A COMBINATION OF THE MOST PROBABLE NUMBER TECHNIQUE AND COLORIMETRIC BACTERIOPHAGE-BASED ASSAY FOR QUANTITATIVE DETECTION OF *ESCHERICHIA COLI* O157:H7 IN CATTLE MANURE

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SUMMARY

A quantitative detection method of *Escherichia coli* O157:H7 using a combination of the most probable number technique and a colorimetric phage-based assay was developed. Firstly, a recombinant phage PP01ccp carrying the *ccp* gene was constructed and examined the detection of *E. coli* O157:H7 in broth. The oxidation activity towards the chromogenic substrate cytochrome c was demonstrated by the cytochrome c peroxidase (CCP) produced from the PP01ccp genome. The color change caused by the oxidation of the substrate could be visually perceived. Secondly, a combination of the detection method based on PP01ccp phage and the most probable number technique (MPN-phage assay) was developed and successfully applied to quantify two *E. coli* O157:H7 concentrations of 10^2 and 10^5 cells g^{-1} in cattle manure.

Keywords: Quantitative detection, colorimetric, bacteriophage, *Escherichia coli* O157:H7, cattle manure.

INTRODUCTION

Escherichia coli O157:H7 has been recognized as an important pathogenic *E. coli* from the first foodborne outbreak associated with the eating of undercooked hamburgers at a fast-food restaurant chain (Riley *et al*., 1983). *E. coli* O157:H7 causes approximately 73,000 illnesses in USA annually with the main epidemiological symptoms of severe diarrhea and hemolytic uremic syndrome (HUS). The *E. coli* O157:H7 outbreak has been also reported in many other countries (Isaacson *et al*., 1993; Chapman *et al*, 1989; Armstrong *et al*., 1996; Watanabe *et al*., 1999). Cattle and other ruminants have been considered as natural reservoirs for *E. coli* O157:H7. Previous studies revealed that prevalence of *E. coli* O157:H7 among cattle on farm or slaughter ranges from 1% to 9% (Wells *et al*., 1991; Zhao *et al*., 1995; Omisakin *et al*., 2003). The direct or indirect contact with cattle feces on farms or slaughter sites causes the *E. coli* O157:H7 incidents (Locking *et al*., 2001; Omisakin *et al*., 2003). In addition, *E. coli* O157:H7 can transmit from the original feces to human by many routes such as meats, dairy products, vegetables, water or human-to-human contact (Chekabab *et al*., 2013).

Therefore, quantitative detection of *E. coli* O157:H7 in fecal samples is important for the risk assessment and prevention of the diseases caused by *E. coli* O157:H7 (McCarthy *et al*., 1998; Kuboniwa *et al*., 2004; Fu *et al*., 2005). Direct plating method based on Sorbitol-MacConkey (SMAC) agar medium are commonly used in quantitative detection of *E. coli* O157:H7 which are based on sorbitol-negative characteristic of *E. coli* O157:H7 (Farmer & David, 1985; March & Ratnam, 1986). However, Hussein *et al* (2008) demonstrated that the sorbitol-negative characteristic is not unique for *E. coli* O157:H7 strains by showing several Shiga toxin-producing *E. coli* strains are also sorbitol-negative. It implied the inaccuracy of the direct plating method based on selective media although the method is simple and inexpensive. In addition, the direct plating method could be only applicable in enumeration of samples with high *E. coli* O157:H7 concentration due to its detection limit of 25-30 CFU per standard plate (Zhao *et al*., 1995; Goldman & Green, 2009). The quantitative detection of *E. coli* O157:H7 based on real-time PCR has been applied successfully to various environmental and food samples (Ibekwe *et al*., 2002; Fu *et al*., 2005; Elizaquível *et al*., 2012). However, the real-time PCR method enables quantitative detection of *E. coli* O157:H7 in soil, manure, feces with a high detection limit of 3.5×10^4 CFU g^{-1} (Ibekwe *et al*., 2002). In order to decrease enumeration limit of the above methods, most probable number (MPN)-based methods have been investigated. Jenkins *et al* (2009) used the MPN method to quantify *E. coli* O157:H7 concentration in surface waters based on the 4-methylumbelliferyl-β-D-glucuronide (MUG)-negative characteristic of *E. coli* O157:H7. However, Hussein *et al* (2008) demonstrated that not only *E. coli* O157:H7 strains are negative with MUG but also other Shiga toxin-producing *E. coli* own this characteristic. The MPN method combined with immunomagnetic separation (IMS) and SMAC agar was used to enumerate *E. coli* O157 strains in meat samples (Chapman *et al*., 2001) and cattle feces (Fegan *et al*., 2003; Fegan *et al*., 2004). In those researches, however, *E. coli* O157:H7 strains were only finally confirmed by using serotyping kits. In other words, the whole process of the enumeration is time consuming, complicated and costly (Fegan *et al*., 2004; Fedio *et al*., 2011).

