Effect of Hyperosmotic Salt Concentration and Temperature on Viability of *Escherichia coli* during Cold Storage

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*Escherichia coli* cells were suspended in phosphate-buffered saline solutions (pH 7.4) at physiological (0.9 %) and hyperosmotic (3.5, 5.0, and 10.0 %) concentrations of sodium chloride (NaCl) and stored at 5, 10, 15, 20, and 25 °C up to 48 d. During storage at 5 and 10 °C, viable cell counts decreased approximately from 9 log CFU/ml to 6-7 log CFU/ml, and NaCl showed slight protective effect on the decrease. When stored at 15, 20, and 25 °C, the counts decreased with increases in NaCl concentration and/or storage temperature. The cells in 10.0 % NaCl suspension became nondetectable after storage at 25 °C for 28 d. Under some storage conditions (NaCl ≤ 5 %, 20 and 25 °C), the counts approached constant values, indicating possible adaptation to NaCl. Injured cells were observed at 5.0 and 10.0 % NaCl. However, recovery was observed only at 5.0 % NaCl during storage at 20 °C. In addition, more cells were detected on nonselective medium when incubated at 37 °C than at 25 °C. Higher hyperosmotic NaCl solutions at higher storage temperatures reduced more viable cells of *E. coli*.

Key words: Sodium chloride / Hyperosmotic / Storage / *Escherichia coli* / Injury.

1. INTRODUCTION

*Escherichia coli* is an intestinal bacterium in warm-blooded organisms. Most of *E. coli* strains are harmless, but some serotypes are severely poisonous to humans (Sutherland et al., 1995). Accordingly, contamination of *E. coli* in food may cause serious diseases such as diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome or even death (Ding et al., 2010). For instance, a severe outbreak of *E. coli* in pickled Chinese cabbage containing hyperosmotic 2 % NaCl was reported in Sapporo, Japan in 2012, resulting in death of 8 people and food poisoning of 169 (Tabuchi et al., 2015).

Salting or addition of salt (sodium chloride, NaCl) is a traditional method to suppress growth of unfavored microbes in foods. NaCl is added to traditional salty foods at hyperosmotic concentrations. For instance, according to Standard Tables of Food Composition in Japan, 7th Edition (MEXT, 2015), salt content is evaluated as NaCl equivalent for traditional Japanese vegetable pickles at 3.7 ± 1.5 % (27 items; min 1.1 %; max 7.1 %) and for traditional *MISO* (fermented soybean paste) at 10.8 ± 2.7 % (6 items; min 6.1 %; max 14.1 %). The database (MEXT, 2015) lists some natural cheese products containing NaCl equivalent at 0.7 ± 0.5 % (11 items; min 0.1 %; max 1.3 %) at more or less comparable levels to physiological saline (0.9 %) beside salty cheese varieties (3.8 % for parmesan and blue; 2.0 % for Gouda, Cheddar, Edam, and Camembert).

When NaCl concentration is higher than a physiological saline level of 0.9 %, microbes encounter hyperosmotic stress (Burgess et al., 2016). Addition of NaCl can create extracellular hyperosmotic pressure, inducing efflux of intracellular water with increased intercellular ionic strength and consequent inhibition of
cellular enzymatic reactions, depending on the concentration of NaCl (Csonka, 1989; Csonka and Hanson, 1991).

Antibacterial effect of NaCl on E. coli cells may depend on NaCl concentration (Buchanan and Klawitter, 1992; Conner, 1992; Lee and Kang, 2016) and storage temperature (Glass et al., 1992; Buchanan and Klawitter, 1992; Conner, 1992). The cells did not grow at 4 °C regardless of NaCl concentrations ranging from 0 % to 10 % (Conner, 1992). With increased temperature from 4 °C to 10 °C, NaCl sensitivity of E. coli increased, and their growth was inhibited by NaCl at a concentration of 4 % or above (Conner, 1992). NaCl concentration of at least 8 % was necessary for growth inhibition of E. coli at 37 °C (Glass et al., 1992; Conner, 1992). Therefore, it is speculated that viability of E. coli cells as well as their growth inhibition could also be dependent on hyperosmotic NaCl concentration during storage. However, the effect of hyperosmotic NaCl concentration and storage temperature on the viability of E. coli has not been clarified sufficiently.

