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Survival of *Escherichia coli* on Polypropylene Surfaces Fouled with Organic Soil of Different Water Contents

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Knowledge of survival behaviors of microorganisms on solid surfaces is important to assess and control the risk of cross contamination for food being processed on the surface. Here we report the survival behavior of *Escherichia coli* left on polypropylene coupons with or without nutritious soil subjected to drying at room temperature. When *E. coli* cells were left on the coupons with 0.85% saline solution, decrease in the viable cell number was observed in two stages, each of which followed the first order kinetics. The specific death rate was 0.21 h^{-1} initially and reduced to 0.11 h^{-1} after the water content reached 3.9 g-water/g-solid. When *E. coli* cells were left with Luria-Bertani broth, as a model soil, of different water contents, the viable cell number did not show a distinct decrease until the water content reached 3-4 g-water/g-solid. After the water content went down below the threshold, the specific death rate was roughly 0.1 h^{-1} though deviation was large especially at the final stage of drying. *E. coli* survived on the surface even after 2 days, and coexistence of nutrients enhanced the final level of viable cells.

Key words: *Escherichia coli*, survival, polypropylene surface, soil, drying

1. Introduction

Surfaces of equipment and utensils for food processing should be kept clean. Improper cleaning would leave food residues on their surfaces as soil, which could help survival or growth of microorganisms and give a chance for cross contamination of food being processed afterwards. To assess and control the risk of cross contamination, knowledge of survival behaviors of microorganisms on solid surfaces is essential.

Recently, several reports have been published on the survival of microorganisms on solid surfaces. Kusumaningrum *et al.*[1] studied survival of *Salmonella enteritidis*, *Staphylococcus aureus*, and *Campylobacter jejuni* inoculated with physiological saline on stainless steel surfaces at different initial contamination levels. They showed that portions of the pathogens remain viable on dry stainless steel surface at room temperature for considerable periods of time. Rose *et al.*[2] studied survival of *Yersinia pestis* not only on stainless steel but also on polyethylene, glass, and paper. Møretrø *et al.*[3] and Cools *et al.*[4] studied survival of *Salmonella spp.* and *C. jejuni*, respectively, inoculated with a culture broth on solid surfaces. Wilks *et al.*[5] reported that *Escherichia coli* O157 survived on stainless steel surface for over 28 days at refrigeration and room temperatures. Milling *et al.*[6] studied survival of *E. coli* and *Enterococcus faecium* on wood in comparison with that on polyethylene. In these studies, solid surfaces artificially contaminated with a bacterial suspension were used as test materials. When the survival experiment is started with a wet surface like in most of the above studies, dehydration of the surface occurs, which can affect the survival course because water content or its activity is an important factor for survival of bacteria. However, no report has ever taken into consideration the change in water content of bacterial environment during the survival course on solid surfaces.

Here we report the survival behavior of *E. coli* left on a wet solid surface with or without organic soil in relation to the progress of drying. We took polypropylene (PP) as an example of surface material since it is often used for food containers and such utensils as cutting boards. We took a liquid medium, Luria-Bertani (LB) broth, as a model organic soil. The use of a culture broth, having sufficient nutrients for bacterial growth, is expected to give a worst case of bacterial survival. We put *E. coli* cells suspended in LB broth of different water contents on PP coupons and left them at room temperature to study the courses of viable cell number and water content on PP surface. To avoid

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microbial contamination without making the ambient conditions entirely different from an indoor atmosphere, all the survival experiments were done in a clean bench, consequently without control of ambient conditions.

2. Materials and Methods

PP coupons (50×50 mm) were washed with an alkaline detergent (Cica Clean MI, Kanto Chemical Co., Inc., Tokyo), rinsed with water, and stored in 70% ethanol at least for one day. Just before use, the PP coupons were taken out and dried under UV light in a clean bench.

