Original Article

Evaluation of bacterial multiplication in cleaning cloths containing different quantities of organic matter

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Abstract

Introduction: To determine a proper length of time for cleaning cloth usage, the present work aimed to evaluate bacterial multiplication in artificially contaminated cleaning cloths containing different amounts of organic matter.

Methodology: Cloths containing 1%, 5%, and 10% of bovine albumin were contaminated with *Salmonella enteritidis* 3091/05, *Escherichia coli* ATCC 25972, *Staphylococcus aureus* ATCC 25923, and *Shigella sonnei* CC07. They were incubated for different time periods at 30°C. Microbial multiplication was evaluated by bacterial counts and the ATP bioluminescence increase was monitored at sampling points. An ampicillin-resistant recombinant HSα *E. coli* strain was used as a pathogen surrogate to investigate the potential of microbial cloth dispersion.

Results: None of the strains showed expressive growth up to two hours of incubation. At three hours, the microorganisms demonstrated a slight increase, with *E. coli* ATCC 25972 showing a significant increase in cells ($p < 0.05$). The ATP bioluminescence did not increase during the incubation period and confirmed the microbial count results, demonstrating that the amounts of organic matter tested did not interfere with bacterial multiplication during the first three to four hours of incubation. The dispersion experiment indicated that a cleaning cloth contaminated with 10^4 CFU/cm^2 was able to spread 10^2 CFU/cm^2 of recombinant *E. coli* onto a stainless steel surface.

Conclusion: Based on these results, we suggest that an appropriate period of time for using disinfected cleaning cloths is around two hours, not exceeding three hours of usage.

Key words: cleaning cloths, ATP bioluminescence, bacterial multiplication

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Introduction

Food-borne outbreaks are increasing worldwide and the magnitude of this problem is certainly underestimated [1]. Cross-contamination is considered as the direct or indirect transference of microorganisms from utensils, raw materials, food handlers, surfaces or objects to foods, and its prevention is an important issue in the avoidance of food-borne diseases. According to Ryan *et al*. [2], cross-contamination contributed to approximately 28% of the domestic outbreak cases that occurred in the United Kingdom.

The use of cleaning cloths in domestic kitchens and food services remains very frequent, even though it may contribute to cross-contamination. It is well established that adequate disinfection methods can reduce microbial populations; however, the maximum time of usage of cleaning cloths is still an important issue to be investigated. Cleaning cloths are largely used in washing-up processes and because of this they frequently become contaminated and may easily spread pathogens throughout kitchen sites and equipment [3]. The washing-up process is important in preventing cross-contamination [4] and serves to physically remove organic matter and microorganisms from kitchen sites, objects, and equipment. Some authors [5,6] have reported cleaning cloths as being especially contaminated when used for multiple purposes, such as wiping down draining boards, surfaces, and equipment. Scott and Bloomfield [7] demonstrated that when contaminated cleaning cloths are rubbed onto surfaces, microorganisms are invariably transferred to the surfaces or hands of the food handlers in sufficient numbers to cause food-borne infection. Several researchers reported high contamination levels of microorganisms in cleaning cloths. For example, Scott and Bloomfield [3,7] demonstrated counts varying from 10^2 to 10^6 CFU/cm^2 in cleaning cloths, and Kusumaningrum *et al*. [8] recorded a microbial contamination of 10^8 to 10^9 CFU in cloth samples of 18 × 18 cm^2. Because cleaning cloths can become very contaminated, their usage is not recommended; however, if they are essential, they

should be adequately cleaned and disinfected. As reported by several authors [9,10,11,7], cleaning alone is ineffective at inactivating microbial contamination and disinfection procedures must be performed to eliminate microorganisms. According to Rusin *et al*. [11] the combination of regular cleaning with an adequate disinfection schedule could significantly reduce bacterial contamination from cloths. For example, sodium hypochlorite was found to be effective against *S. aureus*, *S.* Typhi, and *E. coli* present in cleaning cloths [12].

Even though several food safety regulations do not recommend the use of cleaning cloths, they are still extensively used in domestic kitchens and commercial food services. Their frequent use can be explained because disposable cloths are more expensive and it is very difficult to find a substitute to cleaning cloths. Although adequate cleaning and disinfection procedures were found to significantly reduce the microbial counts present in cleaning cloths, further studies are necessary to determine their maximum time of usage without compromising food safety. The aim of the present study, therefore, was to evaluate bacterial growth in cleaning cloths containing different quantities of organic matter.

