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Note

Survival of Pathogenic *Yersinia enterocolitica* in Vacuum-packed or Non-vacuum-packed Pork at Low Temperature

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Pathogenic *Yersinia enterocolitica* serotypes O:3, O:5,27, O:8 and O:9 were inoculated into sliced and ground pork, and the samples were stored under vacuum or aerobic conditions at 2 and 7°C. All serotypes survived for 5 weeks in pork with or without vacuum packing without any discernable increases in their population. In sterilized pork with or without vacuum packing, there was no evident growth of *Y. enterocolitica*. In pork broth in which the pH had been artificially adjusted to 6.8, the growth of *Y. enterocolitica* was faster than that at 5.7. It is suggested that the growth of *Y. enterocolitica* in pork with or without vacuum packing may be inhibited by pH but not by the microflora or lactic acid bacteria in pork.

Key words : *Yersinia enterocolitica* / Fate / Pork / Vacuum packing / Aerobic.

Yersinia enterocolitica is able to grow under low temperature conditions (Kapperud, 1991; Tsubokura et al., 1975). The serovars O:3, O:5,27, O:8, and O:9 out of the entire group of 60 serovars cause foodborne infections. The *Y. enterocolitica* serovars O:3, O:9 and O:5,27 are distributed in most parts of the world (Tsubokura et al., 1975), while serovar O:8 is found in North America (Kay et al., 1983) and Japan (Hayashidani et al., 1995). Wild rodents are an important natural reservoir for *Y. enterocolitica* O:8 (Hayashidani et al., 1995). Pigs are known as a major reservoir of *Y. enterocolitica*, and the bacteria have been frequently detected in pork (Bhaduri et al., 2005; Duffy et al., 2001; Fredriksson-Ahomaa and Korkeala, 2003; Fredriksson-Ahomaa et al., 2004; Gurtler et al., 2005; Johannessen et al., 2000). The major serovar isolated from pork is O:3, but the serovars O:5,27 and O:9 have also reportedly been isolated (Fukushima, 1986, Fukushima et al, 1987; Schieman, 1989; Tsubokura et al., 1975). The growth of *Y. enterocolitica* on meat stored aerobically has been investigated previously. There have been noticeable differences among the findings such as in the contrasting reports of increases (Borch and Lindberg, 1995; Nissen et al., 2001) and no increase (Fukushima, 1986; Fukushima and Gomyoda, 1986) in populations of *Y. enterocolitica*. The survival or growth of *Y. enterocolitica* in pork is still unclear. In addition, the growth or lack of growth in of *Y. enterocolitica* on meat packed by vacuum-packing, which is a method used worldwide to prolong the storage time of meat, also needs to be clarified. In this study, the growth of *Y. enterocolitica* O:3, O:5,27, O:8, and O:9 inoculated in sliced and ground pork

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was studied during storage under vacuum or aerobic conditions at 2 or 7℃. Furthermore the effects of microflora and the pH of pork on the survival of *Y. enterocolitica* were investigated.

Y. enterocolitica serotype O:3 (NYSD1416-11 and NY0112001); O:5,27 (NY6401001 and Pa9571); O:8 (NY9306089 and APCCY9314); and O:9 (314-2, Pa177, and H739) (Hayashidani et al., 1995) were used in this study. Serotyping of the strains was accomplished with commercial antisera (Denka Seiken Co., Ltd., Tokyo, Japan). The pathogenicity of these strains was confirmed with the temperature-dependent calcium requirement according to Gemski et al. (1980) and temperature-dependent autoagglutination according to Laird and Cavanaugh (1980). The presence of pathogenic plasmids was also confirmed with a method reported by Kado and Liu (1981).

Loaves of pork were purchased from a retail shop in Tokyo. The loaves were immediately sliced in 1 cm thin sections and ground with a sanitary knife and mixer. In order to prepare sterilized sliced pork, the surface of a part of the loaves was burned with a burning device and then the burned surface was cut off with a sterilized knife. This sterilization of the loaf surface was repeated twice. Furthermore, the sterilized loaves were sliced with a sterilized knife. In addition, broth from pressed pork slices was filtrated with a filter (poresize:0.45 μm) and the pH was adjusted to 5.7 or 6.8 with 0.1 N NaOH.

