INFLUENCE OF RAW MEAT NATURAL BACKGROUND FLORA ON GROWTH OF ESCHERICHIA COLI O157:H7 IN GROUND BEEF

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ABSTRACT

Escherichia coli O157:H7 is a foodborne pathogen of increasing importance. It has been involved in several threatening outbreaks, most of them associated with meat products. In this study, the influence of some bacteria from the natural background flora of raw meat over E.coli O157:H7 in ground beef stored under refrigeration and at room temperature was evaluated. Different levels of E.coli O157:H7 (10^1-10^2, 10^3-10^4 and 10^6-10^7 CFU/g), inoculated in ground beef samples, were challenged with strains of non-pathogenic E.coli, Pseudomonas putida or Leuconostoc sp. Growth of the pathogen was monitored using standard cultural methods and an ELISA-type rapid method. Non-pathogenic E.coli, Pseudomonas putida and Leuconostoc sp. did not affect growth of E.coli O157:H7 in ground beef, both under refrigeration and at room temperature. Based on these findings, the low occurrence of E.coli O157:H7 in raw meat may not be attributed to antagonistic effects of bacteria from the natural background flora.

Key words: Escherichia coli O157:H7, antagonism, ground beef

INTRODUCTION

Enterohemorrhagic Escherichia coli is a foodborne pathogen of increasing importance. It was identified as a human pathogen in 1982, when E. coli serotype O157:H7 was associated with two outbreaks of hemorrhagic colitis (20). Since then, many outbreaks have been reported, culminating in 1996 in Japan with a foodborne outbreak that affected at least 6,309 children from 62 Sakai schools (9, 23). Most confirmed E. coli O157:H7 outbreaks have been associated with the consumption of undercooked ground beef and less frequently, other types of foods like unpasteurized milk and apple cider (6).

Geographically, the focus of attention on E. coli O157:H7 has been largely on the North American continent. However, recent reports reveal that E. coli O157:H7 and other serotypes of enterohemorrhagic E. coli are responsible for human disease in other parts of the world as well. The apparent geographic clustering of E.coli O157:H7 may be due to awareness by physicians and testing laboratories (13, 24). Some reports have addressed on infections caused by Shiga-toxin producing E. coli and its presence in food in developing countries like Argentina, Chile and Thailand (5, 15, 22). Moreover, in Argentina E.coli serotype O157:H7 has been associated with 2 to 18% hemolytic uremic syndrome.
E. coli 0157:H7 in ground beef

(HUS) patients and with 4.5 to 7% of children with bloody diarrhea (14).

So far, there is no report on occurrence of foodborne outbreaks due to Shiga-toxin producing E. coli in Brazil. Even the involvement of this pathogen in human cases of hemorrhagic colitis in this country is not known. Gomes et al., 1994, observed that Shiga-toxin producing E. coli was present in only 0.4% of children diarrheic stool samples, but none of the isolated strains was O157:H7 (10). The occurrence of E. coli O157:H7 or of other Shiga-toxin producing E. coli in cattle has also been very low (3).

Many explanations for the low occurrence of E. coli O157:H7 in raw meat may be considered. Studies showed that the carriage of E. coli O157:H7 in cattle is transient and seasonal and the prevalence of this pathogen in animals is low (1, 2). Besides this, interactions between microorganisms in raw meat are believed to be important in the selection of the microflora (7). Antibacterial activity of lactic acid bacteria (LAB) and Pseudomonas spp. over other microorganisms is well known (4, 17).

This study was conducted to observe possible antagonism between bacteria that are part of the background flora of raw meat and E. coli O157:H7 in ground beef samples kept under refrigeration and at room temperature. The study was carried out through challenge tests done with strains of non-pathogenic E. coli, Leuconostoc sp. and Pseudomonas sp. isolated from Brazilian raw meat.

MATERIALS AND METHODS

Bacterial strains

Escherichia coli O157:H7 strain EDL 933 was isolated from a hamburger outbreak (17). Non-pathogenic Escherichia coli, Pseudomonas putida and Leuconostoc sp. were isolated from Brazilian fresh raw meat products, purchased in local supermarkets of the city of São Paulo, Brazil. The E. coli strain selected for this study was isolated using methods recommended by APHA (12) and characterized as non-pathogenic using DNA probes (16). The Pseudomonas putida strain was isolated using cetrimide-fucidin-cephaloridine agar (CFC agar – pseudomonads agar base type CM 559 with selective suplement SR 103; Oxoid), incubated at 30°C for 48 hours, and identified as Ps. putida using the VITEK system (bio-Mériéux). The Leuconostoc sp. strain was isolated using MRS agar (MRS broth plus 1.5% agar) for lactic acid bacteria, with incubation at 30°C for 48 hours, and identified as Leuconostoc sp. according to Schillinger and Lücke, 1987 (21).

