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# Detection and frequency of *Cronobacter* spp. (*Enterobacter sakazakii*) in different categories of ready-to-eat foods other than infant formula

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ABSTRACT

Two hundred sixty eight samples of ready-to-eat foods from retail shops were screened for the presence of *Cronobacter* by selective enrichment followed by plating on three chromogenic agars (ESIA, ESPM and DFI). *Cronobacter* was isolated from 14/23 samples of sprouts and fresh herbs/salads (60.9%), 7/26 samples of spices and dried herbs (26.9%) and 3/42 confectionery samples (7.1%). In cases where repeat samples were available, foods positive for *Cronobacter* were retested twice. In total, 54 *Cronobacter* isolates from 24 foods were recovered and genetic fingerprint patterns generated using PFGE. Identical PFGE-profiles were generated for *Cronobacter* isolates from five samples of two confectionery products obtained from a particular bakery shop over a period of 11 months. This may indicate a persistent contamination of the production site. For all other isolates, no clustering by phylogenetic analysis of PFGE-profiles was observed, indicating the sporadic nature of *Cronobacter* in ready-to-eat foods. Enterobacterial counts varied from a maximum value of $2.9 \times 10^7$ CFU/g (in dill) to a minimum value of <10 CFU/g (in confectionery and dried herbs/spices). There was no correlation between Enterobacterial count and the presence of *Cronobacter*. *Cronobacter* may be regularly imported into private households via ready-to-eat foods.



## 1. Introduction

*Cronobacter* (*Enterobacter sakazakii*) is an opportunistic pathogen mainly associated with infections in neonates (Lai, 2001; Mullane et al., 2007a). However, adult infections have also been reported (Lai, 2001; Ray et al., 2007; See et al., 2007; Gosney, 2008). Although outbreaks are rare, infected neonates can develop severe symptoms such as septicaemia and meningitis (Muytjens et al., 1983). Neonatal infections have in some cases been associated with consumption of contaminated dried infant milk formulae (Himelright et al., 2002), which are products traded on a global level. For this reason, international risk management measures were taken such as the revision of a Codex Alimentarius guideline and the implementation of specific food-safety criteria in the European Community (Anonymous, 2007a). Although several scientific studies provided data for risk assessments in relation to powdered infant formula, a need for further research was identified (Anonymous, 2007b). As information about the ecology of *Cronobacter* is still incomplete, the occurrence of these organisms in foods other than infant formula needs to be addressed (Friedmann, 2007). In the present work, samples of commercially available ready-to-eat foods were screened for the presence of *Cronobacter* and other Enterobacteriaceae. It has been reported that *Cronobacter* can persist in food production environments (Kandhai et al., 2004; Mullane et al., 2007b). Therefore, further samples of products that were found positive for the presence of *Cronobacter* were analyzed and isolated strains compared using pulsed-field gel electrophoresis (PFGE) to detect possible persisting contamination of production plants or retail areas.

## 2. Materials and methods

### 2.1. Food samples

From March 2007 to January 2008, 268 samples of ready-to-eat foods were purchased in retail shops. The samples comprised; 42 confectioneries; 39 soft cheeses from raw milk; 42 meat products; 37 delicatessen salads; 27 ice creams; 32 powdered milk and dessert powders; 23 sprouts and fresh herbs/salads; and 26 spices and dried herbs. Products found to be positive for *Cronobacter* were re-sampled by obtaining further lots of the same product from the retailer either once or twice within a period of approximately two to eleven months after the first analysis.

### 2.2. Reference strains

*C. sakazakii* strain DSM 4485$^T$ was used for preliminary spiking experiments and performance testing of selective plates. *C. muytjensii*

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**Table 1**
Occurrence of *Cronobacter* in various ready-to-eat food stuffs after enrichment in selective broth and relative performance of three chromogenic agar media.

| Category of food | Number of samples | Number of samples yielding confirmed *Cronobacter* isolates | | | Overall % positive samples |
|---|---|---|---|---|---|
| | | ESPM | DFI | ESIA | |
| Confectionery | 42 | 3 ($1^a/2^b$) | 3 | 3 | 7.1 |
| Soft cheese from raw milk | 39 | 0 | 0 | 0 | 0 |
| Meat products | 42 | 0 | 0 | 0 | 0 |
| Delicatessen salads | 37 | 0 | 0 | 0 | 0 |
| Ice cream | 27 | 0 | 0 | 0 | 0 |
| Sprouts and fresh herbs/salads | 23 | 12 ($2^a/10^b$) | 13 | 14 | 60.9 |
| Milk- and dessert powders | 32 | 0 | 0 | 0 | 0 |
| Spices and dried herbs | 26 | 6 ($4^a/2^b$) | 7 | 7 | 26.9 |
| Total | 268 | 21 ($7^a/14^b$) | 23 | 24 | 8.9 |

ESPM, *Enterobacter sakazakii* plating medium (R & F Laboratories, Illinois, U.S.A); DFI, *Brilliance Enterobacter sakazakii* agar (DFI formulation, Oxoid, Basingstoke, UK); ESIA, *Enterobacter sakazakii* isolation agar (AES Laboratoire, Bruz, France). [a]Black colonies (typical); [b]green colonies (atypical).

strain ATCC 51329$^T$ (Iversen et al., 2008a) was used as a reference strain to ensure conformity between gels in PFGE-typing experiments.

