H7-free food samples

[1]*Sangadkit, W., [2]Kaewboonruang, S. and [3]Wattanachaisaereekul, S.

[1]Food Safety Management, Division of Technology Fermentation, School of Food Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand
[2]Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand
[3]Division of Technology Fermentation, School of Food Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

Article history

Received:
9 May 2024
Received in revised form:
18 August 2024
Accepted:
27 August 2024

Keywords

ornithine decarboxylase,
Escherichia coli O157:H7,
phenol red,
novobiocin,
early detection,
foodborne pathogens

Abstract

Enrichment step is a cell amplification step essential for all detection of foodborne pathogens. The present work developed an early identification of finished food samples free from *Escherichia coli* O157:H7 contamination. Our work exploited the activity of ornithine decarboxylase (ODC) along with its pH indicator and a selective inhibitor. In the presence of the target bacteria, the enrichment broth changed colour significantly due to the diauxic growth during sugar fermentation and ODC activity. Among all conditions, phenol red and 0.028 g/L novobiocin were found to be optimal and selective for determining the presence or absence of *E. coli* O157:H7. When tested on real food samples, the proposed method yielded results that agreed well with the conventional method (Cohen's kappa = 1.00). The present work provided a new method for quickly determining if finished food products are free from *E. coli* O157:H7 contamination.

DOI
https://doi.org/10.47836/ifrj.31.5.16

Introduction

Enterohaemorrhagic *Escherichia coli* serotype O157:H7 is a foodborne pathogen with a low infectious dose (10 - 100 cells), and can exert fatal effects (MacDonald *et al*., 1988; Zhang *et al*., 2016; Saeedi *et al*., 2017). Patients infected with *E. coli* O157:H7 often have bloody diarrhoea and haemolytic uremic syndrome (Jay, 2000; Karch *et al*., 2005). Different types of food have been associated with *E. coli* O157:H7 outbreaks, such as rice cake, mayonnaise, dried fermented sausage, and uncooked meat (Han and Linton, 2004; CDC, 2013). The outbreaks have immense economic effects such as medical expenses, missed productivity, and losses from early death (CDC, 2019). In addition to illnesses and deaths, food industries are affected when contaminated products must be recalled (McKenzie and Thomsen, 2001; Alocilja and Radke, 2005).

Therefore, there is an urgent need for the detection of *E. coli* O157:H7 using a cost-effective, high-throughput approach to prevent the outbreak of epidemics (Conrad *et al*., 2014; Wang *et al*., 2017). The "gold standard" method starts with selective enrichment, followed by separation on agar media, and then serological or biochemical validation (Abdel-Latef and Zeinhom, 2014). These steps require a minimum of three days to yield results (Murakami, 2012; Niessen *et al*., 2013; Feng *et al*., 2016). It is impractical to use such lengthy, multistep processes to screen many food samples (Shelef and Tan, 1999). Further, these methods frequently yield false negative results due to excessively high concentrations of inhibitory agents. Moreover, the false negative results may occur in cases with a small number of cells or injured cells (Vimont *et al*., 2006). Despite these disadvantages, numerous regulatory and standard bodies for food have generally adopted

*Corresponding author.
Email: wipavadee.sa@kmitl.ac.th

these processes. Alternatively, there have been many approaches for screening, including PCR (Angus *et al*., 2015; Bian *et al*., 2015), ELISA (Arbault *et al*., 2000; Sunwoo *et al*., 2006; Song *et al*., 2016), PCR-ELISA (Amani *et al*., 2015), a microelectrode array biosensor (Alocilja and Radke, 2005; Varshney *et al*., 2007), and indirect immunofluorescence detection (Chen *et al*., 2015; Song *et al*., 2016). Nevertheless, these methods require costly supplies and complicated facilities that may not be suitable for the food industry. Often, the probability of contamination in finished food products, which are processed through rigorous food-safety control, is much lower than that in raw food materials. In this case, a screening test was performed to confirm that the samples were contamination-free. In the present work, we employed a key biochemical reaction associated with *E. coli* O157:H7 during enrichment, the absence of which implied that *E. coli* O157:H7 was not present.

To be practical for food production lines, methods must be able to detect pathogens at low infectious doses (Miller *et al*., 2011; Hossain *et al*., 2012; Song *et al*., 2016), identify contamination as early as possible, and handle a high volume of samples. Our strategy was to move a confirmation step (usually at the end of the methods) to an enrichment step (Sangadkit *et al*., 2020). Ornithine decarboxylase (ODC) activity has long been used as a biochemical test to confirm the presence of *E. coli* O157:H7. The bacteria contain ODC, which metabolises the amino acid ornithine to putrescine, increasing the broth pH (Cowan and Steel, 2004; FDA, 2005; Özogul and Özogul, 2007). On the basis of the unique biochemical activity of *E. coli* O157:H7, we observed colorimetric changes in the enrichment media using pH indicators, which were further validated using spectrometry. Our proposed method began with approximately 4 h of pre-enrichment to sufficiently grow cells to a sufficient cell density. Then, the ODC-activity assay was performed for another 18 - 20 h. From this testing, if the samples showed no ODC activity or a negative result, we identified the samples as "*E. coli* O157:H7-free." However, samples with positive ODC activity required further testing on selective or chromogenic agars as they could be false positives, including those from other Gram-negative Enterobacteriaceae. To the best of our knowledge, this colorimetric analysis was excluded in standard methods during the *E. coli* O157:H7 enrichment step.

Materials and methods

Culture preparation

E. coli O157:H7 (DMST 23156) and competing bacteria were obtained from the Department of Medical Sciences Thailand (DMST, Bangkok, Thailand) or the Thailand Institute of Scientific and Technological Research (TISTR, Bangkok, Thailand). The Gram-negative, ODC-positive, competitive bacteria, included *Serratia marcescens* (DMST 8845), *Proteus mirabilis* (TISTR 100), *Yersinia enterocolitica* (DMST 8012), *Enterobacter aerogenes* (DMST 8216), *Enterobacter cloacae* (DMST 15289), *Shigella sonnei* (DMST 561), *Salmonella* Enteritidis (DMST 15673), and *Salmonella* Typhimurium (TISTR 292). *Proteus vulgaris* (DMST 22842) represented Gram-negative, ODC-negative competitors. *Listeria monocytogenes* (DMST 17303), *Enterococcus faecalis* (DMST 4736), and *Staphylococcus aureus* (TISTR 808) were also included as ODC-negative pathogenic bacteria (Arroyo and Arroyo, 1995). All pure cultures were maintained on tryptic soy agar (TSA, Lab M, UK). Each strain was placed in one loop into 10 mL of tryptic soy broth (TSB, Lab M, UK) in a glass tube, and the mixture was cultured for 24 h at 37 ± 1°C in an isothermic environment. Serial dilutions were conducted in buffered peptone water with 0.1% (w/v) (BPW; Difco Laboratories, Sparks, MD, USA) to obtain a final cell density of approximately 2 - 3 log CFU/mL.

Media testing and pH indicators

ODC broth (ODB) containing tryptone (Difco Laboratories, Sparks, MD, USA), 4.5 g/L; soytone (USBiological, Salem, MA, USA), 3 g/L; D-glucose (Merck KGaA, Darmstadt, Germany), 1 g/L; sodium chloride (Merck KGaA, Darmstadt, Germany), 5 g/L; and L-ornithine, (USBiological, Salem, MA, USA) 5 g/L, was adopted from TSB (Lab M, UK). The four pH indicators namely bromothymol blue (BB; Acros Organics, Fair Lawn, NJ, USA), 0.065 g/L; thymol blue (TB; Acros Organics, Fair Lawn, NJ, USA), 0.065 g/L; bromocresol purple (BP; Fisher Scientific, Fair Lawn, NJ, USA), 0.02 g/L; and phenol red (PR; Acros Organics, NJ, USA), 0.08 g/L, were added to make ODB-BB, ODB-TB, ODB-BP, and ODB-PR, respectively, to test for the optimal pH indicator for the colorimetric detection of ODC activity. All ODBs were adjusted to pH 7.0 using NaOH (Carlo Erba, France) at 1 N using a pH electrode (Mettler Toledo,

uPlaceTM, NY, USA) and pH/ion meter (Mettler Toledo, S220 SevenCompactTM, NY, USA). Before use, all media were sterilised by filtering through a sterile nylon syringe filter membrane (13 mm dimension and 0.22 µm pore size; Filtrex, Bangkok, Thailand).