Preparation of meat

Samples of bovine semitendinosus muscle were purchased in local supermarkets of the city of São Paulo, Brazil. Under aseptic conditions, the external layer (approximately 0.5 cm thick) of the muscle was removed and internal portions were grounded in a sterile meat grinder. The ground meat was divided into portions of 25 g in sterile plastic bags and kept frozen until used.

Preparation of cultures

The E. coli O157:H7 and the non-pathogenic E. coli strains were cultivated in TSB at 35°-37°C for the time needed to reach 10⁸-10⁹ CFU/ml, determined through a spectrophotometric calibration curve. The Ps. putida and the Leuconostoc sp. strains were grown in TSB at 25°C and in MRS broth at 30°C, respectively, for the time needed to reach 10⁸ CFU/ml, also established through a spectrophotometric calibration curve. The bacterial cultures were serially diluted in 0.1% peptone water and 0.1 ml of each dilution was plated onto TSA or MRS agar plates (MRS broth plus 1.5% agar), for determination of the exact number of CFU/ml.

Challenge tests

Portions of 25 g of ground beef were inoculated with 2.5 ml of the E. coli O157:H7 and the challenge cultures, using proper dilutions in order to get the following combinations:

- E. coli O157:H7 (0, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴ or 10⁻⁵ CFU/g) and non-pathogenic E. coli (0, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴ or 10⁻⁵ CFU/g);
- E. coli O157:H7 (0, 10⁻¹, 10⁻², 10⁻³ or 10⁻⁴ CFU/g) and Pseudomonas putida (0, 10⁻¹, 10⁻², 10⁻³ CFU/g);
- E. coli O157:H7 (0, 10⁻¹, 10⁻², 10⁻³ or 10⁻⁴ CFU/g) and Leuconostoc sp. (0 or 10⁻³ CFU/g).

Six equal samples were prepared for each inoculation level and combination.
homogenization of the inoculated meat samples by hand massaging of the plastic bags, four samples were kept under refrigeration (8.5°C) and analyzed after 24, 48, 72 and 96 hours. The two remaining samples were kept at room temperature (25°C) and analyzed after 24 and 48 hours. Negative controls, consisting of non-inoculated meat portions and of meat portions inoculated with only one of the microorganisms at each inoculation level, were also included.

Analysis of inoculated meat samples

Each ground beef sample was homogenized with 225 ml of 0.1% peptone water in a Stomacher (Seward Medical Ltd.) and subsequent decimal dilutions were made using the same diluent. Portions of 0.1 ml of each dilution were plated onto MacConkey-sorbitol agar (MCS, Difco) for enumeration of E. coli O157:H7 (sorbitol negative colonies) and non pathogenic E. coli (sorbitol positive colonies), onto cetrimide-fucidin-cephaloridine agar (CFC agar, Oxoid) for enumeration of Pseudomonas sp., or onto MRS agar (MRS broth plus 1.5% agar) for enumeration of lactic acid bacteria. The temperature and time of incubation for MCS agar was 35-37°C for 18-24 hours, for CFC agar, 25°C for 48 hours and for MRS agar, 30°C for 48 hours. Colonies of E. coli O157:H7 on MacConkey-sorbitol agar were identified using suitable biochemical (glucose, lactose and sorbitol fermentation, production of gas, H₂S, indol, urease and lysine descarboxilase and motility) and serological tests, according to Ewing, 1986 (7) and Toledo et al., 1982a,b (25, 26).

Enumeration of E. coli (non-pathogenic) was also performed on Petrifilm™EC plates (3M Microbiology, St. Paul, MN), with incubation for 18-24 hours at 35°-37°C. For the enumeration of E. coli O157:H7, the Petrifilm™ kit HEC (3M Microbiology, St. Paul, MN) was used. This kit is based in a ELISA-type test, carried out with colonies grown on Petrifilm EC plates. The colonies are transferred from the plate to a reactive disc and O157:H7 antigens, if present, are used to capture enzyme-labeled anti-O157 antibodies (i.e., conjugate) in the first development step. The antibody location is detected in the second development step when the bound enzyme converts an identifying substrate to a permanent black spot on the disc. Each spot indicates an O157:H7 presumptive-positive colony.