Y. enterocolitica was cultured on trypticase soy agar (TSA; BBL, Cockeysville, Md, USA) at 25℃ for 24h. The colonies were suspended in PBS (pH 7.2) and diluted in PBS to approximately 10^7cfu/ml. A portion (0.1ml) of the dilution was inoculated into 100 g of a pork sample (10^4cfu/g). *Y. enterocolitica* O:3, O:5,27, O:8 and O:9, and serovars O:3 and O:8 were inoculated into sliced pork and ground pork, and sterilized sliced pork, respectively. The inoculums were spread on the entire surface of the sliced pork with a sterilized spreader and mixed in ground pork with a sterilized stick. As controls, uninoculated samples were also prepared.

To pack samples under vacuum, sliced pork was placed in a low oxygen trans plastic bag (20 × 30 cm, O_2 transmission rate of 2 ml/m^2 per 24 h/atm at 20℃, Ozaki Keikagaku, Tokyo, Japan) that was vacuumed by a vacuum packaging machine (Tospack V-220, Tosei Denki Co. Ltd., Shizuoka, Japan). To store samples under aerobic conditions, sliced and ground pork were placed in a sterilized Petri dish. The samples were incubated at 2 and 7℃. After bacterial inoculation, pork samples packed under vacuum and kept in aerobic conditions were tested during the storage period for up to 5 weeks and at 6 days, respectively.

After a pork sample was shredded with a sterilized knife, 1 g of the sample was homogenized in 9 ml of PBS with a sterilized homogenizer. The homogenate was serially diluted 10-fold with PBS and 0.1 ml of the homogenate and the dilutions were spread onto cefusulozin-irgasan-novobiocin agar medium (CIN; OXOID, Hampshire, UK) and virulent *Yersinia enterocolitica* selective agar medium (VYE) (Fukushima 1987) to isolate *Yersinia*, and TSA to determine the total bacterial count. In addition, BL agar medium (Eiken Chemical Co. Ltd., Tokyo, Japan) with 5% horse blood was used for the total bacterial count, including the lactic acid bacteria of pork under the vacuum packaging condition. CIN, VYE and TSA agar media, and BL agar medium with 5% blood were incubated at 25℃ for 48 h and at 4℃ for 1 week, respectively. Colonies suspected of *Y. enterocolitica* were tested for slide agglutination with commercial antiserum (Denka Seiken Co., Ltd., Tokyo, Japan).

Before counting the bacterial populations, pork samples were measured for pH with a contact electrode (pH I 10, Beckman Instruments, Inc., Fullerton, CA, USA). In addition, the color and odor of pork samples were evaluated as "acceptable" or not by more than two of the investigators. Significant differences between the initial bacterial population and the bacterial population during the course of storage over time in sliced pork under vacuum at 2 and 7℃ were analyzed by the Student's *t*-test.

In vacuum-packed pork, the population of each serotype of *Y. enterocolitica* was maintained at a similar level during storage for 5 weeks at 2 and 7 ℃ (Fig. 1). However the number of total bacteria, including lactic acid bacteria at 2 and 7℃, increased rapidly to 10^8 CFU/g during the period of two to five weeks. The pH value slowly decreased from 5.4-5.8 at the initial time point to 5.0-5.3 at five weeks (data not shown). At 4 weeks, pork samples stored at 2℃ emitted an acceptable odor, but those at 7℃ generated a strong smell of H_2S.

In sliced pork kept in aerobic conditions, the population of each serotype of *Y. enterocolitica* remained at similar levels at 2℃, but increased by nearly 1 log cycle at 7℃ during storage for 6 days (Fig. 2). The number of total bacteria increased from 10^5 to 10^9 CFU/g for 6 days at 2 and 7℃. The pH value at 2 or 7℃ increased from 5.4-5.8 to 5.7-6.2 or to 6.1-6.5, respectively. All the samples emitted some amount of disagreeable odor on day 4 of storage. In ground pork kept in aerobic conditions, the population of each serotype of *Y. enterocolitica* slowly increased by nearly 1-2 log cycles during storage for 5 days at