The application of bacteriophages for the detection of specific bacteria is advantageous owing to the high specificity of the bacteriophages in host recognition (Richter *et al*., 2018; O'Sullivan *et al*., 2020). In the current study, a combination of the MPN method and colorimetric phage-based assay (MPN-phage assay) was introduced to specifically enumerate *E. coli* O157:H7 in cattle manure.

MATERIALS AND METHODS

Bacterial strains and bacteriophage

E. coli O157:H7 ATCC 43888 that does not produce Stx1 and Stx2 toxins was used as the host for the PP01 phage. The source of the *ccp* gene was *S. cerevisiae* IAM 4178. The general protocol for cloning involved the use of *E. coli* DH5α as host.

Activity evaluation of a recombinant phage

Construction of a recombinant phage PP01ccp carrying the *ccp* gene was previously described (Hoang & Le, 2015). For evaluation of the activity of CCP produced by the PP01ccp, *E. coli* O157:H7 was cultivated at 37°C until an OD_{600} of 0.5 (~1 x 10^8 CFU mL^{-1}) was attained. Then, the culture was divided into three aliquots, of which two aliquots were mixed with either PP01ccp or PP01wt phage lysate at M.O.I of 5.0. One aliquot was left blank without phage addition. The aliquots were incubated at 37°C for 1 h and then, they were passed through a 0.45-μm membrane filter to obtain filtrates. In addition to the filtrates, the LB medium was also used for the assay. The filtrates or the LB medium was mixed with buffer and substrate to obtain a 10-fold dilution. The mixture was incubated at 30°C and the ABS_{550} of the reaction solution was measured every minute using a spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan). All the enzyme assays were conducted in triplicates. Evaluation of the activity of CCP produced by the PP01ccp was referred the procedure shown by Hoang *et al* (2014) in which *E. coli* K12, T4ccp and T4wt were replaced by *E. coli* O157:H7, PP01ccp and PP01wt, respectively. The final concentrations of cytochrome c and H_2O_2 were 0.7 μM and 360 μM, respectively.

Enumeration of *E. coli* O157:H7 in cattle manure

In cattle manure, many background bacteria co-exist with the target *E. coli* O157:H7 and those background bacteria can outcompete with *E. coli* O157:H7. In order to suppress the growth of background bacteria, four types of antibiotics, i.e., novobiocin (N), cefixime (C), vancomycin (V) and potassium tellurite (T) were tested. *E. coli* O157:H7 was cultivated in BHI broth with addition of antibiotics as follows: BHI containing novobiocin (BHI-N) or BHI containing cefixime and vancomycin (BHI-C-V) or BHI containing cefixime, vancomycin and potassium tellurite (BHI-C-V-T) or BHI containing novobiocin, cefixime, vancomycin and potassium tellurite (BHI-N-C-V-T). Concentrations of potassium tellurite, novobiocin, vancomycin and cefixime were 2.5, 20, 40 mg L^{-1} and 50 μg L^{-1}, respectively. The *E. coli* O157:H7 cultures were incubated at 37°C, 200 rpm. OD_{600} was measured every hour.

Combinations of antibiotics, BHI-N and BHI-C-V that allowed the growth of *E. coli* O157:H7 in pure cultivation were examined whether they can enable the growth of *E. coli* O157:H7 in fecal sample by suppressing the background bacteria. Each one gram of fecal sample was inoculated by approximate 10^2 and 10^5 CFU g^{-1} *E. coli* O157:H7 that was examined by agar plate method. The sample was homogenized in 9 mL of BHI using a homogenizer (ACE Homogenizer; Nihonseiki Kaisha Ltd., Tokyo, Japan) for 10 minutes at 10,000 rpm. In the MPN-phage assay used to quantify *E. coli* O157:H7 concentration is the cattle manure, BHI medium plus with the suitable antibiotics were used in the incubation at 37°C, 200 rpm for 15 h. The resulting cultures were diluted 10 times and used for the phage infection and the enzyme assay as described above.

