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# Survival of *Escherichia coli* O157:H7 and Non-O157 during Post-Processing Stage of Yogurt

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**Abstract:** The presence of *Escherichia coli* O157:H7 including Shiga toxin-producing *E. coli* serotypes other than O157 (non-O157) strain in foods is a food safety risk since they could produce severe illnesses in human. Among virulence characteristics of *E. coli* O157:H7, the acid tolerance enables bacteria to pass through stomach and reach intestine, the site of pathogenesis. In this study, we investigated the survival ability of four *E. coli* O157:H7 and non-O157 strains isolated from environment in Thailand, in yogurt during post-processing stage at two temperatures, 6°C and 10°C. With the starting bacterial inoculum size of $1.5 \times 10^5$ CFU/g yogurt, the results revealed that within 6 h of incubation, the bacterial populations sharply decreased to approximately 3 log CFU/g in all experimental strains at both temperatures. Subsequently, they were decreased slowly in the following time points. At the end of incubation period, 102 h, the comparable amount of all four Thai *E. coli* strains ranged from 1.0 to 2.82 CFU/g was detected. Whereas, *E. coli* O157:H7 strain EDL933, a control strain, focusing at 6°C, was under the detection limit after 78 h of incubation. In this study, the observed results probably indicated that Thai *E. coli* O157:H7 and non-O157 from environment are more tolerant to acidic condition than clinical strain. This study suggested the acid tolerant capability of *E. coli* O157:H7 and Shiga toxin-producing *E. coli* non-O157 strain obtained from Thai environment.

**Key words:** *E. coli* · O157 · Shiga toxin · Yogurt · Survival · Acid tolerance

## INTRODUCTION

*Escherichia coli* O157:H7 was first considered as a pathogen in 1982 [1,2]. It can be a contaminant with different kind of foods. After the arrival of bacteria to the human gut, it is able to colonize and may lead to the severe symptoms, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Although foods with a pH value of less than 4.6 are normally regarded as low risk in the aspect of food safety, one of the striking abilities of *E. coli* O157:H7, it could tolerate acidic conditions [3]. Thus, acidic foods may be a vehicle for *E. coli* O157:H7.

Yogurt is one of acidic foods which are widely consumed worldwide. In Turkey, this type of dairy product was consumed approximately two million tons a year [4]. In the processes of yogurt fermentation, lactic acid bacteria such as *Lactobacillus acidophilus* plays a role in lactic acid production, led to the generation of distinct characteristics of yogurt, the flavour and the texture [5]. In addition, lactic acid is clearly shown to provide the antagonistic activity against other food-borne pathogens [6]. Nevertheless, certain studies have reported the ability of *E. coli* O157:H7 to survive in yogurt and other acidic foods in different temperatures [7-9]. Important evidence has linked the infection by *E. coli* O157:H7 to yogurt consumption. During September to November 1991, *E. coli* O157:H7 infection due to the consumption of locally produced yogurt was reported from North Western area of England. Sixteen cases were infected by *E. coli* O157:H7 identified as phage type 49. This outbreak was the first report of O157:H7 infection resulted from the consumption of yogurt [10].

Although contamination of *E. coli* O157:H7 can be occurred at several stages of yogurt production, Dineen

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*et al*. [11] suggested that the presence of *E. coli* O157:H7 in yogurt was most likely to be due to the post-processing contamination. This reason encouraged us to examine the survival of *E. coli* O157:H7 including non-O157 during the post-processing stages of yogurt. This study provides information about acid tolerance ability of *E. coli* O157:H7 and non-O157 Shiga toxin-producing *E. coli* obtained from Thai environment, to survive in yogurt. The significance to public health concern was discussed.

## MATERIALS AND METHODS

**Bacterial Strains:** Four *E. coli* O157:H7 and non-O157 strains survival behaviors were examined in yogurts. They are *stx*-carrying strains. The information and genotypic characteristics of the strains were shown in Table 1. A working bacterial culture was prepared by growing a single bacterial colony in 3 mL of tryptic soy broth (TSB) for 6 h at 37°C with 150 rpm orbital shaking. Bacterial cells were initially adjusted to 0.5 McFarland turbidity standards (approximately $1.5 \times 10^8$ CFU/mL) in normal saline solution (NSS) by McFarland Densitometer (Biosan, Latvia). They were subsequently performed a decimal dilution to be a $1.5 \times 10^7$ CFU/mL for bacterial survival experiment.

**Yogurts:** Plain yogurts were collected at retail level. It was noted that the quality of yogurts were critical as the good condition before experimental initiation. All of these yogurts were observed as freshly produced. The yogurt's properties such as textures, odour and other visualized characteristics of yogurts were also observed in the desirable condition before experiment.