On the other hand, E. coli can adapt to salty environment such as seawater (3.5 % NaCl) due to its osmotic adaptability (Arense et al., 2010; Rozen et al., 2002). When E. coli was suspended in salty nutrient solutions, viable cell counts remained constant up to 7 % of NaCl concentration (Doudoroff, 1940). Adaptation of Gram-negative E. coli to hyperosmotic environment has been intensively studied. When Gram-negative bacterial cells were transferred into hyperosmotic environments (up to 2.3 %), the cells responded to balance the intracellular osmotic pressure through cell shrinkage (Csonka and Hanson, 1991). Simultaneously, osmoprotectants such as K+ glutamate, proline, and glycine betaine were accumulated inside the cells from the culture medium. Meanwhile, the cells in hyperosmotic environment synthesized trehalose while they released toxic components such as putrescine (Dinnbier et al., 1988; Nagata et al., 2005; Sasaki et al., 2009; Weber et al., 2006). Thus, those osmotic responses may help E. coli cells survive NaCl stress.

In addition, injured bacteria are often found after sublethal treatment such as salting (Hajmeer et al., 2001; Wu, 2008), freezing (Wu, 2008), drying (Wu, 2008), high hydrostatic pressure (Koseki and Yamamoto, 2006; Wu, 2008). Due to injury, the persistence of bacteria may be suppressed, but they are still viable (McKenzie et al., 2014). For detecting injured cells of bacteria such as E. coli, simultaneous plating on selective and nonselective media has been applied conventionally (McKenzie et al., 2014): injured cells can form colonies only on nonselective medium, while healthy cells can be detected both on nonselective and selective media. Furthermore, colony forming ability of injured cells may also be affected by incubation temperature of the selective and nonselective plates (Ray and Speck, 1973; Klein and Wu, 1974; Gnanou Besse et al., 2000; Kimura et al., 2017).

In this study, the effect of hyperosmotic NaCl concentration and storage temperature (5, 10, 15, 20, and 25 °C) on the viability of E. coli was investigated using nutrient-free model liquids of phosphate-buffered solutions containing NaCl at 3.5 %, 5.0 %, and 10.0 %, which would represent NaCl equivalent levels of average Japanese vegetable pickles, their salty products, and MISO, respectively. The viability was evaluated on selective agar at 37 °C and nonselective agar at 25 and 37 °C to differentiate total viable, healthy, and injured (or sublethally-inactivated) cell populations.

2. MATERIALS AND METHODS

2.1. NaCl phosphate-buffered solutions

Phosphate-buffered (PB) solutions at NaCl concentrations of 0.9, 3.5, 5.0, and 10.0 % were prepared, respectively. All solutions were adjusted to pH 7.4 by mixing 0.1 M NaH₂PO₄ and 0.1 M Na₂HPO₄ solutions containing NaCl at each level. All the reagents were of analytical grade.

2.2. Bacterial strain

A commercial strain of Escherichia coli (ATCC25922, Manassas, VA, USA) was employed in this study. The strain was suspended in 0.68 % NaCl solution containing 20 % glycerol (0.9 % NaCl solution / 80 % glycerol = 3 / 1; autoclaved) and kept at -80 °C. The frozen stock was taken out and thawed at room temperature before preculture.

2.3. Bacterial suspension and storage

A portion (100 µl) of the thawed stock culture was inoculated into 100 ml trypticase soy broth (TSB, Wako Pure Chemical, Osaka, Japan) and cultured with shaking at 37 °C for 18 h to achieve a stationary phase. The precultured cell suspension was transferred into two 50 ml sterile tubes, and centrifuged at 2300 x g, 15 °C for 10 min. Cell precipitate was washed twice using phosphate-buffered saline (PBS, pH 7.4) by centrifugation at 2300 x g, 15 °C for 10 min and subsequently suspended in 20 ml PB solution at each NaCl concentration. The cell suspension was diluted tenfold in a sterile beaker with a stirrer bar using each PB solution. The viable cell counts of diluted suspensions were referred to as initial counts. Diluted suspensions were immediately dispersed into sterile tubes (50 ml), capped, and stored for 48 d at temperatures of 5, 10, 15, 20, and 25 °C, respectively.
2.4. Enumeration of viable cells

Desoxycholate agar (Eiken Chemical Co. Ltd., Tochigi, Japan) and standard method agar (Nissui Pharmaceutical, Tokyo, Japan) were used as selective (S) and nonselective (NS) media to detect healthy and total viable cells, respectively. These two media have been applied in past studies (Sinskey and Silverman, 1970; Morimatsu et al., 2019). In terms of selective agent for the selective media, sodium desoxycholate was the bile salt, most inhibitory to the growth of damaged (or injured) E. coli cells (Scheusner et al., 1970).

