A Research Note

Survival of Escherichia coli O157:H7 in Mayonnaise and Mayonnaise-Based Sauces at Room and Refrigerated Temperatures

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ABSTRACT

Three Escherichia coli O157:H7 (EHEC) strains were inoculated separately into portions of commercially prepared mayonnaise held at 25 or 7°C, then examined periodically for survival of detectable EHEC. Four mayonnaise-based sauces including: a) mayonnaise-mustard sauce, b) blue cheese dressing, c) thousand island dressing and d) seafood sauce, were each inoculated with one EHEC strain. Samples of these sauces were held at 5°C, and assayed periodically for survival of detectable EHEC. Both direct plate count and selective enrichment recovery were employed as assay procedures. Escherichia coli O157:H7 strains, when inoculated and mixed into mayonnaise and stored at 25°C, became undetectable after 72 h storage when assayed by direct plating or by selective enrichment. The same strains inoculated into mayonnaise and stored at 7°C were detectable up to 35 days when assayed by direct plating or by selective enrichment. Escherichia coli O157:H7 inoculated into mayonnaise-based sauces and held at 5°C were detectable past 35 days in three of the four sauces. Loss of EHEC culturability occurred within 3 days in mayonnaise-mustard sauce.

Key Words: Escherichia coli O157:H7; mayonnaise, mayonnaise-based sauces, room and refrigerated temperatures

Enterohemorrhagic EHEC was first recognized as a foodborne pathogen in 1982 during outbreaks that occurred in Oregon and Michigan (7,10,11). This organism is responsible for illness marked by abdominal cramps, bloody diarrhea and in limited cases, hemolytic uremic syndrome (HUS). This syndrome, which occurs most often in children under 5 years, is fatal in 3 to 5% of cases and is the most common cause of acute kidney failure in children (5). Foods most commonly linked to outbreaks through epidemiological investigations are undercooked beef and raw milk (7-9). One outbreak was linked to apple cider consumption (2). Early in 1993 a series of outbreaks of foodborne diarrheal illness occurred in Oregon among customers of two outlets of a restaurant chain specializing in steaks and self-serve salad bar food. There were an estimated 300 people affected. Escherichia coli O157:H7 was found to be the causative organism. Eighteen cases were confirmed by stool culture and a further 39 presumptive cases were identified. The most likely foods to have been contaminated were mayonnaise and mayonnaise-based dressings and sauces (Personal communication, William Keene, MD, Oregon State Health Dept.). The concern that low pH foods could serve as a fomite for EHEC prompted the current study to examine the survival of EHEC in mayonnaise and mayonnaise-based sauces held at room and refrigerator temperatures.

MATERIALS AND METHODS

Mayonnaise and mayonnaise-based sauces

Commercially prepared mayonnaise epidemiologically implicated in a recent E. coli outbreak (Spring, 1993) was used for this study. The mayonnaise pH measured 3.65 and ingredients included liquid soybean oil, eggs, corn syrup, water, vinegar, egg yolk, salt and lemon juice. Sauces prepared from the suspect mayonnaise also were studied. The sauces and respective pH values were: a) mayonnaise-mustard (pH = 3.68), b) blue cheese dressing (pH = 4.44), c) thousand island dressing (pH = 3.76) and d) seafood sauce (pH = 4.38).

All samples of mayonnaise and mayonnaise-based sauces used in the study were analyzed for the presence of EHEC by the method of Hill et al. (6) and were not found to contain detectable EHEC.

Inocula preparation

Three strains of E. coli O157:H7 were used as inocula: strain SEA-6318, a Shiga-like toxin (SLT) producer of both toxins I and II, isolated from raw hamburger meat; strain SEA-6370, a human isolate and producer of SLT-II; and strain SEA-6371, a human isolate and producer of both SLT-I and SLT-II. Strains 6370 and 6371 were provided by Oregon Public Health Laboratories. Stock cultures were maintained at -70°C in 10% glycerol. Each strain was grown separately in modified trypticase soy broth (mTSB) (3) at 37°C overnight. Cells were centrifuged, and resuspended in sterile Butterfield's phosphate-buffered dilution water (BPBW) (6). Cultures were diluted decimally in BPBW and appropriate dilutions assayed by spread plating on tryptic soy agar-yeast extract (TSA-Ye).
Inoculation of foods and assay for EHEC