**Survival of E. coli O157:H7 and non-O157 in Yogurts:** In order to monitor the survival of the bacteria in yogurt, four strains of *E. coli* O157:H7 and non-O157 were spiked into the commercial plain yogurts (sucrose 6%, total fat 5% and pH value of 4.0) and monitored their fates at 10 time points (0, 6, 18, 30, 42, 54, 66, 78, 90 and 102 h). In brief, the bacterial culture was grown as stated above. Each bacterial strain was examined individually by diluting the bacterial culture in NSS and inoculation of 1 mL ($1.5 \times 10^7$ CFU/mL) into 99 grams of yogurt was carried out (a final bacterial concentration of $1.5 \times 10^5$ CFU/g was obtained). Yogurts were thoroughly homogenized and held at 6°C or 10°C throughout the study. Subsequently, the bacteria were enumerated by a viable count on Sorbitol MacConkey agar for O157 strains and on MacConkey agar for non-O157 strains [12]. Before enumeration, yogurts were mixed thoroughly using the magnetic stirrer. *E. coli* O157:H7 strain EDL933 which was isolated from clinical sample and demonstrated the highly pathogenic ability, was used as a control strain. The experiment was performed in duplicate.

## RESULTS AND DISCUSSION

The existence of *E. coli* O157:H7 and non-O157 in Thai environment was frequently reported [13-16]. These reports mostly involved with the bacterial isolation from meats especially beef and beef products, leading to the plausibility of O157 including non-O157 strains contamination in unpasteurized milks. The contaminated bacteria in unpasteurized milk have the propensity to survive through pasteurization temperature [12] and the temperature for yogurt fermentation [9]. In order to assess the fate of *E. coli* O157:H7 and non-O157 isolated from Southern Thai environment in yogurt during the post-processing stage, four *E. coli* O157:H7 and non-O157 strains were used as the surrogates. The experiments were established in commercial plain yogurts at two different temperatures, 6°C and 10°C.

A bacterial starting inoculum size of $1.5 \times 10^5$ CFU/g was applied to both temperatures. Focusing at 6°C, *E. coli* O157:H7 strain EDL933, a control strain, exhibited the

Table 1: Bacterial strains used in this study

| | | | Genotype | | | | |
|---|---|---|---|---|---|---|---|
| Strain | Origin | Year of isolation | $stx_1$ | $stx_2$ | *eae* | O157/non-O157 | Reference |
| PSU1 | Beef | 2012 | + | + | - | -/+ | [12] |
| PSU2 | Beef | 2012 | - | + | + | +/- | [12] |
| PSU17 | Beef | 2012 | - | + | - | -/+ | [12] |
| PSU132 | Beef | 2012 | - | - | - | +/- | [12] |
| EDL933 | Human | 1982 | + | + | + | +/- | [25] |

Table 2: Survival of *E. coli* O157:H7 and non-O157 in yogurts during storage at 6°C

| Time after inoculation | Log CFU/g | | | | |
|---|---|---|---|---|---|
| | PSU1 | PSU2 | PSU17 | PSU132 | EDL933 |
| 0 h | 5.18±0.01 | 5.20±0.01 | 5.18±0.04 | 5.21±0.01 | 5.19±0.02 |
| 6 h | 3.48±0.11 | 3.36±0.03 | 3.38±0.03 | 3.48±0.07 | 3.40±0.20 |
| 18 h | 3.38±0.04 | 3.25±0.06 | 3.29±0.02 | 3.12±0.17 | 3.15±0.06 |
| 30 h | 3.52±0.17 | 3.48±0.05 | 3.48±0.07 | 3.24±0.09 | 2.78±0.00 |
| 42 h | 3.36±0.11 | 3.47±0.08 | 3.24±0.09 | 2.84±0.09 | 2.00±0.00 |
| 54 h | 3.18±0.05 | 3.05±0.01 | 3.18±0.09 | 2.87±0.18 | 1.89±0.27 |
| 66 h | 3.00±0.02 | 3.04±0.05 | 3.07±0.15 | 2.31±0.19 | 1.24±0.33 |
| 78 h | 2.96±0.01 | 2.82±0.13 | 2.80±0.09 | 2.51±0.11 | 0 |
| 90 h | 3.19±0.03 | 2.92±0.04 | 3.00±0.11 | 2.63±0.04 | 0 |
| 102 h | 2.81±0.10 | 2.82±0.22 | 2.78±0.05 | 2.30±0.43 | 0 |

Data are expressed as mean ± standard deviation.

Table 3: Survival of *E. coli* O157:H7 and non-O157 in yogurts during storage at 10°C

