Food Control 30 (2013) 106–110

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

The effects of ultraviolet light irradiation and drying treatments on the survival of *Cronobacter* spp. (*Enterobacter sakazakii*) on the surfaces of stainless steel, Teflon and glass

Lin-Shuan Kuo, Be-Jen Wang, Yu-Syuan He, Yih-Ming Weng*

Department of Food Science, National Chiayi University, 300 University Road, Chiayi 600, Taiwan, ROC

ARTICLE INFO

Article history:
Received 21 February 2012
Received in revised form
31 May 2012
Accepted 5 June 2012

Keywords:
Cronobacter spp. (*Enterobacter sakazakii*)
Surface contamination
Stainless steel
Teflon
Glass

ABSTRACT

Cronobacter spp. (*Enterobacter sakazakii*) are opportunistic food-borne pathogens that might cause severe consequences in infants and neonates. The outbreaks are associated with the consumption of powdered infant formula. Possible contamination sources of these particular microorganisms include the processing equipments and the operation environments. The present research was carried out to study the survival of *Cronobacter* spp. on the surfaces of stainless steel, Teflon and glass under ultraviolet (UV) irradiation and at different temperatures. The results show that *Cronobacter* spp. were susceptible to UV light but could survive at 60 or 70 °C for up to 120 min. The study contributes to a better understanding of the growth behavior of *Cronobacter* spp. on food contact surfaces, thereby enabling the development of more effective strategies and interventions for the control.

1. Introduction

Cronobacter spp., formerly *Enterobacter sakazakii*, are opportunistic Gram-negative food-borne pathogens that might cause severe consequences in infants and neonates (Nazarowec-White & Farber, 1997; Pagotto, Lenati, & Farber, 2007). The *Cronobacter* genus was originally defined as an *E. sakazakii* species (Farmer, Asbury, Hickman, Brenner, & the Enterobacteriaceae Study Group, 1980); but *E. sakazakii* has recently been reclassified as six species in the new *Cronobacter* genus (Iversen et al., 2008). In accordance with the new taxonomic change, the designation "*Cronobacter* spp." is used consistently in the subsequent part of this paper with the exceptions when a specific strain is mentioned.

The outbreaks of *Cronobacter* spp. are associated with the consumption of powdered infant formula (Drudy et al., 2006; Iversen & Forsythe, 2004). Because the powdered infant formula is not sterile, low levels of microorganisms including *Cronobacter* spp. have been detected (Lenati, O'Connor, Hébert, Farber, & Pagotto, 2008; Rosset, Noel, & Morelli, 2007). Since *Cronobacter* spp. cannot survive the pasteurization heat treatment (Breeuwer, Lardeau, Peterz, & Joosten, 2003; Iversen, Lane, & Forsythe, 2004; Reich König, von Wiese, & Klein, 2010), possible contamination sources of these particular microorganisms include the addition of non-thermal-treated ingredients (such as vitamins and minerals) and the post-thermal treatment contamination from the processing equipments and environments (Kuda et al., 2012; Mullane et al., 2006; Reich et al., 2010). Therefore, even though significant improvements have been achieved in the processing technology and in the disinfecting protocol on machinery and facility, contamination of *Cronobacter* spp. has not been completely eradicated from powdered infant formula and continues to pose threat to the health of susceptible population (Adekunte et al., 2010).

The microbial contamination on food contact surfaces of processing equipments and from the environments might be caused by accidental splash/spill or air fallout. In fact, *Cronobacter* spp. were detected in the environmental samples in a powdered infant formula processing plant (Reich et al., 2010). If microbes on the processing equipment surfaces cannot be destroyed completely, the microorganisms might be transferred from food contact surfaces to food products through cross-contamination. The contamination originating from the food contact surfaces of utensils and equipments used in the food plants was reported by Shaker, Osaili, Al-Omary, Jaradat, and Al-Zuby (2007). To establish appropriate methods to control this type of cross-contamination, the survival behavior of *Cronobacter* spp. on food contact surfaces is needed. However, survival studies of *Cronobacter* spp. were almost all conducted in food systems such as powder infant

* Corresponding author. Tel.: +886 5 2717599; fax: +886 5 2717596.
E-mail address: ymweng@mail.ncyu.edu.tw (Y.-M. Weng).

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2012.06.015

formula, infant cereal, fresh-cut produce etc. (Adekunte et al., 2010; Al-Holy, Lin, Al-Qadiri, & Rasco, 2008; Lin & Beuchat, 2007; Nazarowec-White & Farber, 1997; Rosset et al., 2007; Shaker et al., 2007). Little information can be obtained in the literature to reveal the survival capability of *Cronobacter* spp. on the food contact surfaces.

Although many materials can be found in food processing plants, the present research was carried out to study the survival of *Cronobacter* spp. on the surfaces of stainless steel, glass and Teflon. Stainless steel is the most common material for constructing food processing machines, while Teflon is used as many machinery parts. On the other hand, glass is used in building windows and is the most common material for laboratory apparatus. In the practical operation, food processing equipments are washed, disinfected and rinsed after food manufacture. The wet equipment surfaces are either dried at room temperature for a long period of time or dried at elevated temperature (around 60–70 °C) with or without air flow for a relatively short time duration. Also, ultraviolet (UV) light is commonly used in food plants to reduce microbial load in the environments and on the equipment surfaces. Collectively, the present research was conducted to investigate the effect of UV light on the survival of *Cronobacter* spp. on these three types of surfaces. The survival of *Cronobacter* spp. on the surfaces either at elevated or room temperatures was also studied.