E. coli NBRC3301 was maintained on a plate of LB agar, consisting of 10 g/l tryptone (Becton Dickinson, Sparks, MD, USA), 5 g/l yeast extract (Becton Dickinson), 10 g/l NaCl, and 15 g/l agar. A loopful of *E. coli* cells on the plate were inoculated into 10 ml of LB broth, having similar composition to LB agar except that no agar was added, and grown at 37℃ for 24 h. The stationary phase culture thus obtained was diluted 10^3 times with one of the diluting agents listed in Table 1 for each experimental run. Run S, using saline solution (0.85% NaCl), represents the condition with a negligible amount of organic soil. Runs 1LB, 5LB, and 10LB were for the cases with organic soil of different initial water contents. An aliquot (0.1 ml) of the dilution, containing $2\times10^4-5\times10^4$ cells, was put on each PP coupon. The coupons were left for various periods of time (0−48 h) in a clean bench. Similarly to Cools *et al.*[4], the viable cell number on the coupons was enumerated by swabbing technique as following. A coupon was swabbed with a sterile cotton bud (Men-tip A1504, JCB Industry Ltd., Tokyo), after being moistened if necessary. The cotton bud was then immersed and vigorously shaken in 1 ml of sterile saline solution to disperse attached cells. An aliquot (0.1 ml) of the dispersion, or of an appropriate dilution if necessary, was spread on a LB plate and incubated at 37℃ for 24 h. Colonies grown on the plate were counted to obtain viable cell number on the PP coupon. All the counts were carried out in duplicates. Preliminary experiments showed that the standard deviation of cell number obtained by the swabbing technique was 11% of the mean value. Survival ratio at time t was defined as $N(t)/N(0)$, where $N(t)$ and $N(0)$ are the viable cell numbers on the coupon at time t and time 0, respectively. For runs 1LB, 5LB, and 10LB, the value of specific death rate at time t was roughly estimated using three successive data of viable cell number, $N(t-\delta_{t-})$, $N(t)$, and $N(t+\delta_{t+})$, as follows:

$$-\left(\frac{\Delta \ln N}{\Delta t}\right)_t = \frac{N(t-\delta_{t-})-N(t+\delta_{t+})}{N(t)\{\delta_{t-}+\delta_{t+}\}} \quad (1)$$

where δ_{t-} and δ_{t+} are swabbing intervals before and after the swabbing at time t.

Temperature was monitored with a thermocouple of type T during the survival course. Water content of the substances on each coupon was estimated by weighing one of the coupons repeatedly and synchronously with swabbing. After the survival experiment, the coupon was finally dried under vacuum to measure the solid weight on the coupon.

Table 1 Composition of diluting agent for microbial culture in each experimental run.

Run	S	1LB	5LB	10LB
Tryptone	0	10	50	100
Yeast extract	0	5	25	50
NaCl	8.5	10	50	100

Compositions are expressed in g/l.

3. Results and Discussion

Figure 1 shows the results of experimental run S, in which *E. coli* cells suspended in saline solution were inoculated on PP coupons. As shown in Fig. 1(a), the ambient temperature was 14.5±1.3℃, relatively constant despite of no temperature control, during the 2-day experiment. As shown in Fig. 1(b), the water content of saline solution decreased almost steadily in the initial 12-h period to reach 3.9 g-water/g-solid. The surface of PP coupons became almost dry after 24 h. Thus the course of drying can be divided into two stages. As shown in Fig. 1(c), the course of survival ratio of *E. coli* can also be divided into two stages. In the initial 12-h period, the survival ratio decreased in an exponential manner with time, indicating that the death of *E. coli* followed the first order kinetics in this period. From the slope of the initial part of the semi-logarithmic plot, the first order rate constant of death (specific death rate) was determined to be 0.21 h^{-1}. The decrease in survival ratio was retarded after 12 h but yet in an exponential relationship with time. The specific death rate was 0.11 h^{-1} at the latter stage.

The first order kinetics is a base for prediction of thermal sterilization. In the prediction, specific death rate or D value is usually assumed as a function of temperature alone unless the other conditions widely change. In the case of run S, the water content around *E. coli* cells was much reduced especially in the initial 12-h period. In spite of such a large change in environmental conditions, the specific death rate was apparently constant in each stage, though the reason is not clear at present.