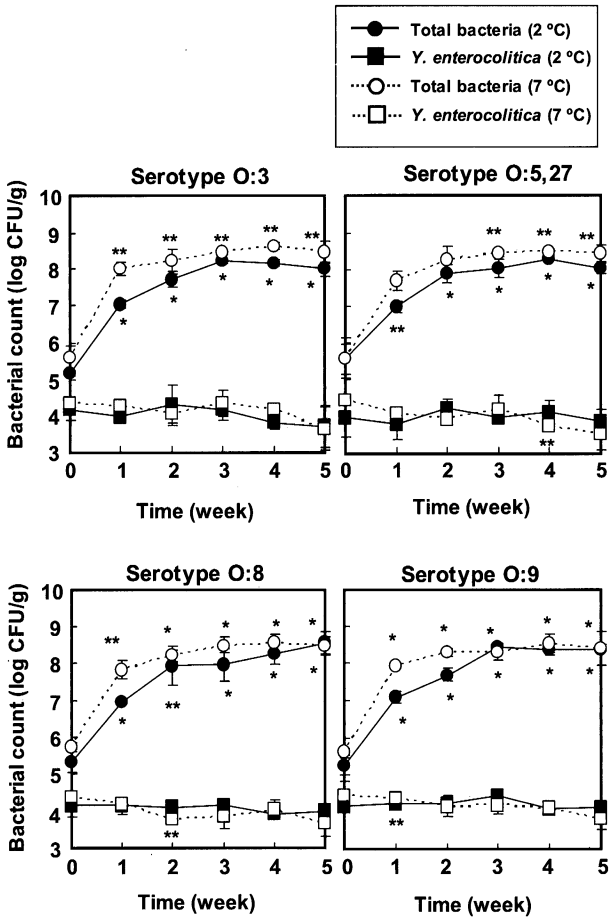

FIG. 1. *Y. enterocolitica* counts in sliced pork during storage under vacuum at 2 and 7℃. The bar in the graph is the standard error. * $p< 0.01$, ** $p< 0.05$.

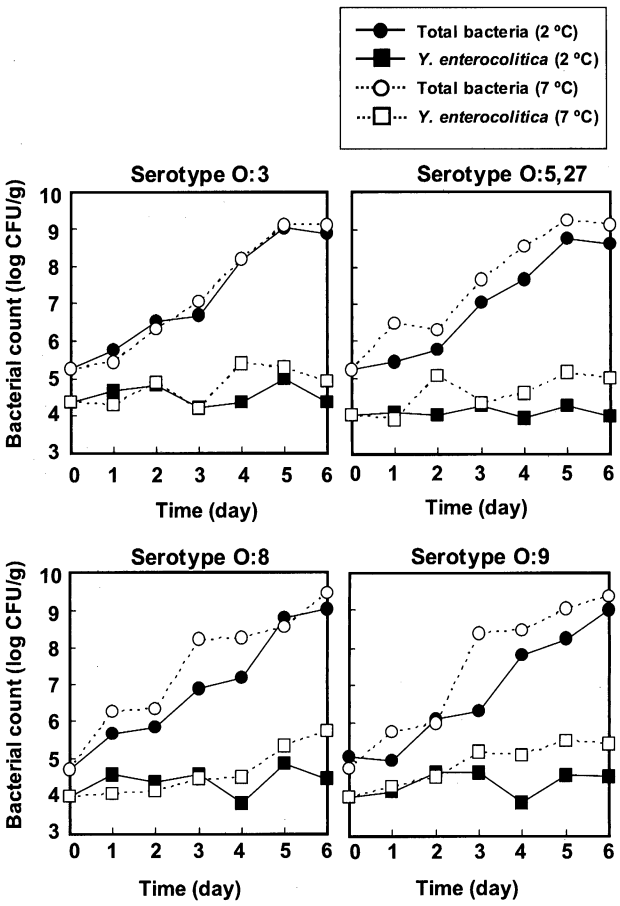

FIG. 2. *Y. enterocolitica* counts in sliced pork during storage in aerobic conditions at 2 and 7℃.

2 and 7℃ (Fig. 3). However, the populations of O:3 and O:5,27 decreased to the initial population, 10^4 cfu/g, at 2℃ on day 6. The number of total bacteria increased to $10^{9\text{-}10}$CFU/g for 6 days at 2 and 7℃ in a similar manner to sliced pork (Fig. 2). The pH value at 2 or 7℃ increased from 5.4-5.8 to 5.9-6.3 or to 6.1-6.5, respectively. All the samples emitted some amount of disagreeable odor on day 4 of storage.

In sterilized pork packed under either vacuum or non-vacuum conditions, the population of each serotype of *Y. enterocolitica* was maintained at a similar level during storage at 2 and 7℃ (Fig. 4). The results in the sterilized pork were similar to those in the non-sterilized pork packed under vacuum (Fig. 1). The pH value was maintained at 5.4-5.8 for 5 weeks. The pork samples were found to have an acceptable odor during storage. In pork broth at pH 5.7, the population of *Y. enterocolitica* was steadily maintained for 6 days except for O:8 at 7℃ (Fig. 5). In pork broth at pH 6.8, the populations of *Y. enterocolitica*, however, increased to 10^7cfu/g and $10^{8\text{-}9}$cfu/g, respectively and the pH value also increased to over 7.5.

