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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

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(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-Meriéux). 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 10^8-10^9 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 10^8 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 10^6-10^7 CFU/g) and non-pathogenic *E. coli* (0, 10^1-10^2, 10^3-10^4 or 10^6-10^7 CFU/g);
- *E. coli* O157:H7 (0, 10^1-10^2, 10^3-10^4 or 10^6-10^7 CFU/g) and *Pseudomonas putida* (0, 10^3-10^4 or 10^6-10^7 CFU/g);
- *E. coli* O157:H7 (0, 10^3-10^4 or 10^6-10^7 CFU/g) and *Leuconostoc* sp. (0 or 10^6-10^7 CFU/g).

Six equal samples were prepared for each inoculation level and combination. After

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, H_2S, 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 transfered 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 10^6-10^7 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.

Figure 1a

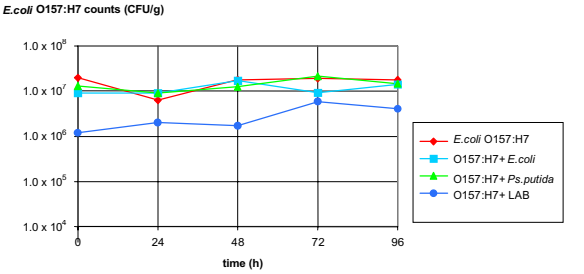
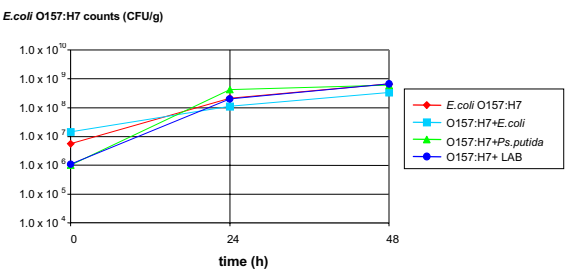


Figure 1b



Figures 1a and **1b** – Counts of *E. coli* O157:H7 in ground meat samples inoculated with *E. coli* O157:H7 and non-pathogenic *E. coli*, *Pseudomonas* spp. (*Ps. putida*) or lactic acid bacteria (LAB – *Leuconostoc* sp.), using an inoculation level of 10^6-10^7 CFU/g. **1a** = meat samples kept under refrigeration; **1b** = meat samples kept at room temperature.

When the intermediate inoculation levels of 10^3-10^4 CFU/g for *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 *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 *E. coli* O157:H7 in the presence of non-pathogenic *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 *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 *E. coli*, *Pseudomonas putida* or *Leuconostoc* sp. did not interfere with the growth or survival of *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 *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 *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 *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 *E. coli* O157:H7 in artificially contaminated ground beef was not influenced by the presence of different concentrations of non-pathogenic *E. coli*, *Pseudomonas putida* or *Leuconostoc* sp. at refrigeration temperature or at room temperature, indicating that this pathogen is a good competitor. Thus, the low occurrence of *E. coli* O157:H7 in ground beef may not be attributed to competition by other microorganisms.

Figure 2a

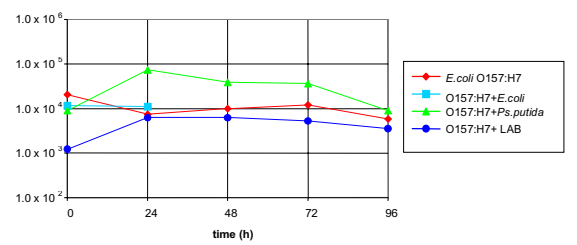
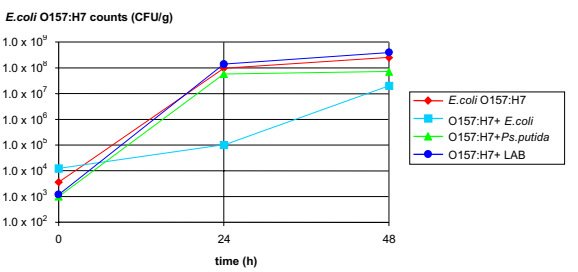


Figure 2b



Figures 2a and **2b** – Counts of *E. coli* O157:H7 in ground beef samples inoculated with *E. coli* O157:H7 and non-pathogenic *E. coli*, *Pseudomonas* spp. (*Ps. putida*) or lactic acid bacteria (LAB – *Leuconostoc* sp.), using an inoculation level of 10^3-10^4 CFU/g for *E. coli* O157:H7 and of 10^6-10^7 CFU/g for the other bacteria. **2a** = meat samples kept under refrigeration; **2b** = meat samples kept at room temperature.

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RESUMO

Influência da microbiota natural da carne na multiplicação de *Escherichia coli* O157:H7 em carne bovina moída

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 cárneos. 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 moída armazenadas em refrigeração e à temperatura ambiente. As amostras foram inoculadas com diferentes níveis de *E. coli* O157:H7 (10^1, 10^3 e 10^6 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 moída, 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 moída

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