Advanced Journal of Microbiology Research Vol. 2012
Available online at http://internationalscholarsjournals.org/journal/ajmr

Short Communication

Growth and survival of *Salmonella zanzibar* in juice and salami stored under refrigerated and room temperature

Chatti Abdelwaheb*, Lamouri Imen and Landoulsi Ahmed

Département des Sciences de la vie, Faculté des Sciences de Bizerte, Zarzouna 7021, Tunisie.
Unité de Biochimie des Lipides et interaction des macromolécules en Biologie, 03/UR/0902.
Laboratoire de Biochimie et Biologie Moléculaire, Faculté des Sciences de Bizerte, Zarzouna 7021.

Accepted 7 February, 2012

The vehicles usually involved in salmonellosis outbreaks are meat, eggs, poultry and milk. Recently, *Salmonella* outbreaks were reported in some vegetables such as tomatoes and unpasteurized juice. After inoculation of *Salmonella zanzibar* onto food samples, growth and survival of *S. zanzibar* in orange juice and salami were monitored. Our results showed that *S. zanzibar* is able to grow and survive in orange juice and also in salami. However, growth in salami was more important than in the case of orange juice. Storage at 4°C caused a difference in the survival curve when compared to samples (also juice and salami) incubated at room temperature. The present study demonstrated that orange juice and salami can provide a favorable environment for survival and growth of *S. zanzibar* in spite of their low pH values.

Key words: *Salmonella*, orange juice, salami, survival, refrigeration.

INTRODUCTION

Salmonella continues to be a major problem to public health in developed and developing countries (Fang, 2005; Lampel et al., 2000). Recently, the number of food borne diseases due to consumption of contaminated food and water has increased (Sivapalasingam et al., 2004; Tauxe et al., 1997). Most of the reported outbreaks have been caused by pathogenic bacteria, especially *Escherichia coli* O157:H7 and *Salmonella* spp. (Beuchat, 1996).

The vehicles usually involved are meat, pork, poultry, eggs and milk (Bryan, 1981). However, it has been well demonstrated that *Salmonella* can disseminate and survive in various environmental niches for long periods of time. They are pervasive in nature and may contaminate animals, vegetables, water and especially food during its production and distribution (Chatti et al., 2007). Several salmonellosis outbreaks associated with unpasteurized juices have been reported (Cody, 1999; Cook et al., 1998). *Salmonella anatum*, *Salmonella hartford*, *Salmonella muenchen* (CDC, 1999), *Salmonella typhimurium* (Chatti et al., 2007) have been identified as causative agents in orange juice and apple cider.

*Corresponding author. E-mail: chattiabdel@yahoo.fr.

Some studies are also interested in *Salmonella* isolated from fruits or plants irrigated by treated waste water. *S. enterica* serotype Zanzibar has been reported frequently in Tunisia and has been isolated from poultry, red meat, cereals, vegetables, fruits, milk and dairy products (Aissa et al., 2007). The present study was initiated to investigate the growth behaviour of *Salmonella enterica* serovar Zanzibar inoculated into juice and on salami, as well as the effectiveness of temperature storage.

MATERIALS AND METHODS

Samples

Orange juice and salami samples were purchased from a local supermarket in Bizerte, Tunisia. All samples were kept at 2 to 4°C between the time of purchase and initiation of experiments.

Bacterial strains

S. enterica serotype Zanzibar, used in this study, was isolated from waste water at the: Institut Pasteur, Tunisia (Chatti et al., 2007).

Preparation of inoculum and inoculation of salami and juice

Salmonella zanzibar was incubated in nutrient broth (Difco) at 37°C

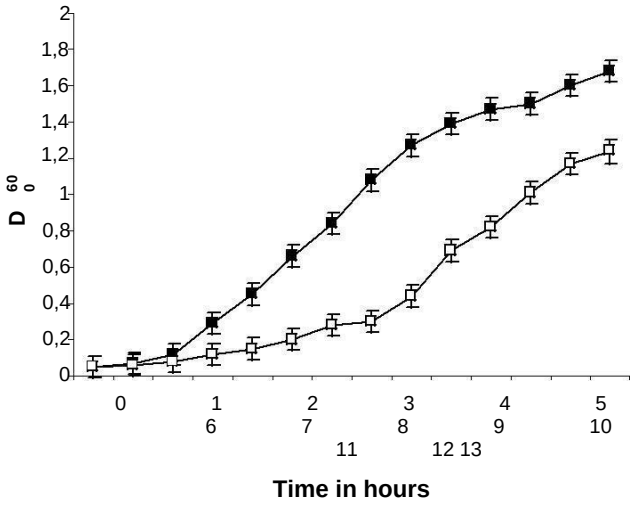

Figure 1. Response of *S. zanzibar*, incubated in Nutrient broth with citric acid ( ) at the pH 3.8. For a control growth curve, *S. zanzibar* was incubated in Nutrient broth pH7 ( ). All values are the averages of three different measures.

for 24 h. The bacterial suspension was diluted in phosphate-buffered saline solution (PBS).