### 2.3. Assessment of selective enrichment media

The following enrichment broths were compared using spiked samples: buffered peptone water (Oxoid CM509) supplemented with 10 µg per mL of filter sterilized vancomycin hydrochloride (Sigma, Buchs, Switzerland); *Enterobacter sakazakii* selective broth (ESSB; AEB611448, AES Laboratoire, Bruz, France); R & F® *Enterobacter sakazakii* Enrichment Broth (R&F; M-1200, R and F Laboratories, Illinois, U.S.A.); and *Cronobacter* broth (CB) comprising: 16 g/l Tryptone (Merck 1.10213); 4 g/l Peptone (Merck 1.07224); 34 g/l NaCl; 10 g/l Saccharose; 0.25 g/l Na-desoxycholate; 60 µM 8-hydroxyquinolone; 2.75 g/l $K_2HPO_4$; and 2.75 g/l $KH_2PO_4$; with a final pH 6.5 ± 0.1. To test the performance of these four media, samples of soft cheese, vanilla cream, minced meat and mung bean sprouts were spiked with approximately 10 and 100 CFU/g of *C. sakazakii* DSM 4485$^T$. The spiked samples were enriched in the various selective broths according to recommended protocols, i.e. ESSB, R&F and buffered peptone water with vancomycin were incubated at 37 °C, while CB was incubated at 42 °C. All broths were incubated overnight (18 ± 2 h) and streaked onto chromogenic agar plates as described below.

### 2.4. Bacteriological analyses of ready-to-eat foods

The screening of market samples was performed as follows: 10 g of food was added to 90 ml of *Cronobacter* broth (CB). After homogenizing in a stomacher for 60 s, samples were incubated aerobically at 42 °C for 24 h. Single colonies were obtained by streaking 10 µl of enrichment broth onto each of the following selective agar plates: *Enterobacter sakazakii* isolation agar (ESIA; AEB520010, AES Laboratoire, Bruz, France); R & F® *Enterobacter sakazakii* Chromogenic Plating Medium (ESPM; M-0700 R and F Laboratories, Illinois, U.S.A.); and *Brilliance Enterobacter sakazakii* agar (DFI formulation) (DFI; CM1055, Oxoid, Basingstoke, UK). All selective plates were incubated aerobically at the following temperatures: ESIA, 44 °C for 21 ± 3 h; ESPM and DFI 37 °C for 24 h). Presumptive colonies (ESIA: blue; ESPM: black; DFI: green) were sub-cultured on sheep blood agar (CM0854 with SR0051, Oxoid, Basingstoke, UK) and confirmed with the ID 32E test system (bioMérieux, Marcy l'Etoile, France). Confirmed isolates were kept at −70 °C until further analysis by pulsed-field gel electrophoresis (PFGE).

Enterobacterial counts were determined according to the international standard method for detection and enumeration of Enterobacteriaceae, ISO 21528-2 (ISO, 2004). This method is a poured-plate technique with violet red bile glucose (VRBG) agar.

### 2.5. Pulsed-field gel electrophoresis (PFGE)

PFGE fingerprint patterns were obtained for a total of 54 *Cronobacter* isolates, using the restriction enzyme Xba1, following a

**Table 2**
Type and origin of ready-to-eat foods contaminated with *Cronobacter*, total enterobacterial counts and incidence of contamination in follow-up samples.

| Food positive for *Cronobacter* | Caterer | Origin of product | Total enterobacterial count (CFU/g) | Follow up samples positive for *Cronobacter* | |
|---|---|---|---|---|---|
| | | | | 1st follow-up | 2nd follow-up |
| *Confectionery* | | | | | |
| Punch balls (chocolate) | A | Switzerland | <10 | − | + |
| Vanilla cream bars | B | Switzerland | $2.3\times10^2$ | + | + |
| Chocolate cake | B | Switzerland | <10 | n.d | + |
| *Sprouts and fresh herbs/salad* | | | | | |
| Mixed salad | C | Switzerland | $4.0\times10^5$ | − | − |
| Parsley | C | Switzerland | $1.5\times10^5$ | − | − |
| Dill | D | Switzerland | $2.9\times10^7$ | − | − |
| Coriander | E | Thailand | $4.6\times10^5$ | + | + |
| Celery | E | Thailand | $2.3\times10^4$ | + | + |
| Basil | E | Thailand | $1.1\times10^4$ | + | + |
| Lemon grass | C | Thailand | $3.2\times10^5$ | + | − |
| Lentil sprouts | D | Switzerland | $1.5\times10^7$ | + | + |
| Onion sprouts | C | Switzerland[1] | $1.0\times10^6$ | + | + |
| Mung bean sprouts | E | unknown | $2.0\times10^7$ | + | + |
| Sprout mixture (alfalfa, ruccola) | C | Switzerland[1] | $6.5\times10^6$ | + | + |
| Sprout mixture (alfalfa, mustard, radish) | D | Switzerland | $5.7\times10^6$ | + | + |
| Sprout mixture (lentil, mung bean, wheat) | C | Switzerland | $3.3\times10^6$ | + | + |
| Sprout mixture (alfalfa, lentil, radish, red cabbage, wheat) | C | Switzerland | $6.4\times10^6$ | + | + |
| *Spices and dried herbs* | | | | | |
| Black pepper, ground | E | India | $2.0\times10^1$ | + | n.d. |
| White pepper, ground | D | Asia | $9.0\times10^1$ | + | + |
| Madras curry | D | America, Asia, Europe | $9.2\times10^3$ | − | − |
| Madras curry | E | India | $3.2\times10^2$ | n.d. | n.d. |
| Mixture of herbs | E | Vietnam | <10 | n.d. | n.d. |
| Dried herb mixture for salads | D | Switzerland | <10 | + | + |
| Dried herb mixture "Provençale" | C | Germany | <10 | n.d. | n.d. |

n.d.: not determined (sample not available); [1]seed: imported.