Measurement of media absorption using various pH indicators

Using a multichannel pipette (Biohit, Bohemia, NY, USA), 180 µL of ODB with various pH indicators at pH 7 were each placed into the microwells of a 96-well flat-bottom microplate (Corning, Tewksbury, MA, USA). To inoculate 20 µL of *E. coli* O157:H7 at 2 - 3 log CFU/mL, 180 µL of each medium was used, and the mixture was then incubated for 24 h at 37 ± 1°C. The absorption of each broth at wavelengths of 340, 405, 450, 490, 550, 600, and 650 nm was measured using a microplate reader (M965, Metertech, Taiwan). The absorption of the corresponding media (pH 7) in the absence of *E. coli* O157:H7 was considered "control." The quality of the colorimetric detection was represented by "the absorption change," which was defined as the difference between the absorption of the media with *E. coli* O157:H7 and that of the control at any wavelength. The higher the absorption change, the more accurate the colorimetric detection. From this experiment, the optimal pH indicators and wavelengths were identified using Eq. 1:

Absorption change = Absorption (media with *E. coli* O157:H7) - Absorption (control) (Eq. 1)

Spectral quantification of ornithine decarboxylation in E. coli O157:H7 and its competitors and correlation with viable cell counts

Absorption measurements of media by E. coli O157:H7 and its competitors

The optimal pH indicators and wavelengths were PR and 550 nm, respectively. Therefore, *E. coli* O157:H7 and its competitors were separately grown in ODB-PR. The inoculum of *E. coli* O157:H7 and each competitor was 20 µL of cell suspension at 2 - 3 log CFU/mL (prepared from single colony recovery in TSB, and diluted to the desired cell density in 0.1% (w/v) BPW). Each cell suspension was inoculated into the 180 µL of ODB-PR in a 96-well microplate. All samples were then incubated at 37 ± 1°C for 24 h. Absorption at 550 nm (A_{550}) was recorded every 3 h using a microplate reader. The microplate was shaken at 840 rpm for 5 s to disperse the cells in the broth before measuring A_{550}. Positive (o) results were defined as A_{550} of the sample, which was greater than A_{550} of the control (the sample without *E. coli* O157:H7). For the positive result, colorimetric detection showed the colour change to pink. Negative (x) values were defined as A_{550} of the sample, which was either less than or equal to A_{550} of the control, and showed no colour change to pink.

Viable cell count

The number of viable cells was counted every 3 h using the modified drop plate technique (MDPT) (Khueankhancharoen and Thipayarat, 2011). Ten-fold serial dilutions of incubated broth samples were prepared. A 10-µL aliquot of each dilution was placed onto 500 µL of solidified TSA (Lab M, UK) in a 96-well microplate. The microplate was incubated at 37 ± 1°C for 12 - 18 h. Visible colonies of viable cells were counted.

Study of inhibitors that suppress competitors but enrich E. coli O157:H7

Selective inhibitor cocktails from standard broth or agar recipes were tested to selectively enrich *E. coli* O157:H7 grown in ODB-PR while suppressing competitors. Enterohaemorrhagic *E. coli* enrichment broth (EHEC) was a modified TSB containing 0.0125 mg/L cefixime (USBiological, Salem, MA, USA), 10 mg/L cefsulodin (USBiological, Salem, MA, USA), and 8 mg/L vancomycin (USBiological, Salem, MA, USA). The modified EC medium (mEC+n) consisted of modified TSB containing 1.15 g/L bile salt (Difco Laboratories, Sparks, MD, USA) and 0.02 g/L novobiocin (Difco Laboratories, Sparks, MD, USA). The standard Sorbitol MacConkey Agar (SMAC-CT) contained 2 mg/L potassium tellurite (Himedia, India), 1.5 g/L bile salt, and 0.05 mg/L cefixime. The commercial selective agar, CHROMagar^{TM}O157, contained 1.5 g/L bile salt. After 24 h of enrichment, viable cells in these broths were counted using the MDPT method.

Optimisation of inhibitor concentrations

It was observed that ODB-PR with mEC+n as the selective inhibitor suppressed the growth of all competitive bacteria, whereas *E. coli* O157:H7 was not suppressed. Therefore, in this section, the optimal concentrations of this inhibitor mixture and its

individual selective agents (bile salt, 1.15 g/L; and novobiocin, 0.02 g/L) were further studied. The inhibitor concentrations in ODB-PR were: 0.862, 1.150, 1.437, and 1.725 g/L for bile salt; 0.015, 0.020, 0.028, and 0.033 g/L for novobiocin; and 0.877, 1.170, 1.462, and 1.755 g/L for the inhibitor mixture. All selective broths were adjusted to an initial pH of 7 (red), and then sterilised by filtration through a sterile nylon syringe filter membrane (13 mm diameter and 0.45 mm pore size; Filtrex, Bangkok, Thailand) before use. The 96-well microplates were loaded with 180 µL of various selective broths as previously mentioned, and each well was inoculated with 20 µL of pure 1 - 2 log CFU/mL *E. coli* O157:H7 or competitors. The microplates were incubated for 24 h at 37 ± 1°C in a stationary environment. The corresponding selective broth media without inoculation were used as controls. A_{550} of the samples and controls were measured using a microplate reader with distilled ionised water as a blank. Bacterial growth (as represented by decarboxylation) was expressed as positive (o) when A_{550} sample > A_{550} control, and negative (x) when A_{550} sample ≤ A_{550} control.

Determination of detection limit and Poisson distribution analysis of samples with low cell concentrations

We prepared samples with different cell densities (1.00, 1.30, 1.47, 2.09, 2.50, 2.90, 3.38, and 3.70 log CFU/mL) using the optimal formula (ODB-PR and 0.028 g/L novobiocin). The samples were incubated for different times: 0, 12, 16, 20, and 24 h. Each type of sample (specific cell density and time) was performed in 10 replicates (*i.e.*, $n = 10$). We also observed colour changes. If no colour change was observed, the sample was regarded as "false negative." The data were plotted in a heat map to indicate the percentage of false negatives, which can be used to indicate the detection limit of the proposed method.

Additionally, we used the Poisson distribution to determine whether the proposed method could be employed in a single-cell system. A number of false negative results were plotted against cell concentration, and the data points were fitted with the Poisson distribution. In this case, if a single cell can be detected, the false negatives, represented by the probability in the Poisson distribution, should be zero (El-Shaarawi *et al*., 1981; McKellar and Knight, 2000; Robinson *et al*., 2001).

Given knowledge of the number of cells required for detection, the cell concentration in the samples can be directly determined from the frequency of empty microwells. For example, regarding the probability of seeing zero cells, if single cells can sense the number of empty cells, they will be fitted by the Poisson distribution. Letting λ be the mean number of cells in the sample, then this probability would be exp (-λ). If two cells are required to be sensed, then one may fit the frequency of empty microwells as a function of concentration with the sum of the Poisson distribution for zero plus one cell: (1 +λ) × exp (-λ). These numbers are important because they represent the probabilities of false negatives as a function of cell concentration (Koyama *et al*., 2016). This method works clearly only in > 0% to < 100% filled microwells.

