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Cronobacter sakazakii in foods and factors affecting its survival, growth, and inactivation

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ABSTRACT

Cronobacter sakazakii has been isolated from a wide range of environmental sources and from several foods of animal and plant origin. While infections caused by *C. sakazakii* have predominantly involved neonates and infants, its presence on or in foods other than powdered infant formula raises concern about the safety risks these foods pose to immunocompromised consumers. We have done a series of studies to better understand the survival and growth characteristics of *C. sakazakii* in infant formula, infant cereal, fresh-cut produce, and juices made from fresh produce. Over a 12-month storage period, the pathogen survived better in dried formula and cereal at low a_w (0.25–0.30) than at high a_w (0.69–0.82) and at 4 °C compared to 30 °C. *C. sakazakii* grows in formulas and cereals reconstituted with water or milk and held at 12–30 °C. The composition of formulas or cereals does not markedly affect the rate of growth. *C. sakazakii* grows well on fresh-cut apple, cantaloupe, watermelon, cabbage, carrot, cucumber, lettuce, and tomato at 25 °C and in some types of produce at 12 °C. Treatment of fresh fruits and vegetables with sanitizers such as chlorine, chlorine dioxide, and a peroxyacetic acid-based solution causes reductions of 1.6–5.4 log CFU/apple, tomato, and lettuce. Cells of *C. sakazakii* in biofilms formed on stainless steel and enteral feeding tubes or dried on the surface of stainless steel have increased resistance to disinfectants. Death of cells in biofilms is affected by atmospheric relative humidity. These studies have contributed to a better understanding of the behavior of *C. sakazakii* in and on foods and on food-contact surfaces, thereby enabling the development of more effective strategies and interventions for its control.

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1. Introduction

Neonatal infections believed to have been caused by *Cronobacter sakazakii*, formerly *Enterobacter sakazakii* (Iversen et al., 2008), were first reported by Urmenyi and Franklin (1961). Numerous cases have been subsequently described. A book (Farber and Forsythe, 2008) and several reviews (Nazarowec-White and Farber, 1997a; Lai, 2001; Iversen and Forsythe, 2003; Lehner and Stephan, 2004; Gurtler et al., 2005; Bowen and Branden, 2006; Drudy et al., 2006; Mullane et al., 2006; Friedemann, 2007) have summarized information on taxonomy, biochemical characteristics, epidemiology, pathogenicity, clinical etiology, and survival and inactivation characteristics of *C. sakazakii* in foods and the environment.

Reconstituted powdered infant formula and powdered milk have been the most common vehicles implicated in neonatal *C. sakazakii* infections. Other unidentified sources of the pathogen were involved in cases of infections in infants, children, and immunocompromised adults having underlying medical conditions (Jimenez and Gimenez, 1982; Pribyl et al., 1985; Hawkins et al., 1991; Emery and Weymouth, 1997; Dennison and Morris, 2002). *C. sakazakii* infections in these age groups raise concerns about the survival and growth characteristics of the pathogen in foods other than powdered and reconstituted infant formula and milk. *C. sakazakii* has been isolated from a wide range of foods and beverages (Table 1), thereby posing some level of safety risk to the consumer. Information about how the behavior of *C. sakazakii* on these foods is affected by conditions to which they are exposed would be meaningful when developing strategies and interventions for its control.

Summarized here is a series of experiments conducted in our laboratories. No attempt is made to review the numerous excellent studies reported internationally. The text evolved from a presentation at an International Meeting on *Cronobacter* (*E. sakazakii*) in Dublin, Ireland, 22–23 January 2009 at which we were invited to give an overview of our *Cronobacter* research. Objectives of our work were to better define the survival and growth characteristics of *C. sakazakii* upon exposure to environments and conditions mimicking those imposed by processes and practices followed in commercial channels

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Table 1
Some foods and beverages other than powdered infant formula, powdered milk, and water from which *C. sakazakii* has been isolated.