Colonies on the plates were enumerated every 4 d during storage and expressed as logarithm of colony forming unit/ml (log CFU/ml). A portion of cell suspensions was aseptically taken out from each tube, and the portion was serially diluted using 0.9 % NaCl solution. The cell dilutions were simultaneously plated on the selective and nonselective media. Selective plates were incubated for 6 d at 37 °C whereas nonselective plates were incubated at both 25 °C and 37 °C. Difference in the viable cell counts between on NS and on S media was referred to as an injured cell population. Experiments were conducted in biological duplicate, and cell suspension was spread on 3 plates for each dilution. Data was expressed as mean ± standard deviation. Significant differences of the data were statistically analyzed by one-way ANOVA in Excel 2016 (Office 365, Microsoft Co., Ltd., Redmond, USA) at a confidence level of p < 0.01.

3. RESULTS AND DISCUSSION

3.1. Viability in the nutrient-free solution

In this study, PB solution was adopted as a liquid model solely containing inorganic compounds such as NaCl, Na₂HPO₄, and NaH₂PO₄. The application of PB solution simplified the experimental system for highlighting the effect of NaCl on the viability of E. coli via minimizing possible interferences of organic compounds as nutrients.

Viability of E. coli at a physiological NaCl concentration of 0.9 % was monitored for 48 d as a reference (Fig. 1). Initial viable cell counts were 9.24 ± 0.04 log CFU/ml (hereafter, log) and 9.26 ± 0.03 log on nonselective medium when incubated at 25 °C (hereafter, NS_25) and 37 °C (hereafter, NS_37), respectively. Meanwhile, initial healthy cell counts were 9.20 ± 0.07 log on selective medium incubated at 37 °C (hereafter, S_37). The counts were not significantly different among the enumeration conditions (p ≥ 0.01), indicating trace or no injured population at the beginning of the storages. During storage at 5 °C, viable cell counts on NS_37 gradually decreased approximately from 9 log to 7 log, whereas the cell counts were slightly lower on NS_25 and S_37 than on NS_37 (p < 0.01), indicating existence of injured populations (Fig. 1).

Since PB solution is a nutrition-free system and NaCl concentration of 0.9 % is comparable to a physiological level which possesses minimum effects on the cells, the reduction of viable cell counts during storage may possibly be ascribed to starvation stress (Hengge-Aronis, 1993) rather than osmotic stress (Csonka, 1989).

Furthermore, the counts during storage at 10 °C (Fig. 2A), 15 °C (Fig. 3A), 20 °C (Fig. 4A), and 25 °C (Fig. 5A) on NS_37 showed similar trends to those on S_37, respectively, approaching constant values of 6.68 ± 0.31, 6.93 ± 0.08, 7.02 ± 0.09, and 6.85 ± 0.09 log, respectively. No notable injured populations were observed after 48 d during storage at 10, 15, 20, and 25 °C in 0.9 % NaCl PB solution. It was indicated that the possible starvation stress, which might have induced injury at 5 °C, could gradually be suppressed during storage at 10 – 25 °C. It was reported that E. coli cells responded to starvation stress and survived through entering a stationary phase where cellular persistence increased (Hengge-Aronis, 1993). On the other hand, according to Lybecker and Samuels (2007), the starvation response was more active at higher storage temperatures. Therefore, it can be speculated that viable cells of E. coli might have entered a stationary phase during storage at 10 – 25 °C where no injured population was observed.