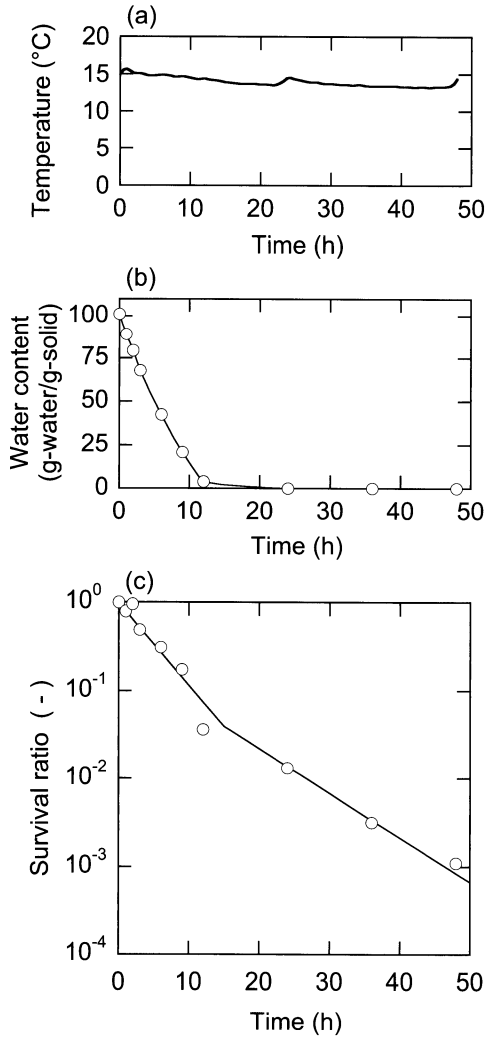

Fig. 1 Results of survival experiments for *E. coli* on PP coupons without organic substances. *E. coli* cells suspended in saline solution were spread on PP coupons and left in a clean bench (run S). Ambient temperature (a), water content on dry basis (b), and survival ratio (c) were measured at appropriate intervals.

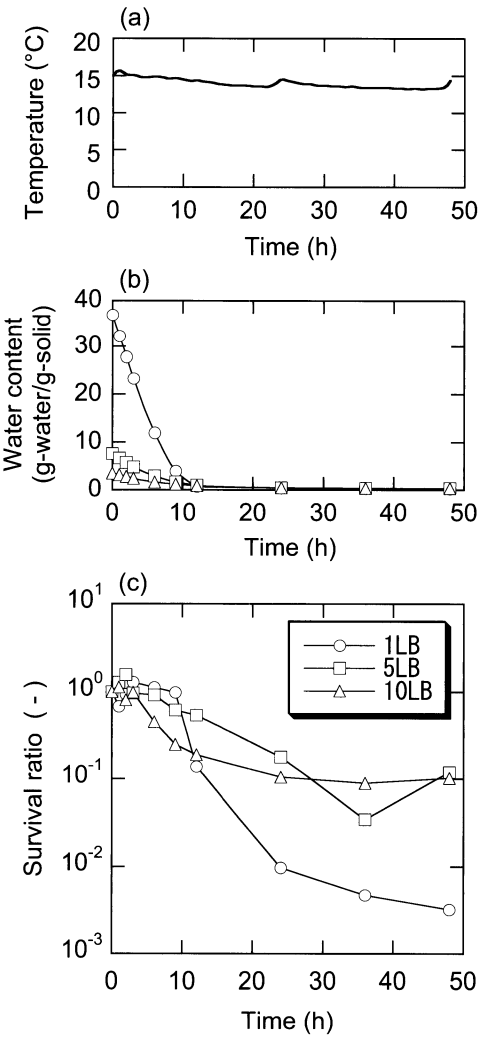

Fig. 2 Results of survival experiments for *E. coli* on PP coupons with organic substances. *E. coli* cells suspended in LB broth of different initial water contents were spread on PP coupons and left in a clean bench. Ambient temperature (a), water content on dry basis (b), and survival ratio (c) were measured at appropriate intervals for runs 1LB (○), 5LB (□), and 10LB (△).

Figure 2 shows the results of experimental rums 1LB, 5LB, and 10LB, in which *E. coli* cells were inoculated on PP coupons with LB broth of different water contents. As shown in Fig. 2(a), the ambient temperature was 13.9± 1.5℃ during the 2-day experiment. As shown in Fig. 2(b), the water content of the broth decreased with time and reached 0.3−0.4 g-water/g-solid at the end of the three runs. Courses of the survival ratio of *E. coli* are shown in Fig. 2(c). Presence of LB broth maintained or slightly increased the survival ratio at the initial stage of the experiment, though duration of the stage depended on the initial water content of LB broth. Subsequently the survival ratio decreased with the progress of drying though the rate of decrease gradually retarded. The final survival ratio tended to be higher when the broth was initially more concentrated.