Salami: 0.1 ml of a suitable dilution was added to 2 g of salami cut into small pieces so that the initial concentration per 1 g were in the order of 6.5×10^3 CFU, as determined by direct plating. Salami with added *Salmonella* was incubated in sealed polyethylene plastic bags at room temperature and at 4°C for 12 days. After incubation, 200 ml of PBS was poured into the plastic bags and mixed. The samples were then diluted in PBS and colony counts were carried out by plating onto Nutrient agar and following incubation at 37°C for 24 h. Triplicate determinations were made of each temperature.

Juice: 0.1 ml of a suitable bacterial dilution was added to 100 ml of juice samples to yield 6.5×10^3 CFU/ml. Juice with added *Salmonella* was incubated at room temperature and at 4°C for 12 days. The samples were then diluted and incubated at 37°C for 24 h on Nutrient agar to enumerate bacteria.

Suspected colonies of *S. zanzibar* were confirmed serologically by slide agglutination using *Salmonella* Typing Sera (Difco) (Wilson et al., 1960).

RESULTS

Acid resistance of *S. zanzibar*

Figure 1 illustrates the growth kinetics of *S. zanzibar* in a Nutrient broth with citric acid added at a concentration of 10%. According to Figure 1, an increase in pH from 3.5 to 7 decreased the lag time of *S. zanzibar*.

Survival of *S. zanzibar* in orange juice

The initial pH of orange juice before inoculation was 3.9. Figure 2 showed survival of *S. zanzibar* in orange juice. These results demonstrate that this isolate is capable of surviving and growing in orange juice. Therefore, our results showed an increase of the number of bacteria during the four first days post-inoculation. After this, the

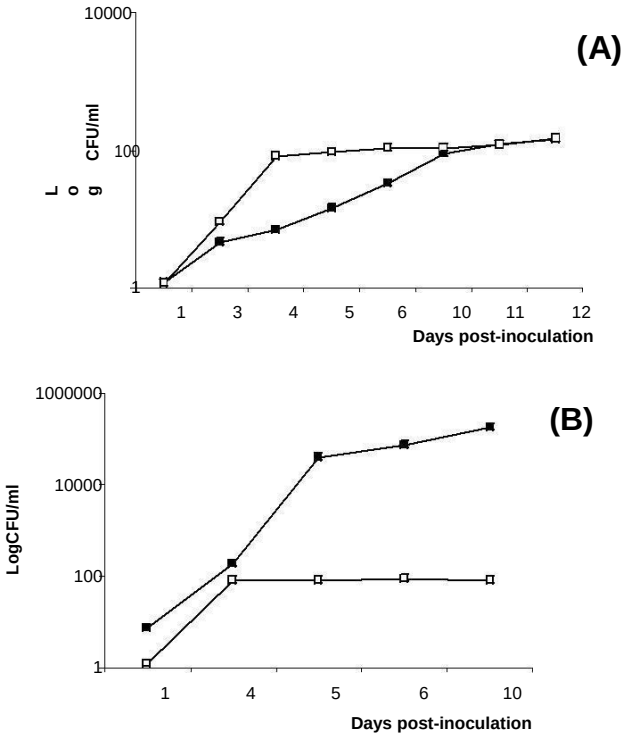

Figure 2. Survival curves of *S. zanzibar* inoculated in orange juice (A) or salami (B) stored at 25°C ( ) and at 4°C ( ). All values are the averages of three different measures.

number remained stable.

To study the effect of temperature on the survival of *S. zanzibar*, we have incubated inoculated juice samples at 4°C. Our results (Figure 2a) showed a difference in the survival curve when compared to samples incubated at room temperature. However, the number of this isolate increased slowly and the lag time was more pronounced than non-refrigerated samples.

Survival of *S. zanzibar* in salami

The same experiments, as above, were conducted and the results (Figure 2b) demonstrated that the growth kinetics of bacteria were different from those observed in the case of orange juice. The results showed an increase in bacterial numbers after inoculation.

In the case of inoculated samples incubated at 4°C, we have shown an increase of the number of bacteria until 4 days post-inoculation. The number then remained constant.

DISCUSSION

Salmonella encounters a wide variety of environments during its life cycle (Stocker and Makela, 1986). One component of the environment that fluctuates widely is pH. In nature, *Salmonella* can experience and survive

dramatic acid stresses that occur in diverse ecological niches. The physiological state of *Salmonella* could influence survival in foods, resistance to food processing and to chemical preservatives.

The vehicles of *Salmonella* usually involved are meat, poultry, eggs and milk (Bryan, 1981; Kristiina and Esko, 1991). However, vegetables are frequently reported to be vehicles of *Salmonella*. In recent years, *Salmonella* outbreaks have been traced back to lettuce (Ercolani, 1976), salads (Garcia-Villanova et al., 1987), sprouts (O'Mahony et al., 1990), tomatoes (Zhuang et al., 1995), and other fruit- and vegetable-containing dishes.

