

CRONOBACTER SPP.

(FORMERLY ENTEROBACTER SAKAZAKII)

THE ORGANISM/TOXIN

Enterobacter sakazakii was recently reclassified into eight distinct taxa of a new genus *Cronobacter* (Iversen *et al.*, 2008). All have been linked retrospectively to clinical cases in adults and infants (FAO/WHO, 2008). To avoid confusion the organism will be referred to here as *Cronobacter* spp. (*E. sakazakii*). The bacterium is Gram-negative, motile, rod shaped, non-spore-forming and will grow in aerobic and anaerobic conditions (FAO/WHO, 2008). It is considered an opportunistic pathogen. Enterotoxin-like compounds are produced by some strains (FAO/WHO, 2004).

THE FOOD

Powdered formulae (PF) can be used to supplement or replace human breast milk. As a powder, it has advantages of cost and storage over the liquid form, however liquid (ready-to-use) infant formula is commercially sterile and is rarely implicated in human illness. PF includes all types of powdered formulae for infants and young children, including:

- Powdered infant formulae (PIF) and infant formulae for special medical purposes;
- Follow-up formulae (FUF);
- Human milk fortifiers used to supplement breast milk.
(CAC, 2008)

In general, PF products have been identified as high-risk foods for the growth of *Cronobacter* spp. (*E. sakazakii*) although only PIF has been implicated in cases of *Cronobacter* spp. (*E. sakazakii*) infection.

PIF is intended for newborns to weaning infants. Its composition closely resembles human breast milk. It is subject to stringent hygiene controls and microbial criteria in its manufacture. Current international standards (CAC, 2008) require *Cronobacter* spp. (*E. sakazakii*) to be absent in 30 samples of 10 grams.

Follow up formula (FUF) is a liquid food (derived from milk and/or other constituents of animal/plant origin) that is suitable for weaning infants from their 6th to 12th month. FUF may contain a wider variety of dry-mix ingredients that diversify the diet, e.g. cocoa powder, fruit/vegetable powders or flakes and flavours. FUF generally has a higher protein, iron and mineral content and a higher renal solute load compared to PIF (MoH, 2008a).

International evidence suggests that FUF has been consumed by infants <6 months old, and occasionally <1 month old (FAO/WHO, 2008). A general consensus has been reached by the Codex Alimentarius Commission not to establish a microbial criterion for *Cronobacter* spp. (*E. sakazakii*) in FUF (CCFH, 2009). This is mostly due to a lack of evidence associating illness with FUF, but also because feeding FUF to infants <6 months old contradicts manufacturers' directions. Unintended use or misuse of FUF has led to calls for clearer labelling and education of caregivers and healthcare professionals regarding the appropriate preparation and use of PIF and FUF.

NZFSa and the Ministry of Health have produced the following advice regarding *Cronobacter* spp. (*E. sakazakii*) and formula preparation:

<http://www.nzfsa.govt.nz/consumers/food-safety-topics/recalls-and-product-advice/infant-formula-sakazakii/index.htm>

Further advice on formula preparation is available from the Ministry of Health (MoH, 2008b):

<http://www.healthed.govt.nz/uploads/docs/HE1521.pdf>

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GROWTH AND CONTROL

Growth

Temperature

- Range 5.5-45°C (Nazarowec-White, 1998).
- Optimum 39.4°C (Kandhai et al., 2006).

Generation time 5 h at 10°C, 40 min at 23°C (Lambert and Bidlas, 2007), 20 min at optimum. It has been shown to grow in breast milk and breast milk with fortifiers (calorie and/or nutrient supplements) at 23°C and 37°C. The addition of fortifiers slowed growth at both temperatures, the effect was especially pronounced at 10°C (Lenati et al., 2008).

pH

- Minimum 3.89 (Lambert and Bidlas, 2007).
- Optimum 5-9.
- No maximum value found in the literature.

Atmosphere

Grows in aerobic and anaerobic conditions.

Water activity

Maximum salt concentration permitting growth: 9.1% (Lambert and Bidlas, 2007).

Survival

Favoured in PIF at low a_w and temperature.