RESULTS AND DISCUSSION

Activity of CCP expressed from PP01ccp genome

The ABS_{550} change during the enzyme assays is shown in Figure 1. Since the results of the assay using the lysate obtained by the PP01wt infection of *E. coli* O157:H7 and the filtrate of the *E. coli* O157:H7 culture without phage addition were almost identical to the result obtained using LB medium without any bacterial inoculation, it was confirmed that the presence of *E. coli* O157:H7 or the lysis of *E. coli* O157:H7 by the infection of PP01wt did not affect the oxidation of substrate. In contrast, using the lysate obtained by the PP01ccp infection of *E. coli* O157:H7 in the assay resulted in a significant change in the ABS_{550}, and it was suggested that the CCP expressed from the PP01ccp genome contributed substantially to the oxidation of cytochrome c.

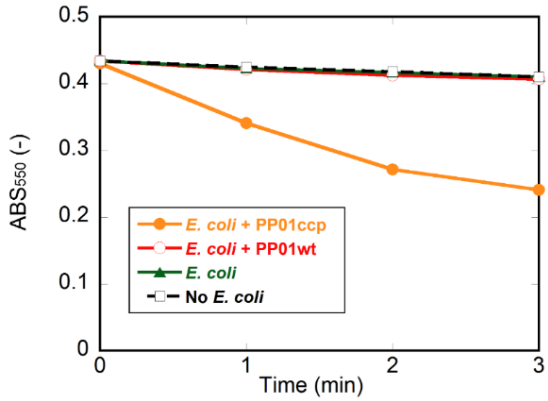

Figure 1. Change in the ABS_{550} during the enzyme assay and color change with lysate obtained from PP01ccp or PP01wt infection.

Therefore, detection of *E. coli* O157:H7 in broth could be conducted by using the PP01ccp phage. Next, the MPN-phage assay was applied to quantitatively detect *E. coli* O157:H7 in fecal sample.

Selection of antibiotics

Detection of *E. coli* O157:H7 in fecal samples is more difficult than the detection in apple juice, milk or other food samples since many other background bacteria co-existing with the *E. coli* O157:H7 in the fecal samples may inhibit the growth of *E. coli* O157:H7 (Hussein, Bollinger, 208). Therefore, antibiotics are generally used in detection of *E. coli* O157:H7 in fecal samples (Hussein *et al*., 2008). The antibiotics should suppress the growth of the background bacteria but do not affect growth of *E. coli* O157:H7. Previous researches showed some proper antibiotics that were used in the detection of *E. coli* O157:H7 in fecal samples. Cefixime was shown to inhibit growths of *Salmonella enterica serovar Typhi* and *Proteus spp*. without negative effects on growth of *E. coli* (Chapman *et al*., 1991). Novobiocin is known to inhibit various *Agrobacterium spp*., *Bacillus mycoides*, *Burkholderia cepacia, Staphylococcus spp.,* and *S. faecalis* but it poorly affects growth of negative bacteria (Hussein *et al*., 2008). Vancomycin inhibit a wide range of gram-positive and gram-negative bacterial species but do not affect *E. coli*. And Tellurite has broad bactericidal spectrum but do not inhibit growth of *E. coli* O157 strains (Hussein, Bollinger, 2008). In this study, combinations made by these four antibiotics were tested to select which combination were suitable for the detection. The four combinations of medium with antibiotics were tested based on previous references (O'Hanlon *et al*., 2004; Lejeune *et al*., 2004; Hussein *et al*., 2008).