Testing food samples using the proposed method

Preparation of food samples for the proposed method

Turbid food samples of sterilised milk, all parts of cheeseburger, dried fermented sausage, and chilled fried rice containing meat were used to evaluate the efficiency of detecting ODC-positive bacteria. They are known as primary sources of *E. coli* O157:H7 contamination from food processing (Buchanan and Doyle, 1997; Han and Linton, 2004). A 25 mL sample of sterilised milk (which did not include any background bacteria) was contaminated with 1 mL of 1 log CFU/mL of (a) *E. coli* O157:H7, (b) *S.* Enteritidis, and (c) *P. vulgaris* (ODC-negative bacteria). Cheeseburger, dried fermented sausage, and chilled fried rice with meat, 25 g each (representing samples naturally contaminated with background bacteria) were spiked with 1 mL of 1 log CFU/mL *E. coli* O157:H7. After adding 225 mL of BPW (Difco Laboratories, Sparks, MD, USA) to each sample, the mixture was quickly homogenised in a stomacher (Interscience, 400 VW, Saint Nom, France) for 10 s at high speed. The same food samples were analysed for *E. coli* O157:H7 contamination using the reference method (USDA/FSIS, 2002) in parallel with the proposed presumptive screening method for *E. coli* O157:H7 based on ODC. The procedure was as follows: 25 g of food and 225 mL of BPW were homogenised and incubated at 37 ± 1°C for 4 h. A 20 µL sample aliquot was mixed with 180 µL ODB-PR and novobiocin in a sterile 96-well microplate. Then, the incubation was maintained at 37 ± 1°C for 20 h. The microplate was shaken for 5 s

at 840 rpm before measuring with an A_{550} microplate reader against pure water. Three replicates were used in all studies.

Testing food samples using the proposed method

The food samples were tested using the conventional method. The conventional method followed the main steps, as shown in Figure 1. First, 25 g of food was mixed with 225 mL of EHEC broth. The mixture was incubated at 37 ± 1°C for 24 h. Then, it was streaked onto SMAC-CT agar, and incubated at 37 ± 1°C for another 24 h. All typical colonies were picked and streaked onto trypticase soy agar in 0.6% yeast extract plates (TSAYE, Lab M, UK). At the end of the agglutination test, if the bacteria were present, they bound to the antibody (latex reagent), yielding a positive result.

In the proposed method (Figure 1), the homogenised samples between 25 g of food and 225 mL of BPW were incubated at 37 ± 1°C for 4 h for pre-enrichment that allowed cells to multiply to at least 1.7 log CFU/mL (in case only a single cell was present). An individual sample aliquot (20 µL) was pipetted into 180 µL of ODB-PR or ODB-PR containing novobiocin in a sterile 96-well microplate. As discussed later, ODB-PR with novobiocin was the optimal enrichment media for detection. All samples were then stationarily incubated at 37 ± 1°C for 20 h. The cells were dispersed throughout the broth by shaking the microplate for 5 s at 840 rpm before measuring with the microplate reader A_{550} against a blank of distilled ionised water. The controls were broth media without samples. The results were positive (o) when A_{550} sample > A_{550} control, and negative (x) when A_{550} sample ≤ A_{550} control.

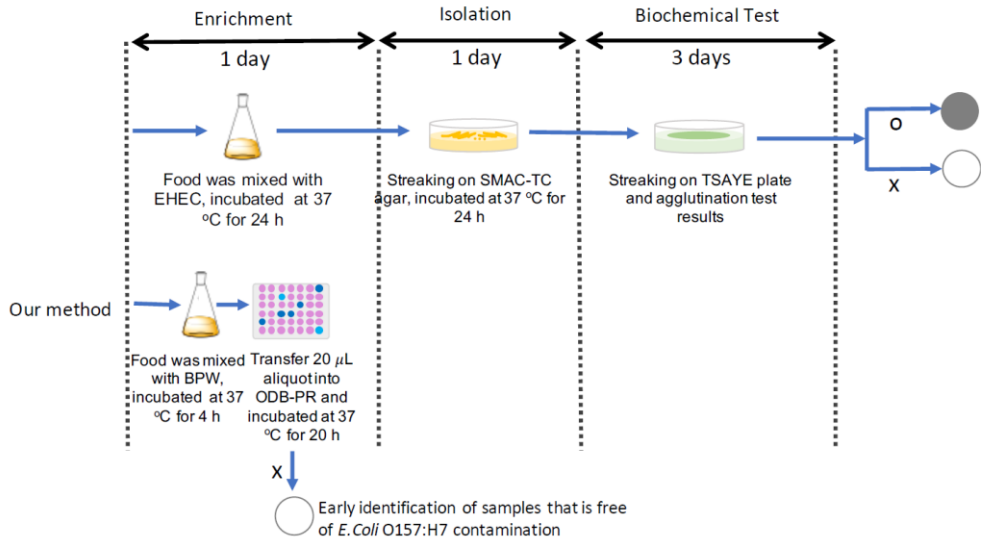


Figure 1. Flowchart of the proposed method compared with the conventional method for detecting *E. coli* O157:H7 in food samples. The symbols (o) and (x) represent positive and negative results, respectively. Dark grey and white circles represent *E. coli* O157:H7 contaminated and uncontaminated samples, respectively.

Confirmation, purification, and biochemical identification of E. coli O157:H7 (USDA/FSIS, 2002; Gulmez and Guven, 2005)

To validate our approach, the following sets of experiments were added to confirm whether *E. coli* O157:H7 was present in all food items examined in this investigation. First, after 20 h of enrichment in ODB-PR (with and without novobiocin), all culture samples were subjected to standard purification and biochemical identification techniques. The cultures were ten-fold diluted, streaked onto selective agar (SMAC-CT), and then incubated at 37 ± 1°C for 24 h. Next, the agar media colonies from the proposed and traditional procedures were streaked onto TSAYE plates. The purified colonies were collected and confirmed using a latex agglutination test (DR0620, Oxoid, UK). Positive results were interpreted as large clumps of agglutination with partial or complete clearing of the background latex within 1 - 2 min.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Analysis

of variance (ANOVA) was used to identify differences between treatments. A significance threshold of $p < 0.05$ was used to identify differences. Additionally, Cohen's kappa test was performed using a 2 × 2 contingency table to evaluate the agreement between the two methods when applied to food samples. Cohen's kappa was calculated and interpreted using the following ranges: poor, < 0.20; fair, 0.21 - 0.40; moderate, 0.41 - 0.60; good, 0.61 - 0.80; and very good, > 0.80 (Nordic system for validation of alternative microbiological methods (NordVal)).

Results and discussion

E. coli O157:H7 and other Gram-negative pathogens possess ODC, which has been used as a biochemical marker of *E. coli* O157:H7 in food and environmental samples. Routinely, suspected colonies were isolated from selective agar after sequential enrichment steps (Davis and Farmer, 1985). Following isolation, the conventional biochemical test required 5 mL of the ODB and two to three days for detectable change of the ODB colour from pale violet to purple (USDA/FSIS, 2002). Unlike clinical or faecal samples, most food samples, especially final or intermediate products, contained minimal or no contamination. The most common problem was false negative results due to strong inhibitors, which were intentionally applied to screen out competing bacteria (Fujisawa *et al*., 2000; 2002). By integrating the measurement of ODC activity into the enrichment step, we can identify early whether food samples are not contaminated with *E. coli* O157:H7.

Optimisation of colorimetric detection of E. coli O157:H7 in ODB

The broth with an initial pH of 7 served as the negative control. In the presence of any bacteria, glucose fermentation occurred, and the by-products of the fermentation caused a decrease in the pH. However, if bacteria with ODC activity were present, decarboxylation continued to occur, and the broth pH increased. Therefore, at the end of the testing, the broth containing ODC-negative bacteria remained acidic, whereas the ODC-positive bacteria decarboxylated, resulting in a basic condition. The colours changed because of bacterial growth in ODB with different pH indicators. Visually, BB in ODB-BB and PR in ODB-PR provided the most discernible colour change. For ODB-BB, the broth with *E. coli* O157:H7 changed from green to blue, whereas that for ODB-PR changed from orange to magenta.