Methodology

Bacterial strains

Different strains were used in the present study. *Salmonella enteritidis* 3091/05 was isolated from a potato salad with homemade mayonnaise that was identified as being responsible for a salmonellosis outbreak that occurred in Rio Grande do Sul (RS) in 2005. *Shigella sonnei* CC07 was isolated from food involved in a food-borne outbreak which occurred in RS in 2007. *Escherichia coli* ATCC 25972 and *Staphylococcus aureus* ATCC 25923 came from the collection of Laboratório de Microbiologia de Alimentos do Instituto de Ciência e Tecnologia de Alimentos/UFRGS. Prior to the experiments, all strains were conserved at -18^{o}C in BHI broth (Merck, RJ, Brazil) containing 50% glycerol (Reagen, RJ, Brazil). With the exception of *Shigella sonnei*, the reason for choosing the named microorganisms was that *Salmonella enteritidis*, *Escherichia coli*, and *Staphyococcus aureus* are responsible for around 50% of the outbreaks in southern Brazil.

Bacterial multiplication in cotton and disposable cloths containing bovine albumin (organic matter)

Bacterial cells were reactivated by transferring a loopful of each individual strain into BHI broth that was incubated at 37°C for 24 hours. The strain inocula were prepared by transferring 1 mL portions of the activated cultures, separately, into 9 mL of 0.1% sterile peptone water [Nuclear, Diadema, Brazil], preparing a ten-fold dilution. Serial dilutions were carried out and a 100 µL portion containing approximately 10^4 CFU was inoculated on sterilized cotton and disposable cloth strips [5 cm × 1 cm], placed inside sterile petri dishes. The cloths were purchased in a local supermarket and the disposable cloth did not contain any antibacterial agent in order to avoid interference with the results. Before inoculation, 0.5 mL of 1% bovine albumin solution (Sigma, St. Louis, USA) was added to each strip to simulate the presence of organic matter [13]. Petri dishes containing the cloth strips were incubated for 0, 1, 2, 3, and 4 hours at 30°C. At each sampling time point, one cotton and one disposable cloth strip contaminated with each of the microorganisms were sampled and placed into 10 mL 0.1% sterile peptone water inside a sterile glass tube that was vigorously agitated for one minute. The temperature of 30°C was chosen for incubation because this temperature is frequently found in food service kitchens in Rio Grande do Sul throughout the year. After sampling, aliquots of 20 µL were taken in triplicate, plated on BHI, and incubated overnight at 37°C. *Salmonella enteritidis*3091/05 was also inoculated in cotton cloth strips without bovine albumin and enumeration was performed under the same experimental conditions as described above for the other microorganisms. All the bacterial counts were carried out in triplicate and the count procedure was performed as described by Silva *et al*. [14]. All the experiments were repeated at least twice and the results were expressed as CFU/cm^2.

ATP bioluminescence experiments

Adenosine triphosphate [ATP] bioluminescence was used to evaluate the influence of different quantities of organic matter on microbial growth in cloths. Three cotton cloths [20 cm × 20 cm], previously sterilized in an autoclave (121^{o}C for 15 minutes at 1 atm), were rubbed on a naturally contaminated tile surface to become contaminated. Afterwards, the cloths were placed separately into 100 mL of 0.1% sterile peptone water containing 1%, 5%, and 10% of bovine albumin in an attempt to add different concentrations of organic matter to the cloths. After the addition of organic matter, the cloths were squeezed by gloved hands and rubbed onto a 70% ethylic alcohol disinfected surface. After periods

of 0, 1, 2, 3, 4 and 20 hours, the ATP bioluminescence was verified using Ultrasnap™ ATP swabs and an ATP Luminometer (Hygiena, SistemSURE II, CA USA). During sampling, the swabs or the insides of the sampling device were not touched with fingers. Sample collection was performed by swabbing the entire 15 cm × 15 cm area of tile surface, in three different directions. After swabbing, the swab was placed back into the swab tube and immediately read in the ATP Luminometer. Between sampling times, the cloths were incubated at 30°C inside sterile plastic bags to allow bacterial growth. The results were expressed in relative luminescence units (RLU) and a surface reading of less than 10 RLU was considered a clean surface, while readings between 11 to 29 RLU and greater than 30 RLU were classified as not adequately cleaned surfaces and dirty surfaces, respectively, according to Hygiena USA criteria.