3.2. Hyperosmotic stress

The cells of E. coli were suspended in hyperosmotic NaCl PB solutions and stored at 5 °C (Fig. 1). As in the cases in 3.5 % NaCl PB solution (Fig. 1B), the initial level of healthy cell counts on S_37 was 8.86 ± 0.05 log, which was comparable (p ≥ 0.01) with that on NS_25 (8.97 ± 0.09 log) or that on NS_37 (9.06 ± 0.09 log). In 5.0 % NaCl PB solution (Fig. 1C), the initial healthy cell counts (S_37) were 8.96 ± 0.07 log, whereas total viable cell counts were 9.16 ± 0.06 log (NS_25) and 9.19 ± 0.03 log (NS_37). In 10.0 % NaCl PB solution (Fig. 1D), viable cell counts were 8.80 ± 0.21 log (S_37), 9.19 ± 0.06 log (NS_25) and 9.19 ± 0.06 log (NS_37). In 5.0 % and 10.0 % NaCl PB solutions (Figs. 1C and 1D), the healthy cell counts (S_37) were slightly lower than the total viable cell counts (NS_25 and NS_37) (p < 0.01). The slight but significant differences in 5.0 and 10.0 % NaCl PB solutions may indicate that the cells were slightly injured immediately after suspending E. coli cells in the hyperosmotic solutions, probably due to osmotic shock (Csonka, 1989).

Hyperosmotic NaCl PB solutions reduced the viable cell counts of E. coli during storage, indicating hyperosmotic NaCl stress to the cells, and the decreasing
trends depended on the storage temperature and NaCl concentration (Figs. 1-5). The decreases during storage at 5 °C (Fig. 1) and 10 °C (Fig. 2) were suppressed with elevated NaCl concentration, indicating slight protective effect of hyperosmotic NaCl against the decreases in the viable cell counts. The protective effect might be explained by osmoregulation, which regulates intracellular osmotic pressure for strengthening cell persistence of *E. coli* against such as starvation (Munro et al., 1989) and antibiotic stresses (McMahon et al.,

**FIG. 1.** Viable cell counts in NaCl PB solutions during storage at 5 °C. The counts were evaluated on nonselective medium incubated at 25 °C (NS_25) and 37 °C (NS_37) and on selective medium incubated at 37 °C (S_37). Each data point was obtained by triplicate plating and expressed as mean ± standard deviation in two independent trials.

**FIG. 2.** Viable cell counts in NaCl PB solutions during storage at 10 °C. The counts were evaluated on nonselective medium incubated at 25 °C (NS_25) and 37 °C (NS_37) and on selective medium incubated at 37 °C (S_37). Each data point was obtained by triplicate plating and expressed as mean ± standard deviation in two independent trials.

**FIG. 3.** Viable cell counts in NaCl PB solutions during storage at 15 °C. The counts were evaluated on nonselective medium incubated at 25 °C (NS_25) and 37 °C (NS_37) and on selective medium incubated at 37 °C (S_37). Each data point was obtained by triplicate plating and expressed as mean ± standard deviation in two independent trials.
2007) after being transferred to a hyperosmotic solution. In addition, after 48 d storage of the suspensions, the counts in the hyperosmotic solutions were comparable on NS_25, NS_37, and S_37 ($p \geq 0.01$), indicating no substantial difference in the populations of total viable and healthy cells.

On the other hand, the decreases during storage were accelerated at higher temperatures of 15 °C (Fig. 3), 20 °C (Fig. 4), and 25 °C (Fig. 5). The counts in the hyperosmotic solutions (3.5, 5.0, and 10.0 % NaCl) decreased more remarkably than those in physiological saline solution (0.9 % NaCl). Furthermore, the counts decreased more at higher NaCl concentrations and/or higher storage temperatures. The accelerated decreases in viable cell counts may be ascribed to enhanced biological activities due to raised temperature. It has been pointed out that biological activities of bacterial cells may increase when the storage temperature is raised from refrigeration temperature to proper temperatures (Montville and Matthews, 2007; Jorczyk et al., 2011). For instance, growth of E. coli in brain heart infusion broth was not observed at 5 °C but at higher temperatures from 10 to 37 °C (Buchanan and Klawitter, 1992). It can be speculated that osmotic stress might have been enhanced due to promoted biological activities at 15, 20, and 25 °C. The different trends in the effect of hyperosmotic NaCl on the decrease in the counts might be ascribed to osmoregulation which was dominant at 5 and 10 °C and possible promotion of biological activities at higher temperatures.