| Time after inoculation | Log CFU/g | | | | |
|---|---|---|---|---|---|
| | PSU1 | PSU2 | PSU17 | PSU132 | EDL933 |
| 0 h | 5.21±0.02 | 5.20±0.02 | 5.16±0.00 | 5.23±0.02 | 5.17±0.02 |
| 6 h | 3.59±0.00 | 3.55±0.09 | 3.08±0.05 | 3.49±0.02 | 3.31±0.01 |
| 18 h | 3.46±0.02 | 3.38±0.05 | 3.26±0.04 | 3.42±0.08 | 3.36±0.05 |
| 30 h | 3.37±0.04 | 3.39±0.04 | 3.32±0.03 | 2.70±0.00 | 2.69±0.12 |
| 42 h | 3.08±0.25 | 3.10±0.02 | 3.13±0.02 | 2.87±0.12 | 2.15±0.21 |
| 54 h | 3.22±0.05 | 2.82±0.23 | 3.11±0.15 | 3.11±0.10 | 2.72±0.08 |
| 66 h | 3.16±0.00 | 1.81±0.05 | 2.70±0.40 | 2.90±0.08 | 2.45±0.03 |
| 78 h | 2.93±0.07 | 1.30±0.42 | 2.89±0.13 | 2.51±0.13 | 2.01±0.23 |
| 90 h | 3.09±0.04 | 1.24±0.34 | 2.70±0.06 | 2.31±0.33 | 1.54±0.09 |
| 102 h | 2.59±0.02 | 1.00±0.00 | 2.80±0.01 | 2.18±0.31 | 1.00±0.00 |

Data are expressed as mean ± standard deviation.

sharply decrease of bacterial population from 5.19 log CFU/g (mean value) to 3.40 log CFU/g within 6 h. Gradual decrease of bacterial population was observed in the following time points and was under the detection limit after 78 h (Table 2). All four Thai *E. coli* O157:H7 and non-O157 also displayed the similar survival behaviors as EDL933 until 18 h of incubation. However, they exhibited a marked higher bacterial population over EDL933 since 30h of incubation. Distinctly, since 78 h to 102 h of incubation, all four Thai O157:H7 and non-O157 survived ranging from 2.30 to 3.19 CFU/g while EDL933 was under the detection limit (Table 2). Similar trends of *E. coli* O157:H7 and non-O157 survival were observed at 10°C of incubation except that the EDL933 could survive until 102 h of incubation (Table 3).

The depletion of *E. coli* O157:H7 and non-O157 populations in yogurts in this study were thought to be mainly due to the effect of organic acids especially lactic acid, synthesized by lactic acid bacteria during yogurt fermentation and as the results of the experiments mentioned above, all four Thai *E. coli* strains were found to be more tolerant to organic acids than a clinical O157:H7 strain EDL933. This phenomenon was distinctly observed at 6°C (Table 2), probably because of the lower temperature. The inability of *E. coli* O157:H7 survival at low temperature was demonstrated by several reports [8,17,18]. In addition to the presence of organic acids in yogurts, other biological molecules have been shown to be produced by lactic acid bacteria such as hydrogen peroxide, diacetyl and bacteriocin [19-21]. These biological molecules can play a role as antimicrobial inhibitors. Nevertheless, several reports demonstrated that organic acids produced by lactic acid bacteria are main effective biomolecules that cope with the reduction of *E. coli* O157:H7 viability. In this study, pH values of yogurts were determined and revealed the values as approximately 4.0 throughout the experiments (data not shown). Thus, it was thought that organic acids were mainly responsible for *E. coli* growth inhibition in this study.

After the dramatically decrease of *E. coli* O157:H7 and non-O157 populations within first 6 h of incubation at both 6°C and 10°C, *E. coli* populations started decreasing slowly (Table 2 and Table 3). This phenomenon was possibly because of bacterial acid adaptation. Various reports described the ability of *E. coli* O157:H7 in adaptation to acidic conditions [22,23]. Arnold and Kaspar [22] evaluated the acid tolerance of three *E. coli* O157:H7 in three phases of growth. Stationary phase of bacterial culture revealed acid tolerance while mid-log phase showed the opposite result. One study from Leyer *et al*. [23] examined three *E. coli* O157:H7 strains, strain 933, strain 932 and strain 505B, for their survival in lactic acid pH 3.85 after acid adaptation in nutrient broth acidified to pH 5.0 with HCl. The results exhibited the marked increase in lactic acid resistant levels with the highest resistant level in *E. coli* O157:H7 strain 933. Outer membrane structure and possibly specific porins change were found to be the cause of acid tolerant ability gaining [24]. Additionally, cross-protection against other environmental stresses such as salts, heat, or any surface active agents can be established after acid adaptation. This incorporated factor might assist the bacteria to be viable in low temperature [24].

In this current study, it was noted that in some *E. coli* strains, certain time points showed a slight increase of bacterial populations, for instance, strain PSU1 incubated at 6°C for 90 h or strain PSU17 at 10°C for 102 h (Table 1 and Table 2). This may resulted from the bacterial propagation to balance their populations in specific circumstances. The result from Arican and Andic [5] also demonstrated the similar phenomenon in *E. coli* O157:H7 when they examined the bacterial survival behavior in yogurt inoculated with $10^5$ CFU/mL incubated at 4°C for 48 h. The differences in the ability to grow against acidic condition may depend upon individual bacterial strain.

Based upon the information obtained from this present study, we concluded that in the case of *E. coli* O157:H7 and non-O157 isolated from environment in Thailand that could contaminate raw materials used for yogurt production, the bacteria will be able to survive and with their main virulence traits, Shiga toxins, they can make a success in human pathogenesis. This is important to the public health stand point.

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