Microbial cloth dispersion experiment

The cotton cloth was artificially contaminated with ampicillin-resistant HSα *E. coli* [Novagen] transformed by the calcium chlorite method (kindly provided by Prof. Dr Jeverson Frazzon of Instituto de Ciência e Tecnologia de Alimentos/UFRGS). This recombinant microorganism was used as a pathogen surrogate with the aim of evaluating microbial dispersion by a contaminated cloth while avoiding hazardous laboratory contamination. This microorganism was grown in ampicillin-enriched Luria-Bertini broth, (LB, Merck, RJ, Brazil) for 24 hours at 35°C, resulting in a culture of 1.29×10^9 CFU/ml. Then 1 mL of the culture was diluted in 99 mL of 0.1% sterile peptone water and the cloth was placed into the diluted suspension to become contaminated. After this, the cotton cloth contained 2.7×10^4 CFU/cm^2 of the recombinant microorganisms. A stainless steel surface (AISI 316) was cleaned using a sponge and neutral detergent, and rinsed with potable water. Afterward, the surface was disinfected by spraying 70% ethylic alcohol and left to air dry. To evaluate the disinfection, the surface was swabbed (100 cm^2) and tested on Plate Count Agar (control). The contaminated cotton cloth was rubbed several times on the disinfected stainless steel surface, simulating a usual practice of food service. The contaminated stainless steel surface was sampled (100 cm^2) with a cotton swab that was placed into 10 mL of 0.1% sterile peptone water, vigorously agitated, and triplicate aliquots of 20 µL of the homogenate were placed in LB supplemented with 50 µg/mL of ampicillin. The plates were incubated at 35°C overnight and the colonies were counted. The results were expressed as CFU/cm^2.

Statistical analysis

For the statistical analysis, all counts were transformed into $\log_{10}$ and submitted to analysis of variance (ANOVA) by Microsoft Excel (Microsoft Corp. Redmond, WA) with $p < 0.05$ being considered significant.

Results

Figure 1 illustrates microbial growth on the cotton cloth containing 1% of bovine albumin. Bovine albumin was added to the cloths to simulate the presence of organic matter. As shown, the four strains tested did not demonstrate significant growth until two hours of incubation. At three hours, the microorganisms demonstrated a slight increase in counts; *E. coli* ATCC 25972 demonstrated a significant increase of cells ($p < 0.05$). After four hours of incubation, all microorganisms demonstrated significantly ($p < 0.05$) higher counts compared to the initial counts and the numbers at two hours. *S. enteritidis* growing in a cotton cloth without organic matter demonstrated the same behaviour as *S. enteritidis* cultivated with 1% bovine albumin [results not shown].

Figure 2 illustrates microbial growth on the disposable cleaning cloths containing 1% bovine albumin during four hours of incubation. Similar to what happened on the cotton cloth, the four strains hardly increased in number up to two hours. After three hours of incubation, *S. enteritidis* 3091/05

Figure 1. *Salmonella Enteritidis* 3091/05 [♦], *Shigella sonnei* CC07 [■], *Staphylococcus aureus* ATCC 25923 [●], and *Escherichia coli* ATCC 25972 [▲] growth in cotton cloth containing 1% bovine albumin at 30°C.