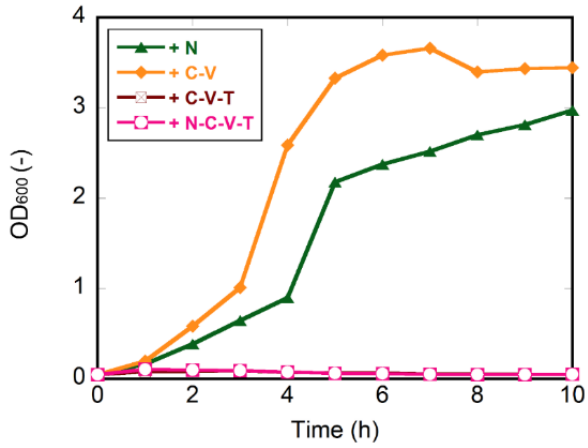

Figure 2. Effect of antibiotics on growth of *E. coli* O157:H7 in broth

The figure 2 shows the effect of the four combinations of antibiotics usage on the growth of *E. coli* O157:H7 based on the OD_{600} measurement. After 10 h incubation, the OD_{600} corresponding to the usage of BHI-N and BHI-C-V increased sharply, on the other hand, there was no increase in OD_{600} corresponding to the usage of BHI-C-V-T and BHI-N-C-V-T. Therefore, the two combinations of BHI-N and BHI-C-V were considered to be proper to favor growth of the *E. coli* O157:H7 in broth.

The two combinations of BHI-N and BHI-C-V were examined by the phage assay with PP01ccp to clarify whether they were suitable for growth of the *E. coli* O157:H7 in fecal sample. As shown on the figure 3, a significant change of ABS_{550} appeared in the case of usage of BHI-C-V. On the other hand, almost no ABS_{500} change appeared in the case of usage of BHI-N. Therefore, the combination of antibiotics C and V were selected to use in detection of *E. coli* O157:H7 in fecal samples.

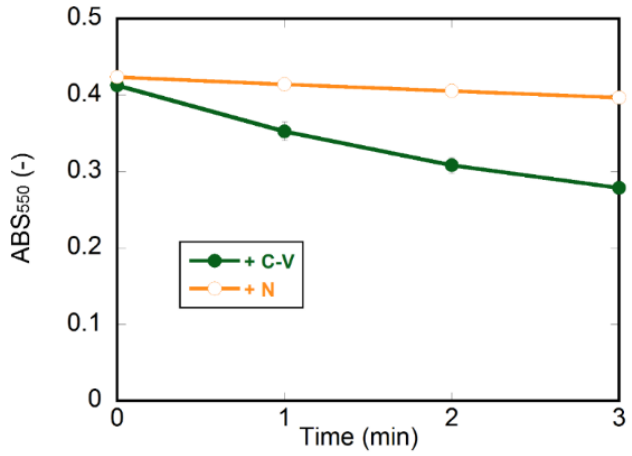

Figure 3. Effect of antibiotics on growth of *E. coli* O157:H7 in fecal sample

In the detection of *E. coli* O157:H7 in fecal samples, the usage of antibiotics together with enrichment media to recover STEC strains has been investigated to inhibit growth of background bacteria. For example, Hussein *et al*. (2008) showed that all 44 STEC strains involving three O157:H7 and 41 non-O157:H7 inoculated into fresh bovine feces were recovered by using BHI medium containing four antibiotics of cefixime, tellurite, novobiocin, and vancomycin at 0.05, 2.5, 20, and 40 mg/L, respectively. However, the same combination could not be applied to promote growth of *E. coli* O157:H7 in this study. Therefore, combination of antibiotics should be clarified with each kind of STEC strains to select suitable antibiotics for the inhibition of background bacteria without effect on the growth of target STEC strains. In addition, on the recovery of *E. coli* O57:H7 in fecal samples shown in previous studies, the IMS was normally employed to enhance the separation efficiency before detection of *E. coli* O157:H7 on selective agar plates (O'Hanlon *et al*., 2004; Lejeune *et al*., 2004; Hussein & Bollinger, 2008). Although in those cases, antibiotics were also used to inhibit growth of background bacteria. It indicated two limitations, firstly usage of the IMS is costly and secondly the whole procedure is time consuming when at least two days are required to accomplish the detection. However, the detection developed in this study based on PP01ccp and usage of antibiotics enabled the detection of *E. coli* O157:H7 within less than 1 day without the need of expensive reagent such as the IMS.