In a long-term survival experiment the organism was inoculated into PIF to achieve a final reconstituted concentration of 10^6 cfu/ml and the PIF stored in screw-capped bottle at room temperature for 2 years. A final concentration of approximately 300 cfu/ml was measured in the reconstituted product (a 3.4 log₁₀ reduction). Most of the reduction occurred in the first 5 months (Edelson-Mammel *et al.*, 2005).

Temperature

Survived 6 months of freezing in reconstituted PIF without a decrease in concentration.

Ten strains did not grow in reconstituted PIF stored at 4°C but could be detected by enrichment 72 h after preparation (Gurtler and Beuchat, 2007a).

pH

Ability to survive moderate acid conditions is pH-dependent. Ten of twelve strains reduced by less than 1 log₁₀ during a 5-hour challenge at pH 3.5 (at 36°C) (Edelson-Mammel *et al.*, 2006).

Water Activity

Survives in PIF ($a_w = 0.2$). Survived better in PIF at a_w 0.25-0.30 than in PIF at a_w 0.43-0.50 at both 21°C and 30°C (Beuchat *et al.*, 2009).

Exponential-phase cells are more sensitive to drying than stationary-phase cells in low water activity environments (Pagotto *et al.*, 2007). Dried stationary phase cells survived 46 days at 25°C and 47°C, reducing by around 2 log₁₀ CFU/ml in the first 20 days then remained stable (Breeuwer *et al.*, 2003).

Inactivation

No synergistic interactions between inhibitory factors such as weak acids, pH, salt and temperature (Lambert and Bidlas, 2007).

Temperature

Substantial diversity in thermal resistance of strains with two distinct heat resistance phenotypes observed. At 58°C the D time varied by almost 20 fold between strains (Edelson-Mammel and Buchanan, 2004).

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Reference temperature	D value in reconstituted PIF (minutes)	Reference temperature	D value in reconstituted PIF (minutes)
52°C	54.8 ¹	60°C	1.1 – 4.4 ^{3,4}
54°C	6.4 – 23.7 ^{1,2}	62°C	0.2 – 0.3 ⁴
56°C	1.1 – 21.1 ^{2,3}	65°C	0.6 ³
58°C	0.27 – 9.9 ^{2,3}	70°C	0.07 ³

¹ Nazarowec-White and Farber 1997; z-value 5.8°C.

² Breeuwer *et al.*, 2003 (in phosphate buffer); z-value 3.1-3.6°C.

³ Edelson-Mammel and Buchanan, 2004 (strain 607 described as most heat resistant in their study); z-value 5.6°C

⁴ Iversen *et al.*, 2004; z-value 5.7-5.8°C.

A >4 log₁₀ reduction of the pathogen in powdered milk and PIF when they are reconstituted with water at ≥70°C) has been demonstrated (Edelson-Mammel and Buchanan 2004; Osaili *et al.*, 2009). However, manufacturers recommend using cooled boiled water. Advice varies regarding the temperature to be achieved but it is generally < 50°C before addition of powder to reduce (1) nutrient loss, particularly vitamin C, (2) clumping of powder and (3) potential for burns/scalds to infants or carer. Higher temperatures may also activate bacterial spores. The Ministry of Health (2008a, 2008b) and the NZFSA advocate cooling water and refrigerating for use on the same day if not used directly. At three months of age, tap water from a town supply can be used. An internet-based risk assessment model (JEMRA, 2007) considers the initial level of contamination, consumption patterns, preparation and handling (including water temperature). This enables comparisons between different preparation and feeding scenarios.

pH

Over a 5-hour challenge at pH 3.0, the decline in 12 strains was 4.9 to >6.3 log₁₀. The rates of inactivation varied considerably between strains (Edelson-Mammel *et al.*, 2006).

Pressure

High hydrostatic pressure as a non-thermal pasteurisation treatment has been reported. Under experimental conditions, a 7-log₁₀ cycle reduction was achieved at 350-400 MPa for 10-15 minutes at ambient 25°C (Pérez *et al.*, 2007).

Disinfectants / Sanitisers

Not applicable.

CLINICAL PICTURE

Incubation: Little information available.

Symptoms: Gastrointestinal symptoms such as diarrhoea. Neurological sequelae include brain liquefaction, seizures, high fever. A full review of symptoms with references is given by Gurtler *et al.*, 2005.