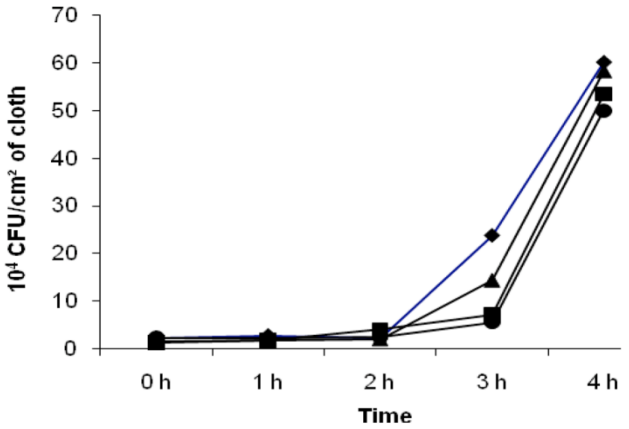
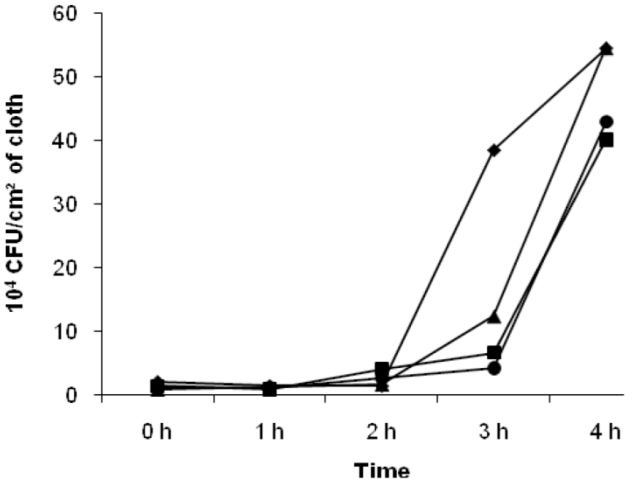


Figure 2. *Salmonella Enteritidis* 3091/05 [♦], *Shigella sonnei* CC07 [■], *Staphylococcus aureus* ATCC 25923 [●], and *Escherichia coli* ATCC 25972 [▲] growth in disposable cloth containing 1% bovine albumin at 30°C.



increased significantly ($p < 0.05$), demonstrating higher counts (3.8×10^5 CFU/cm^2) than the other microorganisms, followed by *E. coli* ATCC 25972 (1.2×10^5 CFU/cm^2), *S. sonnei* CC07 (6.7×10^4 CFU/cm^2), and *S. aureus* ATCC 25923 (4.3×10^4 CFU/cm^2). After four hours, all strains had significantly increased their cell numbers ($p < 0.05$) compared to the results at two hours of incubation.

Comparing the results obtained with the cotton and disposable cloths, *S. enteritidis* 3091/05 was able to grow better than the other strains after three hours of storage ($p < 0.05$), especially in the disposable cloth. *Shigella sonnei* CC07, *S. aureus* ATCC 25923, and *E. coli* 25972 demonstrated similar growth behaviours in both types of cloths; specifically, there was hardly any growth until two hours of incubation, whereas after four hours of incubation they demonstrated rapid multiplication.

The ATP bioluminescence tests confirmed the results presented in Figure 1 and Figure 2; specifically, the RLU levels hardly increased until three hours of incubation at 30°C, suggesting that microbial growth did not occur during this period. However, at four hours, a slight increase in the RLU level was observed and after overnight incubation, the RLU levels increased expressively, indicating microbial development (Figure 3). The results also indicated that the bovine albumin concentration did not influence RLU levels until four hours of incubation; however, after 20 hours with 1% bovine albumin, 262 RLU were generated, while with 5% and 10% bovine albumin, the RLU levels were 518 and 659, respectively.

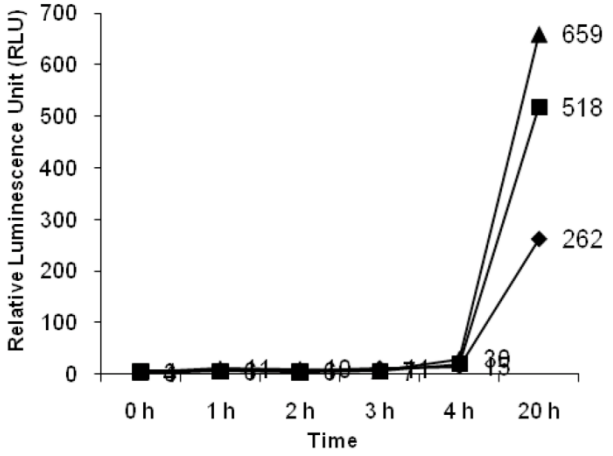
Recombinant *E. coli* was used to simulate the possible microbial dispersion due to cloths minimally contaminated during real usage in food service. The results indicated that a cloth artificially contaminated with 2.7×10^4 CFU/cm^2 was able to spread $5.6 \pm 1.43 \times 10^2$ CFU/cm^2 onto a disinfected stainless steel surface.