Although runs 1LB, 5LB, and 10LB started from different water contents, they had the same proportion of solid-component quantities. Thus the same value of the water content on dry basis indicates that the soils on PP coupons had the same composition though their quantities were different. In this context, we compared the results of the three runs as a function of the water content on dry basis. Figure 3 shows relationship between the survival ratio and the water content. In any of the three runs, the survival ratio did not show distinct decrease until the

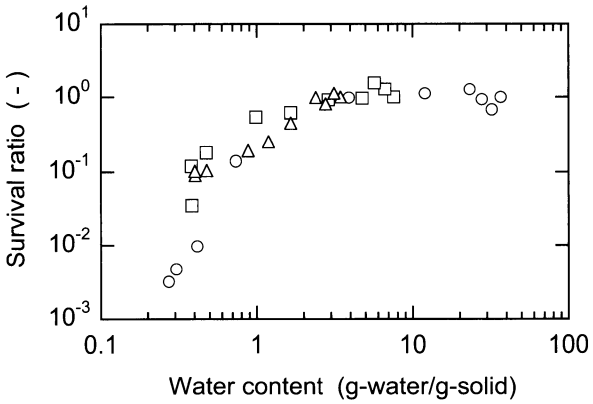

Fig. 3 Survival ratio as a function of water content of broth for runs 1LB (○), 5LB (□), and 10LB (△).

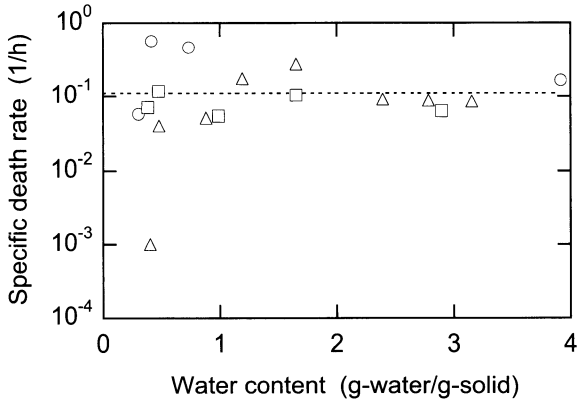

Fig. 4 Specific death rate as a function of water content of broth for runs 1LB (○), 5LB (□), and 10LB (△). The broken line indicates the specific death rate for run S determined from Fig. 1(c).

water content reached 3−4 g-water/g-solid. When the water content decreased further, the survival ratio decreased roughly in a similar tendency though minute differences were found between the courses of the three runs. Figure 4 shows relationship between the specific death rate and the water content. The specific death rate may be taken roughly as 0.1 h^{-1}, which is similar to that of the latter stage in run S. However, difference in the specific death rate among the three runs was relatively large in a range of low water content. Thus, although the water content is considered as an important factor affecting the survival course, additional factors should be taken into account to explain the difference. Dehydration tolerance of the cells is probably important as an additional factor. It has been reported that osmotically induced trehalose accumulation promotes dehydration tolerance in *E. coli* [7]. The cells inoculated with more concentrated broth might have become adapted to drying earlier in the survival course. Growth phase of the cells may be another factor because it is known that *E. coli* cells in the stationary phase are more resistant to drying than those in the exponential phase [8, 9].

This study demonstrated how the viable cell number of *E. coli* on fouled PP surface changed through drying at room temperature. The water content was found as an important factor for the understanding of the survival course. It was also shown that *E. coli* survived on PP coupons for 2 days in the presence and absence of organic soil though the organic soil enhanced the final level of survival ratio. Wilks *et al.*[5] reported that the viable cell number of *E. coli* O157, inoculated with a culture broth on a stainless steel coupon and left at 20℃, decreased by a factor of 10^{-5} in 2 days and was almost constant for the following 26 days. According to Milling *et al.*[6], the survival ratio of *E. coli* pIE639, inoculated with saline solution on a polyethylene tip and left at 21℃, became approximately 10^{-2} after 2 days and 10^{-3} after 6 days. Thus *E. coli* on solid surfaces survives for a substantial period of time under dryness, though the final number of viable cells probably depends on the initial level of inoculation. For further understanding of the survival behavior, more data should be compiled and analyzed in relation to the factors mentioned above as well as the conditions of bacterial environment, such as water activity, temperature, and the type and composition of coexisting soil.

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