Since pork is a food at high risk of being a vehicle for *Y. enterocolitica* infection, we studied the growth of the bacterium in pork packed under vacuum or aerobic conditions. Bodnaruk and Draughon (1998) have reported that a mixture of the *Y. enterocolitica* strains O:3, O:8 and O:20, inoculated into pork at a concentration of 10^3CFU/cm^2, increased to 10^5CFU/cm^2 during storage for 25-days or 15-days under vacuum at 4℃. Nissen et al. (2001) have reported that pathogenic *Y. enterocolitica* serotype O:3 grew in decontaminated and untreated pork during storage for 5 days in air or vacuum at 10℃.

In contrast to these studies, we found that pathogenic *Y. enterocolitica* O:3, O:5,27, O:8 and O;9 did not grow in vacuum-packed pork during a period of 5 weeks at 2 and 7℃. In terms of pH dependence, the present study demonstrates that the pH of pork is an important factor for inhibiting the growth of *Y. enterocolitica* (Fig. 5). Bodnaruk and Draughon (1998) have reported that pathogenic *Y.*

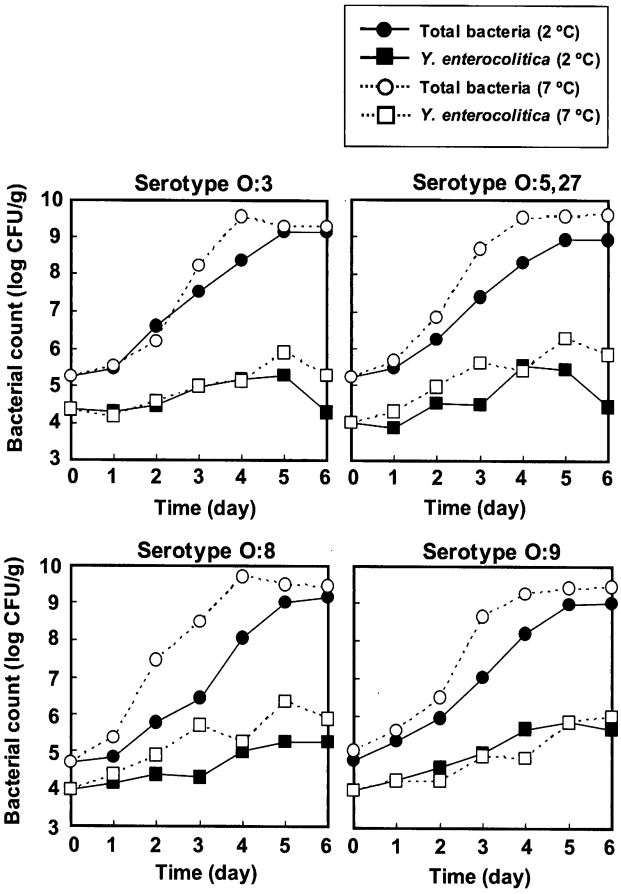

FIG. 3. *Y. enterocolitica* counts in ground pork during storage in aerobic conditions at 2 and 7℃.