a.) Mayonnaise held at 25°C ± 1°C. Samples of mayonnaise were prepared by aseptically transferring 2.5 g portions to sterile 50 ml plastic tubes. Escherichia coli O157:H7 strains were added separately to tubed product, at 5.8 x 10^7 colony forming units (CFU) per gram for strain SEA 6318, 4.2 x 10^7 CFU for strain SEA 6370, and 4.3 x 10^7 CFU for strain SEA 6371, then thoroughly mixed into the mayonnaise. Duplicate samples were assayed after incubation of inoculated mayonnaise for 0, 6, 24, 48 and 72 h at 25°C. Assays for EHEC were performed by homogenization of samples in 22.5 ml mTSB followed by decimal dilutions in BPDW and spread plating of a 0.1 ml portion of appropriate dilutions on TSA-Ye in duplicate. Plates were incubated overnight at 37°C and colonies counted. Representative colonies (five per plate) were confirmed as EHEC by typical growth on HC-MUG agar (6) and on Levine's eosine methylene blue agar (EMB). Homogenates in mTSB were held 2 h at 25°C and then novobiocin supplement was added followed by enrichment at 37°C for 24 h with shaking. Enrichments were appropriately diluted and spread plated (0.1 ml) on HC-MUG agar plates and incubated at 42°C overnight according to the method of Hill et al. (6). Typical colonies were subcultured on EMB agar. Random isolates were confirmed by serology with E. coli O antisera (Difco Laboratories, Detroit, MI). Uninoculated control portions were tested at each assay time point as well.

b.) Mayonnaise held at 7°C ± 0.5°C. Samples were inoculated and assayed as above except that each sample was repeated without any mixing of EHEC inoculum into mayonnaise sample (spot inoculation). Inoculation levels were 1.44 to 1.68 x 10^7 CFU per gram. Sampling time points were 0 time, 1, 2, 3, 4, 6, 7, 14, 21, 28, 35 and 42 days.

c.) Mayonnaise-based dressings and sauces held at 5°C ± 0.5°C. Samples were inoculated and sampled as in a.) above. Inoculation was with EHEC culture 6371, only, at 9.6 x 10^6 CFU per gram. Sampling times were 0 time, 1, 2, 3, 4, 6, 7, 14, 21, 28 and 35 days.

RESULTS AND DISCUSSION

Three cultures of EHEC inoculated and mixed into mayonnaise and stored at 25°C rapidly died off (Fig. 1). When EHEC became undetectable at 72 h by direct plating on TSA-Ye, it was detected in only 2 of 6 samples tested by enrichment method (3). These same EHEC cultures inoculated and thoroughly mixed in mayonnaise then held at 7°C were detectable up to 35 days by direct plating on TSA-Ye (Fig. 2). Spot inoculation of the mayonnaise samples stored at 7°C produced more variability in results but not in the overall rate of survival (data not shown). When four mayonnaise-based sauces were inoculated with one culture of EHEC and held at 5°C, survival in three of four sauces was somewhat longer than in mayonnaise. However, loss of culturability by direct plating on TSA-Ye occurred within 4 days in the mayonnaise-mustard sauce (Fig. 3). When survival of EHEC in mayonnaise and mayonnaise-based sauces was assayed by enrichment method in mTSB, survival times were found to be roughly equivalent to those obtained by direct plating. However, in some instances typical colonies were confirmed on TSA-Ye but the same sampling produced negative results by enrichment in mTSB (data not shown). At each sampling time uninoculated portions of mayonnaise and sauces were assayed and found negative for EHEC (data not shown).

Temperatures chosen for this study were based on the temperature normally used for storage of commercial retail mayonnaise (25°C) and the temperatures at which the mayonnaise and sauces implicated in the EHEC outbreak had been stored at 5 and 7°C, respectively. The comparison of spot inoculation was included to represent the effect on survival of EHEC in mayonnaise if it was inoculated after preparation by cross contamination, such as meat juices dripping into the product during shipping or storage. Our results demonstrating survival of EHEC in mayonnaise for
Figure 3. Survival of one EHEC culture in four mayonnaise-based dressings and sauces when held at 5°C.

less than 3 days at room temperature are consistent with study results of Zhao et al. (12) for EHEC survival in apple cider, another acid food and are similar to results reported for two other pathogens, Salmonella spp. and Listeria monocytogenes, held in mayonnaise products at room temperature (4). The extended survival times of EHEC in mayonnaise at refrigeration temperature also were consistent with results of two prior studies. Zhao et al. (12) found that EHEC seeded in apple cider survived from 10 to 31 days at 8°C. Abdul-Raouf et al. (1) obtained no loss in cell numbers when EHEC was held in beef salads (containing up to 40% mayonnaise) for 72 h at 5°C. In the present investigation, a comparison of results from direct plating on non-selective TSA-Ye to results from enrichment in mTSB showed that as EHEC cells became more physiologically stressed from cold temperature and acid during prolonged storage in mayonnaise, they also became increasingly unable to overcome the selective factors in the enrichment broth. This observation indicates that addition of a resuscitation step or enhancement factors to the enrichment proce-

REFERENCES