The counts reached plateau values in 3.5 % NaCl PB solution during storage at 20 °C (Fig. 4B) and 25 °C (Fig. 5B). Besides, viable cell counts also seemed to limitedly decrease in 5.0 % NaCl PB solution during the late storage at 20 °C (Fig. 4) and 25 °C (Fig. 6). After storage for 48 d, the total viable cell counts were approximate 6 log under above conditions, and there was no notable difference among the counts of NS_37, NS_25 and S_37.

Viable cell counts (total and healthy) decreased to a nondetectable level in 10.0 % NaCl PB solution on 28 d at 25 °C storage (Fig. 5D). The results in this study
agreed with the report on NaCl stress to *E. coli* cells where reduction of viable cells was observed at 8.5 and 10.5 % NaCl (Glass et al., 1992). Meanwhile, faster decreasing trends of the counts were observed at 25 °C (Fig. 5) than at 20 °C (Fig. 4) for NaCl PB solutions containing NaCl at 3.5 % (Fig. 5B vs. Fig.4B), 5.0 % (Fig. 5C vs. Fig.4C), and 10.0 % (Fig. 5D vs. Fig.4D). The trends suggested that high temperature would promote the decreasing trends in the counts.

In 3.5 % NaCl PB solution (Figs. 4B and 5B), the counts approached plateau values after decreasing trends. Since adaptation of *E. coli* to salty environment was reported in seawater (Rozen et al., 2002; Arense et al., 2010) where NaCl concentration is approximately 3.5 %, the plateaus may be explained from a viewpoint of adaptation to NaCl stress at the seawater level. On the other hand, plateaus were also observed in 5.0 % NaCl PB solution (Figs. 4C and 5C), and they might also be ascribed to adaptation. The possible adaptations seemed to occur earlier at higher storage temperatures: 32 d (20 °C) and 16 - 28 d (25 °C) in 3.5 % NaCl PB solution whereas 32 - 40 d (20 °C) and 24 - 28 d (25 °C) in 5.0 % NaCl PB solution.

It was reported that the gene *rpoS* was involved in osmoregulation of *E. coli* (Hengge-Aronis, 1996). When *E. coli* cells were suspended in hyperosmotic solutions (4 % - 12 %), gene expression of *rpoS* was promoted at higher temperatures in the first 3 h (Gawande and Griffiths, 2005). The gene expression induced by hyperosmotic solutions might give some implications on the day-scale slow adaptation at 20 °C in our study.

On the other hand, there seemed no possible adaptation at 20 and 25 °C in 10.0 % NaCl solution (Figs. 4D and 5D). Doudoroff (1940) reported that viable cell counts of *E. coli* cells were constant when exposed to saline nutrient medium containing 7 % NaCl. The report may support possible adaptation of *E. coli* cells to NaCl of 5.0 % and lower in this study.

Significant difference (*p < 0.01*) was observed in the levels of injured cells in 5.0 % (Figs. 4C) and 10.0 % NaCl PB solutions (Figs. 4D and 5D) during storage. The counts of total viable (NS_25 and NS_37) and healthy (S_37) cells in 10.0 % NaCl PB solution decreased as storage period prolonged, and healthy cells became undetectable at 20 and 25 °C after 44 d (Fig. 4D) and 20 d (Fig. 5D), respectively. Furthermore, after storage at 20 °C for 48 d (Fig. 4D), total viable cell counts as a sum of injured and healthy populations on NS were higher at 37 °C (4.70 ± 0.75 log) than at 25 °C (1.37 ± 0.72 log), whereas no healthy cells on S_37 were detected. The trend where more injured cells were detected at higher incubation temperatures agreed with that of freezing-injured *E. coli* cells (Ray and Speck, 1973).

However, opposite trends where less injured cells detected at higher incubation temperatures have been reported. For instance, repair rate of *Listeria monocytogenes* stressed by suspension in acid or salted brain heart infusion broth was faster at an incubation temperature of 30 °C than at 37 or 41.5 °C (Gnanou Besse et al., 2000). Klein and Wu (1974) reported that more microbial population was detected by pour plate technique at warming temperature of 42 and 45 °C than that at 50 °C, indicating that the temperature susceptibility of starvation-stressed *E. coli* cells increased. More *E. coli* cells injured by high hydrostatic pressure were detected at 25 °C than at 37 °C (Kimura et al., 2017).