RESULTS AND DISCUSSION

Figs. 1a and 1b illustrate the growth of E. coli O157:H7 in the samples kept under refrigeration and at room temperature, respectively, when the inoculation level of both E. coli O157:H7 and the challenging microorganisms was 10⁶-10⁷ CFU/g. When E. coli O157:H7 was inoculated individually in the meat samples and kept under refrigeration (Fig. 1a), the counts remained relatively constant throughout the 96 hours of experiment. A similar observation occurred when the other competing microorganisms were also present. At room temperature (Fig. 1b), the counts of all microorganisms increased similarly and were almost identical to that of control treatments in which E. coli O157:H7 was alone.

The curves in Fig. 1a and 1b are almost coincident, presenting counts that didn’t change significantly during the experiment.
When the intermediate inoculation levels of $10^3$-$10^4$ CFU/g for \textit{E. coli} O157:H7 and $10^6$-$10^7$ CFU/g for the challenging microorganism was considered (Figs. 2a and 2b) some differences in the growth curves were noted. In Fig. 2a, they were due to variations in the number of CFU/g of \textit{E. coli} O157:H7 in the inoculum. However, the counts after 96 hours were very similar to the initial ones. In Fig. 2b, a lower count of \textit{E. coli} O157:H7 in the presence of non-pathogenic \textit{E. coli} was observed at 24 hours, probably caused by difficulties to enumerate low numbers of colonies of the pathogen in the presence of high number of colonies of non-pathogenic \textit{E. coli}. These difficulties increased when the lowest inoculation level ($10^1$-$10^2$ CFU/g) was assayed and results were not considered.

These results suggest that the presence of non-pathogenic \textit{E. coli}, \textit{Pseudomonas putida} or \textit{Leuconostoc} sp. did not interfere with the growth or survival of \textit{E. coli} O157:H7 in ground beef samples kept under refrigeration or at room temperature, regardless of the level of contamination. Santos et al., 1995, also observed that \textit{E. coli} O157:H7 counts remained approximately constant in ground meat kept for 12 days at 9.5°C (20). These were less than one log cycle changes in \textit{E. coli} O157:H7 numbers, whereas indigenous Gram negative bacteria increased their counts from the fourth up to the twelfth day at this temperature.

Greer and Dilts, 1995, observed that spoilage bacteria grew on both fat and lean tissue whereas pathogens grew on fat tissue only (11). Therefore, differences in the affinity for different portions of meat by the microorganisms tested in this study may be the cause for the absence of interference over the multiplication of each other.

The correlation between results of enumeration of \textit{E. coli} O157:H7 using the standard cultural method and the ELISA-type rapid method was high (97.2%).

Results of the current study suggest that the growth of \textit{E. coli} O157:H7 in artificially contaminated ground beef was not influenced by the presence of different concentrations of non-pathogenic \textit{E. coli}, \textit{Pseudomonas putida} or \textit{Leuconostoc} sp. at refrigeration temperature or at room temperature, indicating that this pathogen is a good competitor. Thus, the low occurrence of \textit{E. coli} O157:H7 in ground beef may not be attributed to competition by other microorganisms.

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**RESUMO**

Influência da microbiota natural da carne na multiplicação de \textit{Escherichia coli} O157:H7 em carne bovina moída

\textit{Escherichia coli} O157:H7 é um patógeno de origem alimentar de importância crescente, tendo
sido envolvido em diversos surtos ameaçadores, a maioria deles associada ao consumo de produtos carnés. Neste estudo foi avaliada a influência de algumas bactérias da microbiota natural da carne crua sobre _E. coli_ O157:H7 em amostras de carne bovina moida armazenadas em refrigeração e à temperatura ambiente. As amostras foram inoculadas com diferentes níveis de _E. coli_ O157:H7 (10⁵, 10⁴ e 10⁶ UFC/g) e de _E. coli_ não patogênica, _Pseudomonas putida_ ou _Leuconostoc_ sp. A multiplicação do patógeno foi monitorada através de metodologia convencional e através de método rápido do tipo ELISA. _E. coli_ não patogênica, _Pseudomonas putida_ e _Leuconostoc_ sp. não exerceram influência sobre a multiplicação de _E. coli_ O157:H7 em carne moida, tanto em refrigeração como à temperatura ambiente. Assim sendo, a baixa ocorrência de _E. coli_ O157:H7 em carne crua não pode ser atribuída a efeitos antagônicos de bactérias de sua microbiota natural.

**Palavras-chave:** _Escherichia coli_ O157:H7, antagonismo, carne moida

**REFERENCES**