The present study demonstrated that orange juice can provide a favorable environment for growth of *S. zanzibar in* spite of their low pH values. This can be explained by the nature of the major acidulant of juice, citric acid which permits the growth of salmonellae even at low pH values. Growth and survival of *Salmonella* increase particularly if the juice is stored at room temperatures. In this regard storage of juice at low temperatures is considered of great importance.

In summary, results from studies on the behaviours of *S. zanzibar* inoculated onto salami and orange juice confirm observations made by others that *Salmonella* can grow in orange juice (also salami). However, *Salmonella* outbreaks associated with orange juice have been reported (Cody, 1999; Cook et al., 1998).

In addition, some studies have shown that acid adaptation of microorganisms by addition of acidulants or under acidic food environments increases cell resistance against various environmental stresses occurring during food processing (Leyer and Johnson, 1992).

ACKNOWLEDGMENTS

This work was supported by le Ministère de l'Enseignement Supérieur, de la recherche scientifique et de la technologie-Tunisie.

REFERENCES

Beuchat LR (1996). Pathogenic microorganisms associated with fresh produce. J. Food Prot. 315: 204-216.

Ben Aissa R, Al-Gallas N, Troudi H, Belhadj N, Belhadj A (2007). Trends in *Salmonella enterica* serotypes isolated from human, food, animal, and environment in Tunisia, 1994-2004. J. Infect. 55: 324-339.

Bryan LB (1981). Current trends in foodborne salmonellosis in the United States and Canada. J. Food Protect. 44: 394-401.

Centers for Disease Control and Prevention (1999). Outbreak of *Salmonella* sero type Muenchen infections associated with Unpasteurized orange juice. United States and Canada, June 1999 Morb Mortal Wkly. Rep. 48: 582-585.

Chatti A, Daghfous D, Landoulsi A (2007). Acid resistance of *Salmonella* isolated from animals, food and wastewater in Tunisia. Ann. Saudi Med. 27: 195-198.

Cody SH, Glynn MK, Farrar J, Cairns KL, Griffin PM, Kobayashi JM, Fyfe M, Hoffman R, King AS, Lewis JH, Swaminathan B, Bryant R, Vugia D (1999). An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. Ann. Int. Med. 130: 202-209.

Cook KA, Dobbs TE, Hlady WG, Wells MS, Barrett TJ, Puhr ND, Lancette GA, Bodager DW, Toth BL, Genese CA, Highsmith AK, Pilot KE, Finelli L, Swerdlow DL (1998). Outbreak of *Salmonella* serotype Hartford infections associated with unpasteurized orange juice. J. Am. Med. Assoc. 280: 1504-1509.

Ercolani GL (1976). Bacteriological quality assessment of fresh marketed lettuce and fennel. Appl. Environ. Microbiol. 31: 847–852.

Fang, TJ (2005). Bacterial contamination of ready-to-eat foods: Concern for human toxicity. R. Food Nutri. Toxicity. 6: 143-171.

Garcia-Villanova RB, Galvez VR, Garcia-Villanova R (1987). Contamination on fresh vegetables during cultivation and marketing. Int. J. Food Microbiol. 4: 285–291.

Kristiina A, Esko N (1991). The growth of salmonellae in tomatoes. Int. J. Food Microbiol. 13: 177-182

Lampel KA, Orlandi PA, Kornegay L (2000). Improved template preparation for PCR-based assays for detection of foodborne bacterial pathogens. Appl. Environ. Microbiol. 60: 4539-4542.

Leyer GJ, Johnson EA (1992). Acid adaptation promotes survival of Salmonella spp. in cheese. Appl. Environ. Microbiol. 58: 2075-2080.

O'Mahony M, Cowden J, Smyth B, Lynch D, Hall M, Rowe B, Teare EL, Tettmar RE, Rampling AM, Coles M, Gilbert RJ, Kingcott E, Bartlett CLR (1990). An outbreak of *Salmonella saint-paul* infection associated with beansprouts. Epidemiol. Infect. 104: 229-235.

Sivapalasingam S, Friedman CR, Cohen L, Tauxe RV (2004). Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J. Food Prot. 67: 2342-2353.

Stocker BAD, Makela PH (1986). Genetic determinances of bacterial virulence with special reference to *Salmonella*. Curr. Trop. Microbiol. Immunol. 124: 149-172.

Tauxe R, Kruse H, Hedberg C, Potter M, Madden J, Wachsmuth K (1997). Microbial hazards and emerging issues associated with produce; a preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. J. Food Prot. 60: 1400-1408.

Wilson H, Joe OJ, Georgia LM (1960). The Relative Pathogenicity of Some Salmonella Serotypes for Chicks. Avian Dis. 4:103-109.

Zhuang RY, Beuchat LR, Angulo FJ (1995). Fate of *Salmonella montevideo* on and in raw Tomatoes as affected by temperature and treatment with chlorine. Appl. Environ. Microbiol. 61: 2127–2131.