Quantitative detection of *E. coli* O157:H7 in cattle manure

Feces are considered as the original source of the *E. coli* O157:H7. Zhao *et al*. (1995) surveyed prevalence of *E. coli* O157:H7 in dairy herds and reported that about 3.4% of fecal samples is positive with *E. coli* O157:H7 test and the *E. coli* concentration ranged from 10^3 to 10^5 CFU g^{-1}. Concentration of *E. coli* O157:H7 in fecal samples was also revealed in other researches with various ranges from 10^1 to 10^6 CFU g^{-1} (Ogden *et al*., 2002; Omisakin *et al*., 2003). In this study, the MPN-phage assay was tested for quantitative detection of *E. coli* O157:H7 in a cattle manure. The cattle manure was preliminary examined whether it contained *E. coli* O157:H7 by the phage assay with PP01ccp. And the result confirmed that there was no *E. coli* O157:H7 in the original cattle manure sample collected in a cow farm in Japan (data not shown). Next, two concentrations of 10^2 and 10^5 cells/g were inoculated into the cattle manure to test the possibility of the MPN-phage assay. These two concentrations of 10^2 and 10^5 cells/g simulated the low and high concentrations of *E. coli* O157:H7 in cattle manure. The concentrations measured by the MPN-phage assay as well as calculated by the agar method were described in the figure 4. On the Figure 4, the concentration of *E. coli* O157:H7 estimated by the MPN-phage assay is almost identical with that calculated by the agar plate method. The result indicates that *E. coli* O157:H7 in cattle manure could be detected quantitatively by using the MPN-phage assay.

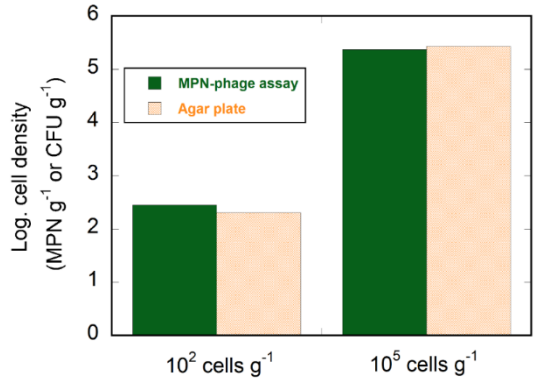

Figure 4. Quantitative detection of *E. coli* O157:H7 in fecal sample

The MPN technique has been applied to enumerate *E. coli* O157:H7 in food and environmental samples. Chapman *et al*. (2001) and Fegan *et al*. (2004) detect *E. coli* O157:H7 by the MPN technique combined with the IMS and CT-SMAC agar. But, as mentioned earlier, the method is expensive and time consuming. Jenkins *et al*. (2009) used MPN method to quantify *E. coli* O157:H7 concentration in surface waters based on the MUG-negative characteristic of *E. coli* O157:H7. However, Hussein *et al*. (2008) demonstrated that not only *E. coli* O157:H7 strains are negative with MUG but also other Shiga toxin-producing *E. coli* own this characteristic. It may decrease the accuracy of the method. The MPN-phage assay developed in this study enabled the quantitative detection of *E. coli* O157:H7 in fecal samples. The method is rapid since it enabled the accomplishment of the detection in less than 1 day. The method in accurate since it is based on the high host specificity of the phage. In addition, the method might be inexpensive since the detection based on the method can be conducted without needing expensive reagent such as the IMS.

Detection of *E. coli* O157:H7 in fecal samples is always difficult with a high background flora (Hussein & Bollinger, 2008). Therefore, antibiotics are generally used in detection of *E. coli* O157:H7 in fecal samples. The antibiotics should suppress the growth of the background bacteria but do not affect growth of *E. coli* O157:H7. Previous researches showed some proper combinations of antibiotics and enrichment media in the detection of *E. coli* O157:H7 (Hussein & Bollinger, 2008). In the current study, the four combinations of medium with antibiotics of C, T, N and V were tested based on previous references (O'Hanlon *et al*., 2004; Lejeune *et al*., 2004; Hussein *et al*., 2008) where the combinations were demonstrated to be successful in recovery and detection of *E. coli* O157:H7 in fecal samples. However, in the current study, among the four combinations, only the combination of BHI-C-V was suitable to support growth and quantitative detection of *E. coli* O157:H7 in cattle manure using the MPN-phage assay. Therefore, combination of enrichment medium and antibiotics should be examined carefully to ensure the effective inhibition of background flora without effect on the recovery of *E. coli* O157:H7 in fecal samples.