Such colorimetric detection can be validated by spectrophotometry. An optimal pH indicator and wavelength were defined as conditions that gave the highest absorption change. As shown in Figure 2 and Table 1, we found that the highest absorption change was observed at 550 nm for ODB-PR. In ODB-PR, *E. coli* O157:H7 caused glucose fermentation, with an initial pH of 7.0 changing to a pH of 4.5. Such acidic conditions activated decarboxylase to convert ornithine to putrescine, an amine derivative, increasing the medium pH. The fact that ODB-PR was the optimal pH indicator indicated that the final pH of the decarboxylation was approximately 6.8 - 8.4, a pH range of PR. This value was similar to that reported previously (pH 8.5 - 9.0). During the selective enrichment of *E. coli* O157:H7, cell development likely produced turbidity, which could alter the broth's absorbance. For spectrophotometric measurements, clear samples are ideal because they reduce the errors caused by scattering, refraction, and reflection. Khueankhancharoen *et al.* (2016), however, reported that the initial *S.* Anatum inoculum (6 log CFU/mL) enriched for 24 h to achieve 8 - 9 log CFU/mL did not produce sufficient cell turbidity to effectively obstruct absorption at 550 nm.

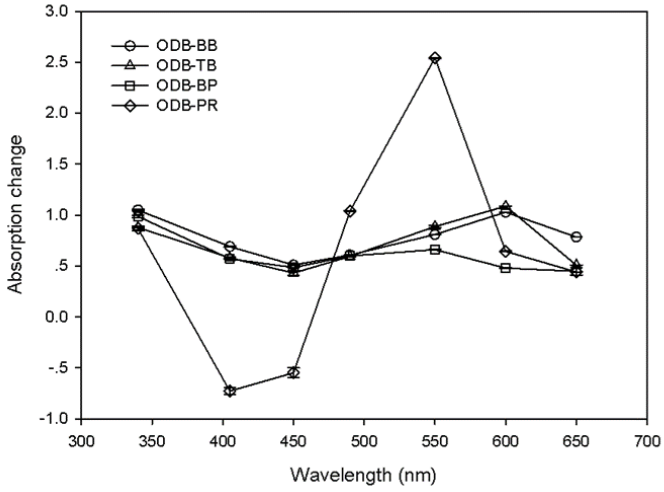


Figure 2. Absorption changes in four broths with different pH indicators: bromothymol blue (o), thymol blue (Δ), bromocresol purple ( ), and phenol red (◊). Each data point represents the mean of triplicates ± SEM.

Table 1. Maximum absorption change for each broth tested.

Broth	λ_{max} Maximum absorption change (nm)	Maximum absorption change
ODB-BB	340	1.05 ± 0.03^{b}
ODB-TB	600	1.08 ± 0.02^{b}
ODB-BP	340	0.98 ± 0.01^{c}
ODB-PR	550	2.54 ± 0.02^{a}

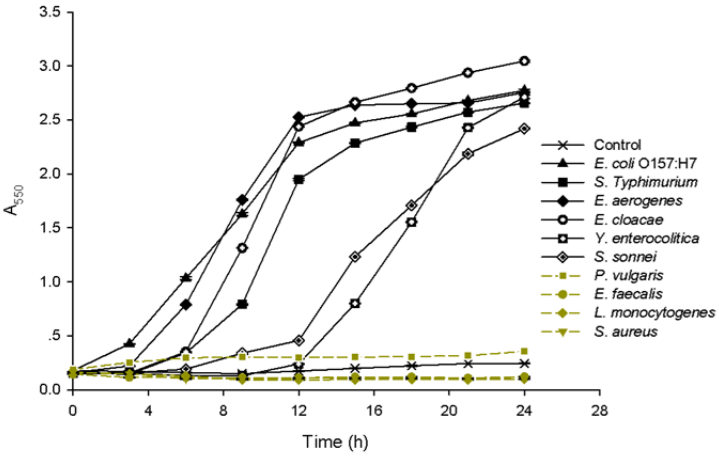
Values with different lowercase superscripts are significantly different ($p < 0.05$).

Therefore, in the present work, we assumed that the spectrophotometric measurement was not interfered with by turbid samples (up to 9 log CFU/mL). We also performed a *p*-value analysis among the samples without bacteria (control), and with ODC-negative and -positive bacteria. Using ODB-PR, we found that the control appeared orange and very similar to the original colour (t = 0 h), with an average absorbance reading of 1.236 ± 0.010. Apparently, due to glucose fermentation, the ODC-negative bacteria assimilated dextrose, decreased the medium pH from its starting value of 7.0 to 4.5, and provided yellow colour with the absorption of 0.568 ± 0.020. Conversely, ODC-positive bacteria showed magenta colour with the absorption of 3.284 ± 0.014. Using *p*-value analysis, we found that the results were significantly different in terms of absorption (ANOVA, $p < 0.05$).

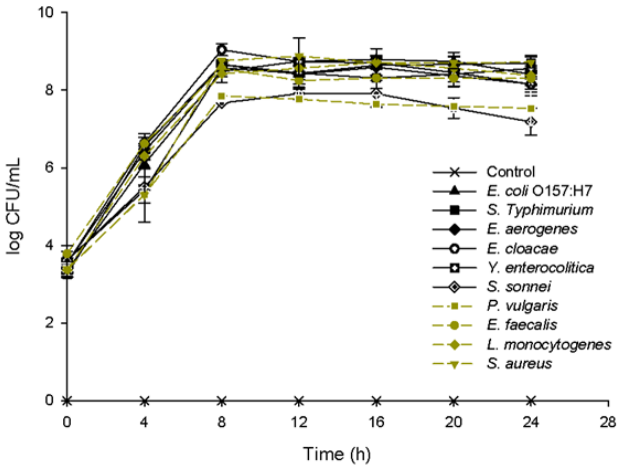
Time-dependent ODC activity and growth of E. coli O157:H7 and its competitors

ODB-PR was used as a broth to study the time dependence of ODC activity, and the growth of *E. coli* O157:H7 and its competitors. The absorption at 550 nm (A_{550}) was used as an indicator of ODC activity (Figure 3a). As shown in Figure 3b, the bacterial growth reached a stationary phase at approximately 3 h for *E. coli* O157:H7 and its competitors. Conversely, 12 h was required for ODC activity to become obvious. Some competitors, including *E. aerogenes*, *E. cloacae*, and *S.* Typhimurium, also showed ODC activity, which might have onsets and absorption values similar to those of *E. coli* O157:H7. However, among these four bacteria, *E. coli* O157:H7 had the earliest ODC activity onset. In contrast, *S. sonnei* and *Y. enterocolitica* exhibited ODC activity onsets later than others.

The long initial delay in absorption change was due to decarboxylation occurring only in the acidic broth following glucose fermentation. Some ODC-positive strains remained acidic longer than others, possibly due to slow ornithine metabolism (Viala *et al*., 2011). *E. coli* O157:H7 showed rapid onset of ODC activity, probably indirectly due to its sensing and adapting abilities to low pH conditions (Viala *et al*., 2011). It was postulated that protection against *E. coli* O157:H7 was provided by systems that included an antiporter and an associated amino-acid decarboxylase against acid shock (Audia *et al*., 2001). Other Gram-negative competitors, *S.* Typhimurium, *E. aerogenes*, *E. cloacae*, *Y. enterocolitica*, and *S. sonnei* possessed ODCs, and increased A_{550} (Figure 3a). *P. vulgaris*, a Gram-negative, but without ODC activity, and other ODC-negative strains (*E. faecalis*, *L. monocytogenes*, and *S. aureus*) showed unchanged A_{550}.



(a)



(b)

Figure 3. Time courses of absorption at 550 nm wavelength **(a)**, and growth curves for *E. coli* O157:H7 and some competitors **(b)** in ODB-PR. Each data point represents the mean of triplicates ± SEM.

Effects of standard inhibitors on cell growth and ODC activity

As earlier discussed, decarboxylation is a useful tool for detecting *E. coli* O157:H7. However, to differentiate *E. coli* O157:H7 from other Enterobacteriaceae, suitable selective inhibitors were added to ODB-PR to suppress the growth of competitive bacteria, and enhance selectivity for *E. coli* O157:H7. First, we needed to determine whether the inhibitors affected *E. coli* O157:H7 itself. However, the growth inhibition of *E. coli* O157:H7 had a significant difference among the four media (ANOVA, $p < 0.05$). Among the four inhibitors, only mEC+n did not affect the growth and ODC activity of *E. coli* O157:H7. Moreover, ODB-PR without inhibitors provided the corresponding results for mEC+n.