Food/beverage	Reference
Animal origin	
Camel	Al-Dughan and Yassiem (2001)
Cheese	Leclercq et al. (2002), Roig-Sagués et al. (2002), Iversen and Forsythe (2004), Ogier et al. (2004), Liu et al. (2005), Chaves-Lopez et al. (2006), El-Sharoud et al. (2008)
Eggs	Cabassi et al. (2004), Musgrove et al. (2004)
Fish and fresh products	Mensah et al. (2002), Nketsia-Tabiri et al. (2003), Miranda et al. (2003), Liu et al. (2005)
Meat products	Kimura et al. (1999), Leclercq et al. (2002)
Milk	Choi et al. (1999), Jayaro and Wang (1999), Lafarge et al. (2004), Ogier et al. (2004)
Pork (dry, raw, cured)	Castano et al. (2001), Molloy et al. (2008)
Poultry	Jimenez et al. (2003)
Sausage	Goullet and Picard (1986), Schalch et al. (1994), Leclercq et al. (2002)
Shellfish	Balebona et al. (1990)
Shrimp	Teuber (2001), Kim et al. (2008b)
Plant origin	
Attiéké (fermented cassava product)	Coulin et al. (2006)
Barley (green malt)	Scheepe-Leberkülne and Wagner (1986)
Biscuits	Liu et al. (2005)
Cereal (adult and infant)	Restiano et al. (2006)
Courgette	Gray et al. (2001)
Cowpea paste	Bulgarelli et al. (1988)
Dry ingredients (almonds, coconut powder, pistachio, lentils, sponge mix, soup, beanfeast, vegetable suet)	Iversen and Forsythe (2004)
Dumpling	Leuschner et al. (2004a,b)
Fufu (pounded cassava)	Mensah et al. (2002)
Grains, flour or meal (corn, rice, soy, wheat)	Iversen and Forsythe (2004), Restiano et al. (2006), Shaker et al. (2007)
Herbs and spices	Iversen and Forsythe (2004), Restiano et al. (2006)
Khamir (fermented sorghum bread)	Gassem (1999)
Laver (red algae)	Jung and Park (2006)
Lettuce	Francis and O'Beirne (1998), Soriano et al. (2001)
Nuts and seeds	Freire and Offord (2002), Iversen et al. (2004a,b,c)
Pea soup powder	Leuschner et al. (2004a,b)
Rice	Cottyn et al. (2001), Jung and Park (2006)
Seed sprouts	Geiges et al. (1990), Robertson et al. (2002), Cruz et al. (2004), Kim et al. (2009)
Sobia (fermented beverage)	Gassem (2002)
Sous (licorice beverage)	Nassereddin and Yamani (2005)
Soy protein, lentils	Iversen and Forsythe (2004)
Spices	Restiano et al. (2006)
Sweets	Liu et al. (2005)
Tea	Tamura et al. (1995), Zhao et al. (1997)
Tempé (fermented soybean)	Denter and Bisping (1994)
Tofu	Fouad and Hegeman (1993), No et al. (2002)
Tomato	Mensah et al. (2002), Jung and Park (2006)
Vegetables	Osterblad et al. (1999), Leclercq et al. (2002)
Vegetables (mixed salad)	Galli et al. (1990), Geiges et al. (1990), Ottaviani et al. (1992), Lack et al. (1999), Weiss et al. (2005)

and in food storage and preparation areas in hospitals, day-care centers, and the home.

2. Recovery of stressed cells

Several differential and selective media have been developed for detecting or enumerating *C. sakazakii* in clinical, food, and environmental samples (Hsing-Chen and Wu, 1992; Iversen et al., 2004b; Leuschner et al., 2004a,b; Oh and Kang, 2004). While these media are promising for recovering the pathogen from various sources, their suitabilities for supporting repair of stressed or injured cells and colony development by these cells were not compared. *C. sakazakii* is known to undergo injury upon exposure to chemical and physical stresses (Breeuwer et al., 2003; Barron and Forsythe, 2007; Al-Holy et al., 2008; Osaili et al., 2008a,b; Shaker et al., 2008).