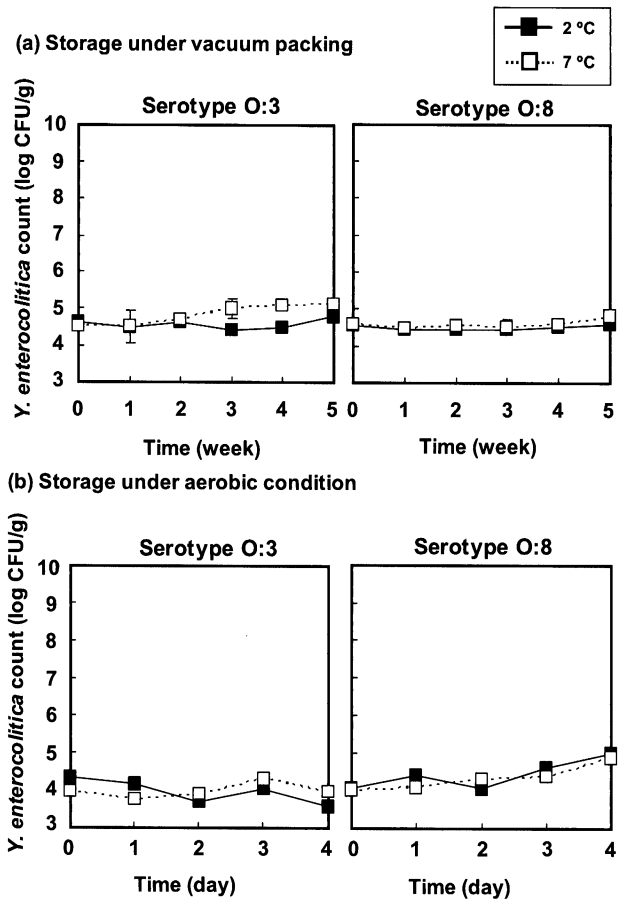

FIG. 4. *Y. enterocolitica* counts in sterilized sliced pork during storage under vacuum and aerobic conditions at 2 and 7℃.

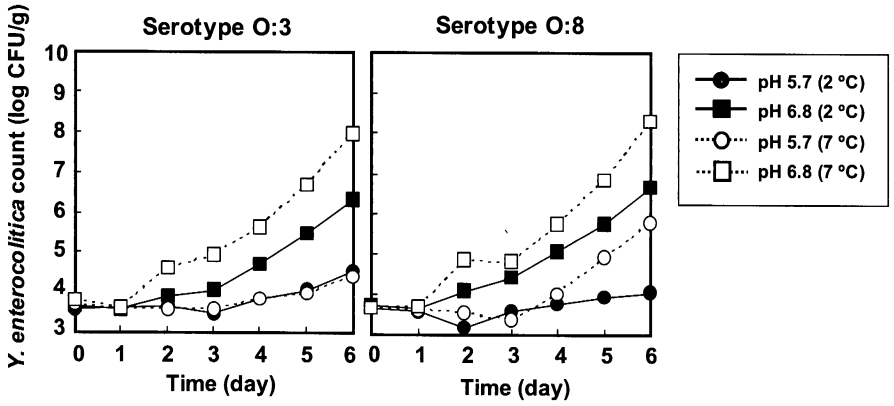

FIG. 5. *Y. enterocolitica* counts in pork broth at pH 5.7 and 6.8 during storage at 2 and 7℃.

enterocolitica in pork grew faster at a level greater than pH 6.0 than at pH lower than 5.8. Grau (1981) has reported that non-pathogenic *Y. enterocolitica* strains grew in beef at a pH greater than 6.0, although the strains did not grow in pork at a pH of 5.4-5.9, as is generally the case for vacuum-packed beef during storage for 5 ℃. We also showed that *Y. enterocolitica* in pork tended to grow if the total bacterial count had reached a plateau and the pH increased to the level the meat was inedible after storage at 2 and 7℃. Although vacuum-packing is one of the storage conditions used to store meat for

a long period of time at low temperatures, it is suggested that an increase in the pH value in meat promotes the growth of resident pathogenic *Y. enterocolitica*.

There have been reports of the inhibition of *Y. enterocolitica* by the natural microflora and lactic acid bacteria on pork (Fukushima and Gomyoda, 1986; Raccach and Henningsen, 1984). However, Nissen *et al.* (2001) have reported no inhibitory effects of the background microflora on the growth of *Y. enterocolitica* based on the findings that the serotype O:3 strain grew in pork decontaminated by steam vacuuming combined with being sprayed with lactic acid during storage at 10℃, as was the case in untreated pork in aerobic and vacuum conditions. Consistent with their findings, we observed that microflora in pork exerted no effect on the growth of *Y. enterocolitica*. The pathogen population was maintained at similar levels in sterilized and untreated pork stored in aerobic or vacuum conditions during storage at 2 and 7℃. There were no differences among the serovors O:3, O:5,27, O:8 and O:9 in terms of the growth of *Y. enterocolitica* in pork during storage under vacuum packing or aerobic conditions.

In conclusion, it has been demonstrated that the pathogenic *Y. enterocolitica* serotypes O:3, O:5,27, O:8 and O:9 survived in pork stored either under vacuum or aerobic conditions at a low temperature. Pork microflora had no discernible effect on the survival of *Y. enterocolitica*. However, in this study, we did not examine the water activity of pork samples examined. In a future study, we should examine the effect of water activity of pork to *Y. enterocolitica* growth on pork because the water activity of pork seems to be an important factor regulating the bacterial growth in meat.

Based on these findings, it seems likely that avoiding *Y. enterocolitica* contamination in slaughter houses or meat factories is important in the prevention of *Y. enterocolitica* infection. Furthermore, the survival of *Y. enterocolitica* for an extended period of time in pork and transmission of the pathogen to other foods may be a critical issue in efforts to insure food safety.

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