Stressed bacteria become sensitive to environmental conditions such as temperature and chemicals due to damages of cell wall and membrane with modified permeability (Gnanou Besse et al., 2000). The different trends in terms of the effect of incubation temperature on the detection of injured bacteria might be related to the level and site of injury (Wu, 2008). It might also be dependent on the bacterial species. It is necessary to investigate further the effect of incubation temperature on the detection of injured bacteria to clarify the different trends.

Furthermore, the counts of injured cells in 10.0 % NaCl PB solution were higher on NS_37 than those on NS_25 (Figs. 3D, 4D, and 5D). This may indicate existence of at least two different populations of injured cells: some populations formed colonies on NS at both 25 and 37 °C, while the others formed colonies only at 37 °C.

During storage at 20 °C in 5.0 % NaCl PB solution (Fig. 4C), viable cell counts on NS_37 decreased monotonically while those on NS_25 and S_37 decreased monotonically down to about 4 log (36 d) and then increased significantly (*p < 0.01*) to a quasi-plateau value of about 6 log (40 d). The difference in the counts between on NS_37 and NS_25/S_37 indicated existence of injured cells which did not form colonies on NS_37.

On the other hand, during storage at 20 °C in 3.5 % NaCl PB (Fig. 4B), viable cell counts on NS_25, NS_37, and S_37 decreased in a comparable monotonous manner down to 5 log (28 d) and then increased significantly (*p < 0.01*) to a plateau value of about 6 log (32 d), respectively. No notable difference among the data on the different plating conditions indicated no detection of injured cells. Similar trends were observed during storage at 25 °C in 5.0 % NaCl PB solution (Fig. 5C): viable cell counts on NS_25, NS_37, and S_37 decreased monotonically down to 4 log - 5 log (20 d) and then increased significantly (*p < 0.01*) to a quasi-plateau value of 5 log - 6 log (24 d).

It can be speculated that some healthy cells might have disorders in colony forming ability, which were not
distinguishable by selective and nonselective detections in this study. The subsequent 1 or 2 log increase might be also ascribed to repair of the disorders in healthy cells, although the underlying mechanisms are not clear. In addition, taking into account the possible cannibalistic growth of *E. coli* at 15 °C and higher storage temperatures in PBS (Morimatsu et al., 2019), it might be possible for the injured and/or healthy cells to have utilized some nutrients derived from the dead cells for growth in this study. However, the growth of *E. coli* in hyperosmotic nutrient-poor solutions in this study may not be probable. Growth of *E. coli* was suppressed in nutrient-rich medium containing NaCl in a range of approximately 1 – 5.8 % (Sasaki et al., 2009), which covers hyperosmotic condition (3.5 % and 5.0 % NaCl) in this study. In addition, bacterial growth would be suppressed more in nutrient-poor system than in nutrient-rich system. For instance, faster growth was observed with *Listeria monocytogenes* in nutrient-rich system (trypsincase soy broth) than in nutrient-poor PBS containing their heat-treated dead cells (Nakaura et al., 2019). Further study will be indispensable for elucidating the mechanisms underlying the trends in the viable cell counts.

**4. CONCLUSIONS**

The effect of NaCl concentration (0.8, 3.5, 5.0, and 10.0 %) and storage temperature (5, 10, 15, 20, and 25 °C) on the viability of *E. coli* was studied during storage up to 48 d. Total viable and healthy cells were evaluated on nonselective and selective media, respectively. Viable *E. coli* cells decreased gradually during storage at 5 and 10 °C, and NaCl showed a slight protective effect on viable cells. When storage temperature was 15 °C and above, decreasing trends in viable cell counts were enhanced with increases in NaCl concentration and storage temperature. The counts approached constant values in 3.5 and 5 % NaCl PB solutions, indicating possible adaptation, while the cells were inactivated to a nondetectable level in 10.0 % NaCl PB solution after 28 d storage. On the other hand, cells were injured by NaCl stress (≥ 5.0 %), and total viable cells were detected more on nonselective medium at incubation temperature of 37 °C than that at 25 °C. In addition, injured cells in 5.0 % NaCl PB solution recovered during storage, but no recovery was observed in 10.0 % NaCl PB solution. NaCl even at high concentration of 5.0 - 10.0 % might not be sufficient for inactivation of *E. coli* in nutrient-free liquid in this study. It is of further interest to study the hyperosmotic effect in nutrient-rich system and the combined effect of NaCl addition and intervention technologies on the viability of *E. coli*.

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