Discussion

It is well documented that cleaning cloths can become very contaminated [15,7,16], but this contamination can be significantly reduced by appropriate cleaning and disinfection methods [17,11]. Therefore, the study of bacterial multiplication in cloths is important to help estimate the proper length of time of usage.

Bacteria attached to fibre cloths may multiply vigorously due to the presence of food residues and humidity, and also because they frequently remain for long periods at room temperature inside domestic kitchens and food services. Expressive microbial contamination, as well as the risk of transferring microorganisms to food handlers or equipment surfaces, was reported by Bloomfield and Scott [17]. In a previous study, Scott and Bloomfield [7] reported the growth of residual survivors when cleaning cloths were stored overnight after disinfection. Cogan *et al*. [18] demonstrated that cloths used to wipe chopping boards that had been used to prepare *Salmonella*-contaminated chickens became immediately contaminated with a mean level of bacterial counts of 4.2×10^5 CFU/200 cm^2. After overnight storage at 20°C, the mean bacterial counts rose to 6.6×10^8 CFU/200 cm^2 and in some cloths, the *Salmonella* counts increased by 3 log. In the present study, the cotton and disposable cloths containing bovine albumin were contaminated by four different strains of microorganisms and the results showed that bacterial counts did not increase after two hours of storage at 30°C. The microorganisms were inoculated at levels of approximately 10^4 CFU/cm^2, after previous studies by our laboratory demonstrated that this quantity was the minimal amount of heterotrophic microorganisms found in used cleaning cloths sampled from food services and also in cloths purchased from supermarkets, without being submitted to any cleaning or disinfection procedures (data not shown). These results can likely be explained by the lag phase of the bacteria inoculated in the cloths since all the

Figure 3. ATP bioluminescence originating from surfaces rubbed with contaminated cotton cloths containing 1% [♦], 5% [■] and 10% [▲] of bovine albumin solution after different periods of incubation at 30°C.



strains tested presented the same behaviour: little growth with or without organic matter. During the lag phase, the bacterial cells adapted to the new environment, activating or deactivating genes, and consequently metabolic pathways, in preparation for the next phase of growth, specifically exponential growth. Malheiros *et al*. [19] reported that different *Salmonella* serovars present a lag phase of around two hours when activated in BHI and inoculated in nutrient broth or homemade mayonnaise. The same authors cited that when bacterial cells are stressed by food treatment or chemical products, the lag phase usually increases. Based on this, and noting that the strains tested in the present work were inoculated in cloths after activation in BHI medium, the lag phase of these microorganisms in cloths under real conditions could be two hours or longer.

Our results showed that all the microorganisms presented significant growth after three or four hours of incubation ($p < 0.05$). These results indicate that long periods of storage at 30°C can increase bacterial growth in cloths and subsequently increase the risk of cross-contamination.

These results were confirmed by ATP bioluminescence, which was used to test whether different amounts of bovine albumin could influence bacterial development during the first few hours of cloth contamination. The luminometer measures adenosine triphosphate (ATP) from organic matter or from animal, plant, bacterial, yeast, and mould cells. It is considered a rapid and sensitive method. In this study, it was used to verify microbial growth with different amounts of organic matter. Our results showed that the ATP counts started to increase after three hours of incubation in cloths with different amounts of bovine albumin, suggesting that microorganisms did not develop during the first three hours in the cleaning cloths.

Surrogate organisms are used to mimic growth and survival patterns of a pathogen and to help explain what occurs with a pathogen during handling and storage [20]. Besides this, researchers also prefer to use a surrogate instead of a pathogen to prevent contamination of an environment/laboratory with harmful organisms. In the present study, a recombinant strain of non-pathogenic *E. coli* was used to determine microbial dispersion caused by artificially contaminated cloths to stainless steel surfaces. The results demonstrated that a cloth containing initial numbers of 2.7×10^4 CFU/cm^2 was able to transfer around 10^2 CFU/cm^2 to the surfaces. Considering that there are few food pathogens with very low infectious doses [20], attention should be given to the cross-contamination potential of cleaning cloths.