Table 2 shows that ODB-PR without inhibitors displayed no selective preference for supporting the growth of Gram-positive or -negative bacteria. All inhibitors inhibited the growth of all Gram-positive bacteria tested (*L. monocytogenes*, *E. faecalis*, and *S. aureus*), and some Gram-negative bacteria (*P. vulgaris*). The optimal selective inhibitors should allow only *E. coli* O157:H7 to grow but not other bacteria (regardless of ODC-positive or -negative). However, some ODC-positive bacteria (*E. aerogenes*, *E. cloacae*, *S.* Enteritidis, and *S.* Typhimurium) remained viable and exhibited positive ODC activity in the mEC+n broths. Subsequently, we further optimised the ratio of individual inhibitors to mEC+n, bile salt, and novobiocin to achieve the desired selectivity.

Table 2. Viable cell count and ODC (shown between the square brackets: [o] = A_{550} sample > A_{550} control; and [x] = A_{550} sample ≤ A_{550} control) in *E. coli* O157:H7, and competitive bacteria grown in ODB-PR with an mEC+n inhibitor, and incubated at 37°C for 24 h. The control was the corresponding broth without culture.

Test strain	Viable cell count (log CFU/mL) ± SEM, *n* = 3 [ODC activity]	
	No inhibitor	**mEC+n**
E. coli O157:H7	8.09 ± 0.35^{a} [o]	8.15 ± 0.40^{a} [o]
ODC-positive		
S. marcescens	7.82 ± 0.24^{a} [o]	4.96 ± 0.42^{b} [x]
P. mirabilis	7.65 ± 0.19^{a} [o]	0.00 ± 0.00^{b} [x]
Y. enterocolitica	8.12 ± 0.46^{a} [o]	3.68 ± 0.22^{b} [x]
E. aerogenes	7.57 ± 0.42^{a} [o]	6.93 ± 0.18^{a} [o]
E. cloacae	7.74 ± 0.35^{a} [o]	7.64 ± 0.22^{a} [o]
S. sonnei	7.60 ± 0.18^{a} [o]	4.16 ± 0.42^{b} [x]
S. Enteritidis	7.25 ± 0.22^{a} [o]	6.99 ± 0.12^{a} [o]
S. Typhimurium	6.69 ± 0.15^{a} [o]	6.12 ± 0.72^{a} [o]
ODC-negative		
P. vulgaris	6.52 ± 0.23^{a} [x]	0.00 ± 0.00^{b} [x]
L. monocytogenes	8.18 ± 0.08^{a} [x]	0.00 ± 0.00^{b} [x]
E. faecalis	7.50 ± 0.16^{a} [x]	0.00 ± 0.00^{b} [x]
S. aureus	7.86 ± 0.21^{a} [x]	0.00 ± 0.00^{b} [x]

[o] = colour change; [x] = no colour change. Values with different lowercase superscripts within similar row are significantly different ($p < 0.05$).

Further modification of mEC+n inhibitor in ODB-PR

To improve the selectivity of the proposed procedure, bile salt and novobiocin were studied as single inhibitors and mixtures with different concentrations. We carefully considered only a set of inhibitors that did not inhibit the ODC activity of *E. coli* O157:H7, but instead suppressed the growth of other ODC-positive competitors. The ODC activities of *E. coli* O157:H7 and other bacteria that were rich in ODB-PR with novobiocin are shown in Table 3. Novobiocin's effectiveness against ODC-positive strains is attributed to its ability to inhibit DNA and RNA synthesis through direct action on template polymerase complexes involved in

Table 3. Effects of individual inhibitors (bile salt and novobiocin) and mixed inhibitors (mEC+n) at different ratios of bile salt and novobiocin on the ODC activity of *E. coli* O157:H7 and other bacteria enriched in ODB-PR. The effects were measured by A_{550} (o, positive, when A_{550} sample > A_{550} control; x, negative, when A_{550} sample ≤ A_{550} control).

Test strain	No inhibitor	Bile salt (g/L)				Novobiocin (g/L)				Bile salt + novobiocin; mEC+n (g/L)			
		0.862	1.150	1.437	1.725	0.015	0.020	0.028	0.033	0.862 0.015	1.150 0.020	1.437 0.028	1.725 0.033
ODC-positive													
E. coli O157:H7	o	o	o	x	x	o	o	o	x	o	o	x	x
S. marcescens	o	o	x	x	x	x	x	x	x	x	x	x	x
P. mirabilis	o	o	o	o	x	x	x	x	x	x	x	x	x
Y. enterocolitica	o	o	o	o	o	o	o	x	x	x	x	x	x
E. aerogenes	o	o	o	o	o	x	x	x	x	o	o	x	x
E. cloacae	o	o	o	o	o	x	x	x	x	o	o	x	x
S. sonnei	o	o	x	x	x	o	x	x	x	o	x	x	x
S. Enteritidis	o	o	o	o	o	x	x	x	x	o	o	o	x
S. Typhimurium	o	o	x	x	x	o	o	x	x	o	o	x	x
ODC-negative													
P. vulgaris	x	x	x	x	x	x	x	x	x	x	x	x	x
L. monocytogenes	x	x	x	x	x	x	x	x	x	x	x	x	x
E. faecalis	x	x	x	x	x	x	x	x	x	x	x	x	x
S. aureus	x	x	x	x	x	x	x	x	x	x	x	x	x

lipopolysaccharide (LPS) transport, which are critical for bacterial growth and division (Utsumi *et al*., 1990). The novobiocin concentrations varied from 0.015 to 0.033 g/L. At 0.028 g/L, *E. coli* O157:H7 strains were more resistant to novobiocin than the most ODC-positive strains. As a result, the colorimetric change was observed. *E. coli* O157:H7 strains exhibited greater resistance to novobiocin due to genetic and phenotypic variations that enhance their survival and adaptability (Hussein *et al*., 2019; Weinroth *et al*., 2023).

The ODC-positive competitors were *S. marcescens*, *P. mirabilis*, *Y. enterocolitica*, *E. aerogenes*, *E. cloacae*, *S. sonnei*, *S.* Enteritidis, and *S.* Typhimurium. Our results were in line with those of previous report (Palzkill, 2001), demonstrating that using novobiocin at an optimal concentration inhibited most ODC-positive bacteria, and it exhibited better selective inhibition than bile salt. For ODC-negative bacteria, they did not undergo decarboxylation; thus, colorimetric changes were not observed, as shown in Table 3. Therefore, novobiocin was an optimal inhibitor for screening the presence or absence of *E. coli* O157:H7.

Determination of detection limit and Poisson distribution analysis for samples with low cell concentrations

We investigated the minimum cell density required to accurately detect *E. coli* O157:H7. For the heat map shown in Figure 4, the proposed method required 2.50 log CFU/mL and 16 h to avoid false negatives. A Poisson distribution curve was fitted to the percentage of false negative data, as shown in Figure 5 and Table 4. The Poisson distribution is utilised in predicting single-cell presence in food samples by assigning varying cell numbers to each sample based on the distribution function (Aguirre *et al*., 2012). This model can help in understanding the distribution of microbial populations in food, aiding in microbiological analysis by predicting cell counts, and assessing the heterogeneity of microbial communities. Fitting the percentage of false negatives and cell density in food samples using the Poisson distribution involves estimating the average rate of occurrence (λ), and applying the Poisson model to predict the distribution of these events. The Independent Poisson Distribution (IPD), advanced statistical tests, and modelling techniques were used to enhance the accuracy and applicability of the

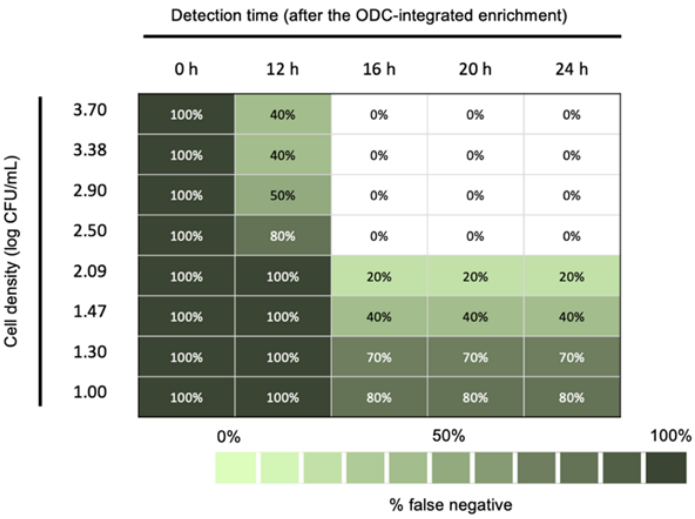


Figure 4. Heat map showing percentage of false negatives for different sets of experiments with different initial cell densities and enrichment times.