We compared the suitability of agar media to resuscitate and support colony development by healthy and heat-, freeze-, acid-, alkaline-, and desiccation-stressed cells of *C. sakazakii* (Gurtler and Beuchat, 2005). Cells of *C. sakazakii* exposed to heat (55 °C for 5 min), freezing (−20 °C for 24 h, thawed, frozen again at −20 °C for 2 h, thawed), acidic pH (3.6 adjusted with lactic acid), alkaline pH (11.3 adjusted with sodium hydroxide), and desiccation in powdered infant formula (a_w 0.25, 25 °C for 30 days) were surface plated on eight test media. Tryptic soy agar supplemented with pyruvate, used as a control, supported colony development by the highest number of stressed cells (Table 2). Overall, Leuschner et al. (2004a) agar performed best for recovering stressed *C. sakazakii*. Test media possessing greater selective characteristics clearly inhibited resuscitation and colony development. The poor performance by Enterobacteriaceae enrichment (EE) agar raises concern about the ability of EE broth, which has been used as an enrichment broth in research and testing laboratories, to provide conditions necessary to recover stressed *C. sakazakii*.

3. Survival and growth in infant formulas and in powdered milk

3.1. Survival in powdered infant formula

Differences in composition of infant formulas, coupled with differences in a_w and storage temperature, are likely to affect the survival of *C. sakazakii* in powdered infant formula and other foods. The pathogen is known to survive for at least two years in powdered infant formula at low a_w (Edelson-Mammel et al., 2005; Barron and Forsythe, 2007). We undertook a study to determine the effects of a_w and storage temperature on the survival characteristics of the pathogen in four commercially manufactured milk-based and two soybean-based powdered infant formulas (Gurtler and Beuchat, 2007c). A mixture of ten strains of *C. sakazakii* isolated from infected infants (five strains), foods (four strains), and the environment (one strain) was spray-inoculated into powdered milk. The dried inoculum was added to the six infant formulas at three a_w ranges (0.25–0.30, 0.31–0.33, and 0.43–0.50) to give low (log 0.80 CFU/g) and high (log 4.66–4.86 CFU/g) populations. Formulas were stored at 4, 21, and 30 °C for 12 months during which samples were analyzed for the presence (by enrichment) and populations of *C. sakazakii*.

Populations in formulas initially containing a high inoculum decreased significantly over time at all a_w and storage temperature combinations. Shown in Fig. 1 are numbers of *C. sakazakii* recovered from a milk-based formula at a_w 0.26, 0.31, and 0.44. Changes in populations in the other five formulas stored under the same conditions were similar. When examining the effects of a_w of formulas, populations of *C. sakazakii* in five of six formulas at a_w 0.43–0.50 were significantly reduced compared to populations in formulas at a_w 0.25–0.30 when storage was at 4 °C for 6 months. Decreases in populations were greater in formulas stored at 21 and 30 °C than at 4 °C, and greater at 30 °C than at 21 °C. In three of the six formulas (a_w 0.43–0.50) stored for 6 months and five of six formulas stored for 9 months at 21 °C, initially high populations decreased to ≤1 log CFU/g. *C. sakazakii* was detectable only by enrichment (detection limit was 1 CFU/10 g) of four of six formulas stored at 30 °C for 3 months. The pathogen was detected by enrichment in only three of six formulas (a_w 0.43–0.50) stored at 30 °C for 6 months.

In powdered infant formulas inoculated with a low population of *C. sakazakii* (0.80 log CFU/g) and stored for 12 months, the pathogen was detected by enrichment of 17 of 18 (94%), 7 of 18 (39%), and 2 of 18 (11%) formula/a_w combinations stored at 4, 21, and 30 °C, respectively. Survival was favored by low a_w and at low storage temperature. Inactivation was generally unaffected by composition of

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