Based on these results, it is possible to conclude that an appropriate period of time for using disinfected cleaning cloths is two hours, not exceeding three hours. These parameters are published in Portaria 78/2009 of Secretaria Estadual de Saúde of Rio Grande do Sul State, and are mandatory for all food services of this Brazilian State. Even though these parameters seem adequate, it is also important to combine the appropriate time period for using cloths with good manufacturing practices and appropriate cleaning and disinfection methods.

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References

1. Jackson V, Blair IS, McDowell DA, Kennedy J, Bolton DJ (2007) The incidence of significant foodborne pathogens in domestic refrigerators. J Food Contr 18: 346-351.
2. Ryan MJ, Wall PG, Gilbert RJ, Griffin M, Rowe B (1996) Risk factors for outbreaks of infectious intestinal disease linked to domestic catering. Commun Dis Rep CDR Rev 13: R179-R182.
3. Scott E, Bloomfield SF (1990a) The survival and transfer of microbial contamination via cloths, hands and utensils. J Appl Bacteriol 68: 271-278.

4. Mattick K, Durham K, Domingue G, Jorgensen F, Sen M, Schaffner DW, Humphrey TJ (2003) The survival of foodborne pathogens during domestic washing-up and subsequent transfer onto washing-up sponges, kitchen surfaces and food. Int J Food Microbiol 85: 213-226.
5. Humphrey TJ, Martin KW, Slader J, Durham K (2001) *Campylobacter* spp. in the kitchen: spread and persistence. J Appl Microbiol 90: 115-120.
6. Kagan LJ, Aiello AE, Larson E. (2002) J Community Health 27: 247-267.
7. Scott E, Bloomfield SF (1990b) Investigation of the effectiveness of detergent washing, drying and chemical disinfection on contamination of cleaning cloths. J Appl Bacteriol 68: 279-283.
8. Kusumaningrum HD, Riboldi G, Hazelger WC, Beumer RR (2003) Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. Int J Food Microbiol 85: 227-236.
9. Cogan TA, Bloomfield SF, Humphrey TJ (1999) The effectiveness of hygiene procedures for prevention of cross-contamination from chicken carcasses in the domestic kitchen. Lett Appl Microbiol 29: 354-358.
10. Josephson KL, Rubino JR, Pepper IL (1997) Characterization and quantification of bacterial pathogens and indicator organisms in household kitchens with and without the use of a disinfectant cleaner. J Appl Microbiol 83: 737-750.
11. Rusin P, Orosz-Coughlin P, Gerba C (1998) Reduction of fecal coliform, coliform and heterotrophic plate counts bacteria in the household kitchen and bathroom by disinfection with hypochlorite cleaners. J Appl Microbiol 85: 819-828.
12. Parnes C (1997) Efficacy of sodium hypochlorite bleach and "alternative" products in preventing transfer of bacteria to and from inanimate surfaces. Environ Health 59: 14-20.
13. Brasil Portaria n. 15 de 23 de agosto de (1988) Aprova produtos saneantes domissanitários com ação antimicrobiana em superfícies onde se manipulem alimentos. Ministério da Saúde – Secretaria Nacional de Vigilância Sanitária de Produtos Saneantes Domissanitários.
14. Silva N, Junqueira VCA, Silveira NFA (2007) Manual de métodos de análise microbiológica de alimentos, 3th edition. São Paulo: Varela, 544p.
15. Mackintosh CA, Hoffman PN (1984) An extended model for transfer of microorganisms via the hands. J Hyg 92: 345-355.
16. Tebbut GM (1986) An evaluation of various working practices in shops selling raw and cooked meats. J Hyg 97: 81-90.
17. Bloomfield SF, Scott EA (1997) Cross-contamination and infection in the domestic environment and the role of chemical disinfectants. J Appl Microbiol 83: 1-9.
18. Cogan TA, Slader J, Bloomfield SF, Humphrey TJ (2002) Achieving hygiene in the domestic kitchen: the effectiveness of commonly used cleaning procedures. J Appl Microbiol 92: 885-892.
19. Malheiros PS, De Paula C M, Tondo EC (2007) Cinética de crescimento de *Salmonella* Enteritidis envolvida em surtos alimentares no RS: uma comparação com linhagens de outros sorovares. Ciênc Tecnol Alim 27: 751-755.
20. James J (2006) Microbial Hazard Identification in Fresh Fruit and Vegetables, 1st edition. Dublin: Wiley-Interscience 312 p.

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