Condition: Meningitis, septicaemia, necrotising enterocolitis.

Dose: A dose-response curve is not available because of a lack of data. Multiplication prior to consumption is required to cause illness. Approximately 1000 cells may be sufficient to cause an infection (Iversen and Forsythe, 2003). Current knowledge suggests that <3 cfu/100g in PIF followed by multiplication after reconstitution can lead to infection (FAO/WHO, 2004).

At Risk Groups: Causes disease in all age groups. However, those at greatest risk are neonates and infants <2 months old, particularly premature, low-birth weight or immuno-compromised infants (FAO/WHO, 2008). Worldwide, 9 cases of adult infection have been documented. Groups with low gastric acid secretion and absence of natural gut flora may be at more risk as the pathogen may survive stomach passage into the intestine.

Long Term Effects: Because of underreporting in most countries, the long-term effects of infection are not clear. Mental retardation and quadriplegia have been reported. Mortality rates vary, historically from 10% to 80% (since 1958), but declining to under 20% in recent years (FAO/WHO, 2004).

Treatment: Antibiotics. There is reported increased resistance to broad-spectrum penicillins and cephalosporins (Lai, 2001).

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SOURCES

Human: Has been recovered from clinical specimens of cerebrospinal fluid, blood, sputum, throat, nose, stool, gut, wounds, bone marrow, eye, ear, stomach aspirates, anal swabs and breast abscess.

Animal: Flies and rodents are reported as source and vector, although a low prevalence (0.2%) in stable flies has been reported (Mramba *et al.*, 2006).

Food: First documented case linked to PIF was reported by Clark *et al.* (1990). The pasteurisation for IF manufactured using a wet-mix process must achieve a minimum 10 log unit reduction for vegetative bacteria but theoretically achieves a 50-80 log unit reduction (FAO/WHO, 2008). This suggests that contamination occurs post-pasteurisation. Current standards do not require PIF to be sterile, but liquid ready-to-feed infant formula is commercially sterile. In 50-80% of cases, PIF is both the vehicle and source (direct or indirect) of illness (FAO/WHO, 2004). The ability to monitor formula has improved in recent years. Cheese products, dried herbs, spices, rice seeds, lettuce, minced beef, sausage meat and vegetables, fermented cassava, mung bean and alfalfa sprouts, and crab meat reported to contain the organism but not linked to cases.

Environment: Isolated from water, dust, soil, plant materials, mud and vacuum cleaners (Pagotto *et al.*, 2007).

Transmission Routes: Two different routes for *Cronobacter* spp. (*E. sakazakii*) to enter PIF; (1) contaminated ingredients added after drying and before packaging (intrinsic contamination) or (2) through reconstitution and handling (external contamination). No evidence of infant-to-infant transmission. Not all infants with infections have been exposed to PIF, suggesting another environmental source. A case documented in Brazil appears to be the first of mother-to-infant transmission via breast milk (Barreira *et al.*, 2003).

OUTBREAKS AND INCIDENTS

NZ Incidence: Became notifiable in 2005 following the death of a premature infant in a Waikato neonatal unit (ESR, 2007). One invasive disease case was notified in 2005; an elderly male with peritonitis who was on a renal ward (ESR, 2007). The Ministry of Health has reported that newborn twins contracted *Cronobacter* spp. (*E. sakazakii*) meningitis in the neonatal intensive care unit at National Women's Hospital in 1991. Both babies survived but one suffered brain damage and spastic quadriplegia (MoH, 2005).

Overseas incidence: A 2002 USA survey estimated the rate of infection among infants as 1/100,000 and the rate among low birth weight neonates as 8.7/100,000 (WHO 2004, citing pers. comm.). Since the first case was documented in 1958 there have been around 120 documented cases worldwide, and at least 27 deaths (to July 2008) (FAO/WHO, 2008).

Overseas outbreaks

Belgium, 1998, necrotising enterocolitis, 12 infants, 2 fatalities. All cases fed PIF (van Acker *et al.*, 2001).

Knoxville, USA, 2001, 49 infants in intensive care (Weir, 2002).

France, 2004, 9 infants, 2 fatalities (Coignard *et al.*, 2006).

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