REFERENCES

Armstrong GL, Hollingsworth J, Morris JG (1996) Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol Rev* 18: 29-51.

Chapman PA, Christine A, Siddons A, Zadik PM, Jewes L (1991) An improved selective medium for the isolation of *Escherichia coli* O157. *J Med Microbiol*, 35: 107-110.

Chapman PA, Cerda´n Malo AT, Ellin M, Ashton R, Harkin MA (2001) *Escherichia coli* O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef and lamb products in South Yorkshire, UK. *Int J Food Microbiol* 64: 139-150.

Chapman PA, Wright DJ, Norman P (1989) Verotoxin-producing *Escherichia coli* infections in Sheffield: Cattle as a positive source. *Epidem Inf* 102: 439-445.

Chekabab SM, Paquin-Veillette J, Dozois CM, Harel J (2013) The ecological habitat and transmission of *Escherichia coli* O157:H7. *FEMS Microbiol Lett* 341: 1-12.

Elizaquível P, Sánchez G, Aznar R (2012) Quantitative detection of viable foodborne *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* in fresh-cut vegetables combining propidium monoazide and real-time PCR. *Food Control* 25: 704-708.

Farmer JJ, Davis BR (1985) H7 antiserum-sorbitol fermentation medium: a single tube screening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol* 22: 620-625.

Fedio WM, Jinneman KC, Yoshitomi KJ, Zapata R, Wendakoon CN, Browning P, Weagant SD (2011) Detection of *E. coli* O157:H7 in raw ground beef by Pathatrix™ immunomagnetic-separation, real-time PCR and cultural methods. *Int J Food Microbiol* 148: 87-92.

Fegan N, Higgs G, Vanderlinde P, Desmarchelier P (2003) Enumeration of *Escherichia coli* O157 in cattle faeces using most probable number technique and automated immunomagnetic separation *Lett Appl Microbiol* 38: 56-59.

Fegan N, Vanderlinde P, Higgs G, Desmarchelier P (2004) The prevalence and concentration of *Escherichia coli* O157 in faeces of cattle from different production systems at slaughter. *J Appl Microbiol* 97: 362-370.

Fu Z, Rogelj S, Kieft TL (2005) Rapid detection of *Escherichia coli* O157:H7 by immunomagnetic separation and real-time PCR, *Int J Food Microbiol*, 99: 47-57.

Goldman E, Green LH (2009) Practice handbook of microbiology 2nd edition, CRC press, FL, USA.

Hoang HA, Abe M, Nakasaki K (2014) A novel colorimetric method for the detection of *Escherichia coli* using cytochrome c peroxidase-encoding bacteriophage. *FEMS Microbiol Lett* 352: 97-103.

Hoang HA, Le TD (2015) Rapid and simple colorimetric detection of *Escherichia coli* O157: H7 in apple juice using a novel recombinant bacteriophage-based method. *Biocontrol Sci* 20(2): 99-103.

Hussein HS, Bollinger LM (2008) Influence of selective media on successful detection of shiga toxin–producing *Escherichia coli* in food, fecal, and environmental samples *Foodborne Pathog Dis* 5: 227–244.

Hussein HS, Bollinger LM, Hall MR (2008) Growth and enrichment medium for detection and isolation of shiga toxin–producing *Escherichia coli* in cattle feces *J Food Protect* 71: 927–933.

Ibekwe AM, Watt PM, Grieve CM, Sharma VK, Lyons SR (2002) Multiplex fluorogenic real-time PCR for detection and quantification of *Escherichia coli* O157:H7 in dairy wastewater wetlands *Appl Environ Microbiol* 68: 4853-4862.

Isaacson M, Canter PH, Effler P, Arntzen L, Bomans P, Heenan R (1993) Haemorrhagic colitis epidemic in Africa. *Lancet* 341: 961

Jenkins MB, Endale DM, Fisher DS, Gay PA (2009) Most probable number methodology for quantifying dilute concentrations and fluxes of *Escherichia coli* O157:H7 in surface waters *J Appl Microbiol* 106: 572-579.