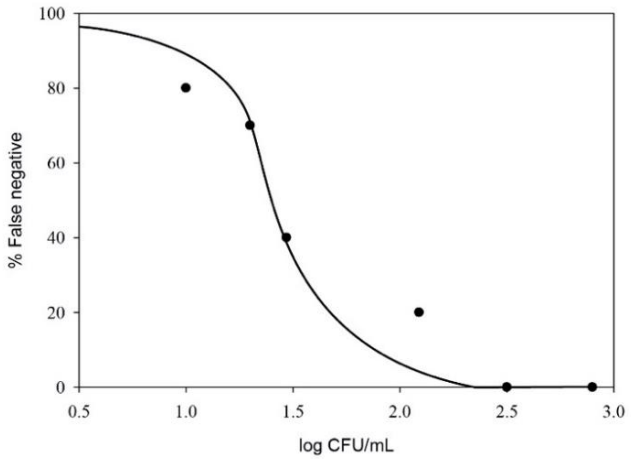


Figure 5. Percentage curve of false negatives and cell densities along with Poisson distribution fit which estimated the mean cell density to be 0.22 ± 0.04 cells/mL at 1 log CFU/mL.

Table 4. Percentage of false negatives at different log CFU/mL levels based on raw and fitted (Poisson distribution) data.

Log CFU/mL based on raw counts	Log CFU/mL based on fitted counts	% False negative result
1.00	0.85	80
1.30	1.27	70
1.47	1.68	40
2.09	2.09	20
2.50	2.50	0
2.90	2.91	0

Poisson distribution (Brizzi *et al*., 2012; Pan *et al*., 2023). The fitting curve assumes that the detection was sensitive to a single cell. The curve fitted quite well to the data point (R^2 = 0.94). Based on the fitting curve, the calculated mean (μ) of the number of cells was approximately 0.22 ± 0.04 at the sample of 1 log CFU/mL. The number was very close to our expectation (0.2 cells) because we performed the detection in a volume of 0.02 mL for each microwell. If the sample has a cell density of 1 log CFU/mL (*i.e*., 10 cells/mL), then, approximately 0.2 cells would be expected in the sample. Therefore, our assumption that the proposed method detected a single cell was verified.

Since it was possible to detect a single pathogenic cell in a sample, the detection limit was governed by the sampling error implied by the Poisson distribution (*i.e*., the probability of the false negatives). Therefore, it is important to develop enrichment for adequate cell growth to eliminate false negatives. In the present work, we investigated the growth of *E. coli* O157:H7 from 0 log CFU/mL (*i.e*., 1 CFU/mL) in BPW. BPW is a commonly used non-selective pre-enrichment broth for the resuscitation of *E. coli* O157:H7 in various food products (Sata *et al*., 2003; Park *et al*., 2011). The cells were able to multiply to 2.50 log CFU/mL within 4 h. As shown earlier, an initial cell density of 2.50 log CFU/mL, and further enrichment for 20 h led to a false negative rate of zero. Regarding the detection of low cell density, this confirmed that the proposed method required 4 h of pre-enrichment in BPW, and another 20 h of ODC-integrated enrichment. The bacterial growth curve was fitted using a modified logistic model (Gibson *et al*., 1987).

Validation of the proposed method using spiked food samples

ODB-PR containing 0.028 g/L novobiocin was tested on real food products spiked with *E. coli* O157:H7. The objective was to show that the proposed method could accurately determine whether food samples were free from *E. coli* O157:H7 contamination. Sterilised and non-sterilised food products were included in this study, as shown in Table 5. For comparison, the proposed method using ODB-PR (with and without novobiocin) and the conventional method were performed on the same batch of food samples. The conventional method involved enrichment in broth containing cefixime, cefsulodin, and vancomycin; then performing analysis in SMAC-CT agar containing potassium tellurite and cefixime (Figure 1). The ODB-PR with 0.028 g/L novobiocin and confirmation tests were in good agreement for the sterilised and non-sterilised samples. All samples that were spiked with *E. coli* O157:H7 showed positive results in all tests. The results for artificial and natural contaminated samples obtained from both detection methods provided the corresponding results when using a 2 × 2 contingency table. This test revealed that the proposed method using with ODB-PR with novobiocin had an "excellent" agreement with the conventional method (Cohen's kappa = 1.00).

Comparison of results from ODB-PR with and without novobiocin is shown in Table 5, columns 5 and 6. Novobiocin selectively prevented the growth of the ODC-positive bacteria, *S.* Enteritidis, from growing in sterilised milk spiked with *S.* Enteritidis, and background bacteria in chilled stir-fried rice containing meat. Therefore, 0.028 g/L novobiocin was an essential supplement to ODB-PR because it might increase the accuracy of the results, and inhibit additional background microorganisms. Additionally, the ODC-negative, *P. vulgaris*, gave negative results in sterilised milk, indicating that our procedure could distinguish ODC-negative from ODC-positive strains in real food products.

Non-sterilised food samples such as non-spiked chilled stir-fried rice containing meat might be naturally contaminated with bacteria, including *E. coli* O157:H7. Here, negative and positive results from ODB-PR, with and without novobiocin broth, were essential to indicate that the samples contained some bacteria, but not the pathogenic *E. coli* O157:H7. Subsequent steps should be further tested for other possible pathogenic bacteria, such as *S. aureus* and *B. cereus*, to cover the targeted pathogens that need to be detected by the food industry. With the proposed method for *E. coli* O157:H7 detection, the samples could be presumptively accepted as *E. coli* O157:H7-free due to the lack of ornithine decarboxylation. To apply this method to the food industry with the pre-enrichment step for better accuracy, it is highly recommended to pre-enrich food samples in BPW for at least 4 h to ensure a cell density of ≥ 2.5 log CFU/mL, and to resuscitate injured cells. ODB-PR with novobiocin was designed to screen the possibility of *E. coli* O157:H7 contamination with a decreased probability of dangerous false negative results through minimal use of selective inhibitors. With the optimal concentration

Table 5. Detection of *E. coli* O157:H7 in real food samples using three different methods: optical measurement of ODCs in simultaneous selective enrichment media (ODB-PR with novobiocin), non-selective enrichment media (ODB-PR), and standard conventional method. Some food samples were spiked with different bacteria, as shown.