2. Materials and methods

2.1. Microbial cultures

Cronobacter sakazakii BCRC14153 (ATCC type strain 12868) was obtained from the Bioresources Collection and Research Center (BCRC), Hsin-Chu, Taiwan. *Cronobacter* sp. 29T was isolated from a rice flour sample and identified with API 20E identification kit (Marcy l'Etoile, France). The bacteria were streaked on tryptic soy agar (TSA) (Merck, Darmstadt, Germany) plate and incubated at 37 °C overnight. To ensure the correctness of bacteria used in following experiments, typical *Cronobacter* spp. colonies on TSA plates were identified and confirmed by yellow pigmentation (Kandhai et al., 2010) and API 20E kit (Al-Holy et al., 2008; Drudy et al., 2006). To prepare overnight culture for further tests, a single colony was selected and inoculated into 50-mL sterilized tryptic soy broth (TSB) (Merck, Darmstadt, Germany) and incubated at 37 °C for about 16 h. Although a wide range of incubation temperature (from 25 to 45 °C) for *Cronobacter* spp. has been reported (Iversen & Forsythe, 2007), relatively fast growth (usually within 1 day) can be obtained at 37 °C (Farmer et al., 1980). Since the growth at 25 °C will take 2–4 days, the incubation temperature of the present study was set at 37 °C in order to meet the cultivation protocol that only 0.5 mL growth medium was applied on material surfaces.

2.2. Effect of UV light on the survival of Cronobacter spp. on the culture medium surfaces

The overnight culture was diluted to cell density of about 10^4 CFU/mL with 0.1% sterilized peptone water. An aliquot of 0.1 mL was evenly spread on the surface of TSA or violet red bile glucose agar (VRBGA, Merck, Darmstadt, Germany) plates with a sterile L-shaped glass rod. The plates were uncovered and placed under an ultraviolet light (TUV 15W/G15 T8, Philips, Holland) at the distance of 20–55 cm for up to 300 s in a laminar flow. Then, the plates were incubated at 37 °C for 2 days and the colonies were counted. The survival rate was calculated against the number of colony detected in plates without UV irradiation.

2.3. Effect of UV light on the survival of Cronobacter spp. on the material surfaces

2.3.1. Inoculation of bacteria on material surface

Because stainless steel, Teflon and glass are among the most commonly used materials for utensils and equipments in food plant and laboratory, the capability of *Cronobacter* spp. to survive on the utensil and equipment surfaces is worth investigating. Therefore, the present study was conducted to investigate the survival of *Cronobacter* spp. on these three types of material surfaces.

The stainless steel plates (SUS301; 60 × 40 × 1.33 mm; Yi-Yuan Co., Douliou, Taiwan), glass slides (76.2 × 25.4 × 1.2 mm; Hon-Li Co., Chiayi, Taiwan) and Teflon plates (76.2 × 25.4 × 1.2 mm; Yi-Yuan Co., Douliou, Taiwan) were carefully cleaned and individually placed in a glass Petri dish (90 mm dia.). The glass Petri dishes containing the testing materials were sterilized in an oven (170 °C, 3 h) and then cooled down to room temperature. The overnight culture was diluted to cell density about 10^4 CFU/mL with sterilized 0.1% peptone water and an aliquot of 10 μL overnight culture was placed on the surface of each stainless steel plate, glass slide or Teflon plate.

2.3.2. Survival of Cronobacter spp. on material surfaces under UV irradiation

After inoculation, the glass Petri dishes containing individual stainless steel plate, Teflon plate or glass slide were uncovered and placed in a laminar flow under the UV light at a distance of 55 cm for up to 300 s. At the end or irradiation, an aliquot of 0.5 mL TSA was carefully placed at the inoculation spot of the material surface. The Petri dishes were incubated at 37 °C for 24 h and the growth of *Cronobacter* spp. was recorded. The results were expressed as the number of plates or slides with microbial growth over the 16 replicates.

2.4. Survival of Cronobacter spp. on material surfaces at different temperatures

2.4.1. Survival of Cronobacter spp. on material surfaces at elevated temperatures

The inoculation of *Cronobacter* spp. culture on stainless steel plates, glass slides and Teflon plates was performed as described earlier (2.3.1). In order to study the survival rate of *Cronobacter* spp. under simulated drying conditions in food processing plants, the Petri dishes were kept in a forced-air convection oven at 60 or 70 °C for up to 120 min. At each time interval, randomly sampled Petri dishes were removed from the oven and left in a laminar flow for about 30 min to cool down to room temperature. After the TSA medium (0.5 mL) was carefully placed at the inoculation spot, the Petri dishes were incubated at 37 °C for 24 h to observe the growth of bacteria. The results were expressed as the number of plates of slides with microbial growth over the 16 replicates.

2.4.2. Survival of Cronobacter spp. on material surfaces at room temperatures

To investigate the survival of *Cronobacter* spp. on material surfaces at room temperature, the Petri dishes containing inoculated stainless steel plate, glass slide or Teflon plate were placed in an incubator at 25 °C or 30 °C for up to 6 days. The Petri dishes were randomly sampled during the storage period. The growth medium application, incubation and observation of bacterial growth were conducted as described earlier (2.4.1). The results were expressed as the number of plates of slides with microbial growth over the 16 replicates.

2.5. Statistical analysis

The statistical analysis was performed with SPSS software (version 10.0; SPSS, Chicago, IL, USA) on a personal computer. Data