Kuboniwa M, Amano A, Kimura KR, Sekine S, Kato S, Yamamoto Y, Okahashi N, Iida T, Shizukuishi S (2004) Quantitative detection of periodontal pathogens using real-time polymerase chain reaction with TaqMan probes. *Oral Microbiol Immunol* 19: 168-176.

Lejeune JT, Besser TE, Rice DH, Berg JL, Stilborn RP, Hancock DD (2004) Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover. *Appl Environ Microbiol* 70: 377-384.

Locking ME, Obrien SJ, Reilly WJ, Wright EM, Campbell DM, Coia JE, Browning LM, Ramsay CN (2001) Risk factors for sporadic cases of *Escherichia coli* O157 infection: the importance of contact with animal excreta. *Epidemiol Infect* 127: 215-220.

March SB, Ratnam S (1986) Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol* 23: 869-872.

McCarthy J, Holbrook R, Stephens PJ (1998) An improved direct plate method for the enumeration of stressed *Escherichia coli* O157:H7 from food. *J Food Prot* 61: 1093-1097.

O'Hanlon KA, Catarame TMG, Duffy G, Blair IS, McDowell DA (2004) Rapid detection and quantification of *E. coli* O157/O26/O111 in minced beef by real-time PCR. *J Appl Microbiol* 96: 1013-1023.

Ogden ID, Hepburn NF, MacRae M, Strachan NJC, Fenlon DR, Rusbridge SM, Pennington TH (2002) Long-term survival of *Escherichia coli* O157 on pasture following an outbreak associated with sheep at a scout camp. *Lett Appl Microbiol* 34: 100-104.

Omisakin F, MacRae M, Ogden ID, Strachan NJC (2003) Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter *Appl Environ Microbiol* 69: 2444-2447.

O'Sullivan L, Bolton D, McAuliffe O, Coffey A (2020) The use of bacteriophages to control and detect pathogens in the dairy industry. *Int J Dairy Technol* 73(1): 1-11.

Richter Ł, Janczuk-Richter M, Niedziółka-Jönsson J, Paczesny J, Hołyst R (2018) Recent advances in bacteriophage-based methods for bacteria detection. *Drug Discov Today* 23(2): 448-455.

Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake AP, Cohen ML (1983) Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med* 308: 681–685.

Watanabe Y, Ozasa K, Mermin JH, Griffin PM, Masuda K, Imashuku S, Sawada T (1999) Factory outbreak of *Escherichia coli* O157:H7 infection in Japan. *Emerg Infect Dis* 5(3): 424-428.

Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN, Downes FP, Martin ML, Griffin PM, Ostroff SM (1991) Isolation of *Escherichia coli* Serotype O157:H7 and Other Shiga- Like-Toxin-Producing *E. coli* from Dairy Cattle. *J Clin Microbiol* 29: 985-989.

Zhao T, Doyle MP, Shere J, Garber L (1995) Prevalence of Enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds *Appl Environ Microbiol* 61: 1290-1293.

KẾT HỢP PHƯƠNG PHÁP SỐ KHẢ HỮU VÀ PHƯƠNG PHÁP THỰC KHUẨN THỂ THAY ĐỔI MÀU ĐỂ ĐỊNH LƯỢNG *ESCHERICHIA COLI* O157:H7 TRONG MẪU PHÂN BÒ

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TÓM TẮT

Phương pháp mới (MPN-phage) phát hiện và định lượng *Escherichia coli* O157:H7 sử dụng sự kết hợp giữa phương pháp số khả hữu (most probable number) và phương pháp sử dụng thực khuẩn thể thay đổi màu sắc đã được phát triển. Đầu tiên, thực khuẩn thể tái tổ hợp PP01ccp mang gene ngoại lai *ccp* được xây dựng và sử dụng phát hiện *E. coli* O157:H7 trong môi trường chuẩn. Sự oxi hóa cơ chất màu cytochrome c bởi enzyme tái tổ hợp cytochrome c peroxidase (CCP) đã được chứng minh. Sự thay đổi màu sắc có thể được nhận biết bằng mắt thường. Tiếp theo, phương pháp mới MPN-phage được phát triển và định lượng thành công *E. coli* O157:H7 ở mật độ 10^2 và 10^5 tế bào/gam phân bò.

Từ khóa: Định lượng, thay đổi màu sắc, thực khuẩn thể, *Escherichia coli* O157:H7, phân bò.

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