Condition	Food sample	Spiked pathogen (number of sample)	Conventional method[1] (Feng *et al.*, 2016)	ODB-PR with novobiocin[2]	ODB-PR without novobiocin[2]	Confirmation test[3]
Sterilised product	Sterilised milk	None (6)	x	x	x	x
		E. coli O157:H7 (8)	o	o	o	o
		S. Enteritidis (8)	x	x	o	x
		P. vulgaris (8)	x	x	x	x
		E. coli O157:H7 + *S.* Enteritidis (8)	o	o	o	o
Non-sterilised product	Cheeseburger	None (6)	x	x	x	x
		E. coli O157:H7 (8)	o	o	o	o
	Dried fermented sausage	None (6)	x	x	x	x
		E. coli O157:H7 (8)	o	o	o	o
	Chilled stir-fried rice with meat	None (6)	x	x	o	x
		E. coli O157:H7 (8)	o	o	o	o

[1]Conventional method (o, positive, grey colonies with smoky centre; x, negative, no grey colonies with smoky centre). [2]Proposed method used ODB-PR (o, positive, A_{550} sample > A_{550} control; x, negative, A_{550} sample ≤ A_{550} control), where the control was the corresponding broth without cells. [3]Confirmation tests (o, positive for large clumps of agglutination; x, negative for no large clumps of agglutination).

of selective agents, the possibility of a false negative result was minimised. Our results showed that all samples (sterilised milk, cheeseburger, dried fermented sausage, and chilled fried rice with meat) spiked with *E. coli* O157:H7 could be detected in our ODB-PR media. Furthermore, the detection could be completed within one day, which was faster than the conventional method that uses a chromatic biochemical indicator, which typically took two to three days. The effectiveness of the media would be primary data helping for selection of the most effective formula. Nevertheless, further tests to validate this method with other *E. coli* strains are required.

Conclusion

ODC activity can be used as a tool for early detection of *E. coli* O157:H7 presence. In the present work, we integrated the ODC assay and enrichment step. Using an optimal pH indicator and selective inhibitors enabled us to determine whether the food samples were free from *E. coli* O157:H7 contamination. ODB employed PR as an optimal pH indicator, and novobiocin at a concentration of 0.028 g/L, an optimal inhibitor allowing the growth of *E. coli* O157:H7, but not other competitors that might lead to false positive results. Statistically, the Poisson distribution verified single-cell detection in the proposed method, which required 4 h of pre-enrichment and 20 h of enrichment. Pre-enrichment allowed sufficient growth of *E. coli* O157:H7 to approximately 2.5 log CFU/mL. At this cell density, we could further enrich the sample for another ≥ 20 h to obtain more accurate detection (*i.e*., false negative results should be avoided). Our experiment with real food samples demonstrated that the proposed method provided results that agreed well with the conventional method, but the detection time was reduced to only 24 h.

Acknowledgement

The authors would like to thank King Mongkut's Institute of Technology Ladkrabang and all the staff members of the division of Food Safety Management for all their support and assistance. The present work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The suggestions and comments from Asst. Prof. Dr. Noppon Weeranoppanant on this manuscript preparation are appreciated.

References

Abdel-Latef, G. K. and Zeinhom, M. M. A. 2014. Public health risk of some milk borne pathogens. Journal of Basic and Applied Sciences 3: 209-215.

Aguirre, J., Llatas, C. B., Ordóñez, J. A. and García de Fernando, G. D. 2012. The Poisson distribution is applied to improve the estimation of individual cell and micropopulation lag phases. Advances in Microbiology 2: 146-161.

Alocilja, E. C. and Radke, S. M. 2005. A high density microelectrode array biosensor for detection of *E. coli* O157:H7. Biosensors and Bioelectronics 20(8): 1662-1667.

Amani, J., Ahmadpour, A., Fooladi, A. A. I. and Nazarian, S. 2015. Detection of *E. coli* O157:H7 and *Shigella dysenteriae* toxins in clinical samples by PCR-ELISA. The Brazilian Journal of Infectious Diseases 19(3): 278-284.

Angus, S. V., Cho, S., Harshman, D. K., Song, J. Y. and Yoon, J. Y. 2015. A portable, shock-proof, surface-heated droplet PCR system for *Escherichia coli* detection. Biosensors and Bioelectronics 74: 360-368.

Arbault, P., Buecher, V., Poumerol, S. and Sorin, M. L. 2000. Study of an ELISA method for the detection of *E. coli* O157 in food. Food Biotechnology 17: 359-368.

Arroyo, G. and Arroyo, J. A. 1995. Selective action of inhibitors used in different culture media on the competitive microflora of *Salmonella*. Journal of Applied Bacteriology 78(3): 281-289.

Audia, J. P., Webb, C. C. and Foster, J. W. 2001. Breaking through the acid barrier: An orchestrated response to proton stress by enteric bacteria. International Journal of Medical Microbiology 291(2): 97-106.

Bian, X., Jing, F., Li, G., Fan, X., Jia, C., Zhou, H., ... and Zhao, J. 2015. A microfluidic droplet digital PCR for simultaneous detection of pathogenic *Escherichia coli* O157 and *Listeria monocytogenes*. Biosensors and Bioelectronics 74: 770-777.

Brizzi, M., Nani, D. and Betti, L. 2012. Poisson distribution and process as a well-fitting pattern for counting variables in biologic models. International Journal of High Dilution Research 11(40): 126-127.

Buchanan, R. L. and Doyle, M. P. 1997. Food disease significance of *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli*. Food Technology 51: 69-76.

Centers for Disease Control and Prevention (CDC). 2013. Multistate outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to ready-to-eat salads (final update). Retrieved on February 2, 2022 from CDC website: https://www.cdc.gov/ecoli/2013/O157H7-11-13/index.html

Centers for Disease Control and Prevention (CDC). 2019. Outbreak of *E. coli* infections. Retrieved on April 14, 2022 from CDC website: https://www.cdc.gov/ecoli/2019/o103-04-19/index.html

Chen, Z. Z., Cai, L., Chen, M. Y., Lin, Y., Pang, D. W. and Tang, H. W. 2015. Indirect immuno-fluorescence detection of *E. coli* O157:H7 with fluorescent silica nanoparticles. Biosensors and Bioelectronics 66: 95-102.

Conrad, C. C., Stanford, K., McAllister, T. A., Thomas, J. and Reuter, T. 2014. Further development of sample preparation and detection methods for O157 and the top 6 non-O157 STEC serogroups in cattle feces. Journal of Microbiological Methods 105: 22-30.

Cowan, S. T. and Steel, K. J. 2004. Theory and practice of bacterial identification. In Barrow, G. I. and Feltham, R. K. A. (eds). Manual for the Identification of Medical Science, p. 46-49. United Kingdom: Cambridge University Press.

Davis, B. R. and Farmer, J. J. 1985. H7 antiserum-sorbitol fermentation medium: A single tube screening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. Journal of Clinical Microbiology 22(4): 620-625.

El-Shaarawi, A. H., Esterby, S. R. and Dutka, B. J. 1981. Bacterial density in water determined by Poisson or negative binomial distributions. Applied and Environmental Microbiology 41: 107-116.

Feng, P., Weagant, S. D. and Jinneman, K. 2016. BAM Chapter 4A - Diarrheagenic *Escherichia coli*. In FDA Bacteriological Analytical Manual. United States: Food and Drug Administration (FDA).

Food and Drug Administration (FDA). 2005. Bacteriological analytical manual (BAM). United States: FDA.

Fujisawa, T., Sata, S., Aikawa, K., Takahashi, T., Yamai, S. and Shimada, T. 2000. Modification of sorbitol MacConkey medium containing cefixime and tellurite for isolation of *Escherichia coli* O157:H7 from radish sprouts. Applied and Environmental Microbiology 66(7): 3117-3118.

Fujisawa, T., Sata, S., Aikawa, K., Takahashi, T., Yamai, S. and Shimada, T. 2002. Evaluation of sorbitol-salicin MacConkey medium containing cefixime and tellurite (CT-SSMAC medium) for isolation of *Escherichia coli* O157:H7 from raw vegetables. International Journal of Food Microbiology 74: 161-163.

Gibson, A. M., Bratchell, N. and Roberts, T. A. 1987. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. Journal of Applied Bacteriology 62(6): 479-490.

Gulmez, M. and Guven, A. 2005. Evaluation of bile salt no.3, novobiocin, and cefixime-tellurite in enrichment and/or selective plating media for detection of *Escherichia coli* O157:H7 from beef. Turkish Journal of Veterinary and Animal Sciences 29(2): 251-256.

Han, Y. and Linton, R. H. 2004. Fate of *Escherichia coli* O157:H7 and *Listeria monocytogenes* in strawberry juice and acidified media at different pH values and temperatures. Journal of Food Protection 67(11): 2443-2449.

Hossain, S. M. Z., Ozimok, C., Sicard, C., Aguirre, S. D., Ali, M. M., Li, Y. and Brennan, J. D. 2012. Multiplexed paper test strip for quantitative bacterial detection. Analytical and Bioanalytical Chemistry 403: 1567-1576.

Hussein, S. H., Samir, R., Aziz, R. K. and Toama, M. A. 2019. Two putative MmpL homologs contribute to antimicrobial resistance and nephropathy of enterohemorrhagic *E. coli* O157:H7. Gut Pathogens 11: 15.

Jay, J. M. 2000. High-temperature food preservation and characteristics of thermophilic microorganisms. In Jay, J. M. (ed). Modern Food Microbiology, p. 347-369. United States: Springer.

Karch, H., Tarr, P. I. and Bielaszewska, M. 2005. Enterohaemorrhagic *Escherichia coli* in human medicine. International Journal of Medical Microbiology 295: 405-418.

Khueankhancharoen, J. and Thipayarat, A. 2011. Application of modified drop plate technique (MDPT) and logistic model to optimize non-selective substrates for *Salmonella* Typhi resuscitation. Asian Journal of Food and Agro-Industry 4(6): 349-358.

Khueankhancharoen, J., Saranak, J. and Thipayarat, A. 2016. Optimized microscale detection of amino acid decarboxylase for rapid screening of *Salmonella* in the selective enrichment step. Food Control 69(6): 352-367.

Koyama, K., Hokunan, H., Hasegawa, M., Kawamura, S. and Koseki, S. 2016. Do bacterial cell numbers follow a theoretical Poisson distribution? Comparison of experimentally obtained numbers of single cells with random number generation *via* computer simulation. Food Microbiology 60: 49-53.

MacDonald, K. L., O'Leary, M. J., Cohen, M. L., Norris, P., Wells, J. G., Noll, E., ... and Blake, P. A. 1988. *Escherichia coli* O157:H7, an emerging gastrointestinal pathogen. Results of a one-year, prospective, population-based study. Journal of the American Medical Association 259: 3567-3570.

McKellar, R. C. and Knight, K. 2000. A combined discrete-continuous model describing the lag phase of *Listeria monocytogenes*. International Journal of Food Microbiology 54: 171-180.

McKenzie, A. M. and Thomsen, M. R. 2001. The effect of *E. coli* O157:H7 on beef prices. Journal of Agricultural and Resource Economics 26(2): 431-444.

Miller, N. D., Davidson, P. M. and D'Souza, D. H. 2011. Real-time reverse-transcriptase PCR for *Salmonella* Typhimurium detection from lettuce and tomatoes. LWT - Food Science and Technology 44(4): 1088-1097.

Murakami, T. 2012. Filter-based pathogen enrichment technology for detection of multiple viable foodborne pathogens in 1 day. Journal of Food Protection 75(9): 1603-1610.

Niessen, L., Luo, J., Denschlag, C. and Vogel, R. F. 2013. The application of loop-mediated isothermal amplification (LAMP) in food testing for bacterial pathogens and fungal contaminants. Food Microbiology 36(2): 191-206.

Özogul, F. and Özogul, Y. 2007. The ability of biogenic amines and ammonia production by single bacterial cultures. European Food Research and Technology 225(3-4): 385-394.

Palzkill, T. 2001. Impending doom: Antibiotic exposure and bacterial gene expression. Genome Research 11(1): 1-2.

Pan, Y., Landis, J. T., Moorad, R., Wu, D., Marron, J. S. and Dittmer, D. P. 2023. The Poisson distribution model fits UMI-based single-cell RNA-sequencing data. BMC Bioinformatics 24: 256.

Park, S. H., Ryu, S. and Kang, D. H. 2011. Improved selective and differential medium for isolation of *Escherichia coli* O157:H7. Journal of Clinical Microbiology 49(1): 405-408.

Robinson, T. P., Aboaba, O. O., Kaloti, A., Ocio, M. J., Baranyi, J. and Mackey, B. M. 2001. The effect of inoculum size on the lag phase of *Listeria monocytogenes*. International Journal of Food Microbiology 70: 163-173.

Saeedi, P., Yazdanparast, M., Behzadi, E., Salmanian, A. H., Mousavi, S. L. and Nazarian, S. 2017. A review on strategies for decreasing *E. coli* O157:H7 risk in animals. Microbial Pathogenesis 103: 186-195.

Sangadkit, W., Nopphon, W. and Thipayarat, A. 2020. An integrated enrichment-detection platform for identification of contamination of *Vibrio parahaemolyticus* in food samples. LWT - Food Science and Technology 119: 108841.

Sata, S., Fujisawa, T., Osawa, R., Iguchi, A., Yamai, S. and Shimada, T. 2003. An improved enrichment broth for isolation of *Escherichia coli* O157:H7, with specific reference to starved cells, from radish sprouts. Applied and Environmental Microbiology 69(3): 1858-1860.

Shelef, L. A. and Tan, W. 1999. Automated detection of *Salmonella* spp. in foods. Journal of Microbiological Methods 37(1): 87 - 91.

Song, C., Liu, C., Wu, S., Li, H., Guo, H., Yang, B., ... and Liu, Q. 2016. Development of a lateral flow colloidal gold immunoassay strip for the simultaneous detection of *Shigella boydii* and *Escherichia coli* O157:H7 in bread, milk and jelly samples. Food Control 59: 345-351.

Sunwoo, H. H., Wang, W. W. and Sim, J. S. 2006. Detection of *Escherichia coli* O157:H7 using chicken immunoglobulin Y. Immunology Letters 106: 191-193.

United States Department of Agriculture and Food Safety Inspection Service (USDA/FSIS). 2002. MLG 5.03, rev. 10/25/02 - Detection, isolation and identification of *Escherichia coli* O157:H7 and O157:NM (nonmotile) from meat products. United States: USDA/FSIS.

Utsumi, H., Shibuya, M. L. and Elkind, E. E. 1990. Novobiocin inhibits the repair of potentially lethal damage but not the repair of sublethal damage. Radiation Research 123(1): 55-60.

Varshney, M., Li, Y., Srinivasan, B. and Tung, S. 2007. A label-free, microfluidics and inter-digitated array microelectrode-based impedance biosensor in combination with nanoparticles immunoseparation for detection of *Escherichia coli* O157:H7 in food samples. Sensors and Actuators B - Chemical 128: 99-107.

Viala, J. P. M., Meresse, S., Pocachard, B., Guilhon, A. A., Aussel, L. and Barras, F. 2011. Sensing and adaptation to low pH mediated by inducible amino acid decarboxylases in *Salmonella*. PLoS One 6(7): e22397.

Vimont, A., Vernozy-Rozand, C. and Delignette-Muller, M. L. 2006. Isolation of *E. coli* O157:H7 and non-O157 STEC in different matrices: Review of the most commonly used enrichment protocols. Letters in Applied Microbiology 42(2): 102-108.

Wang, T., Kim, S. and An, J. H. 2017. A novel CMOS image sensor system for quantitative loop-mediated isothermal amplification assays to detect food-borne pathogens. Journal of Microbiological Methods 133: 1-7.

Weinroth, M. D., Clawson, M. L., Harhay, G. P., Eppinger, M., Harhay, D. M., Smith, T. P. L. and Bono, J. L. 2023. *Escherichia coli* O157:H7 *tir* 255 T > A allele strains differ in chromosomal and plasmid composition. Frontiers in Microbiology 14: 1303387.

Zeinhom, M. M. A. and Abdel-Latef, G. K. 2014. Public health risk of some milk borne pathogens. Journal of Basic and Applied Sciences 3: 209-215.

Zhang, X., Li, Y., Li, B., Mao, Y., Wu, X., Zou, X., ... and Chen, H. 2016. Three supplementary methods for analyzing cytotoxicity of *Escherichia coli* O157:H7. Journal of Microbiological Methods 120: 34-40.