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# MEMBERS OF THE CRONOBACTER GENUS – CHARACTERISTICS AND PREVALENCE IN FOOD

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**Summary.** *Cronobacter* spp. are Gram(–), motile, non-spore forming bacilli from the *Enterobacteriaceae* family. The genus *Cronobacter* consists of seven species, three of which – namely *C. malonaticus*, *C. sakazakii*, and *C. turicensis*, are human pathogens that have been isolated from infected neonates and immunocompromised adults. Surveillance studies detected *Cronobacter* spp. in various environments including households, livestock facilities, and food industry plants. *Cronobacter* spp. had previously been associated only with powdered infant formulas, but many recent studies have demonstrated their prevalence in food products of plant origin. Nevertheless, the main "reservoir" and routes of food contamination are yet to be ascertained. *Cronobacter* spp. have been isolated from different sources ranging from animal-related food sources like powdered infant formulas, milk and milk products, eggs, meat and meat products, fish and seafood, and plant-related food sources like cereals, sprouts, nuts, vegetables, herbs and spices, and ready-to-eat products.

Key words: Cronobacter species, Enterobacter sakazakii, prevalence, food contamination

## INTRODUCTION

*Cronobacter* spp. belong to the family *Enterobacteriaceae*. The current seven species of the genus include: *C. condimenti*, *C. dublinensis*, *C. malonaticus*, *C. muytjensii*, *C. sakazakii*, *C. turicensis* and *C. universalis* [Iversen et al. 2008, Singh et al. 2015].

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These organisms are ubiquitous and have been isolated from a wide spectrum of environmental samples such as water and soil [Singh et al. 2015], kitchen utensils [Kilonzo-Nthenge et al. 2012], and human clinical samples (cerebrospinal fluid, urine, respiratory secretions, digestive tract, and skin wounds) [Lai 2001]. They have also been detected in various raw materials and food products of both plant and animal origin. *Cronobacter* spp. have been found in fresh, ready-to-eat (RTE), frozen, dried, fermented or cooked food products, but the first mentions and interest in these microorganisms concerned the contamination of powdered infant formula (PIF) associated with severe infant infections caused by *Cronobacter* spp. [Iversen and Forsythe 2004, Singh et al. 2015, Berthold-Pluta et al. 2017, Brandão et al. 2017, Vasconcellos et al. 2018].

The three following species: *C. malonaticus*, *C. sakazakii*, and *C. turicensis*, are reported to cause meningitis, bacteremia, and necrotizing enterocolitis in infants and neonates, and also bacteremia cholecystitis and urinary tract infections in elderly or immunocompromised adults, with mortality rates ranging between 10 and 80%. Among the cases, about half of the patients died within one week since the onset of the infections and about 94% of the meningitis survivors exhibited severe neurological complications [Lai 2001, Jaradat et al. 2009, Vasconcellos et al. 2018]. The number of cases of infections induced by *Cronobacter* spp., especially in the adult persons, is probably underestimated as no appropriate methods for their identification have so far been implemented in clinical laboratories [Vasconcellos et al. 2018]. Food contamination with *Cronobacter* spp. may pose threat to the health and life of the immunocompromised persons, neonates, elderly, and persons with severe underlying diseases [Turcovský et al. 2011].

Initially, the bacteria classified to the genus *Cronobacter* have been referred to as yellow pigment-producing *Enterobacter cloacae* (also today one of the stages of *Cronobacter* identification according to ISO 22964:2017 includes determination of the growth of yellow colonies on a tryptone-soy agar). Based on differences in DNA sequence, biochemical traits, resistance to antibiotics, and capability to produce yellow pigment, they have been described and distinguished as a separate species under the name *Enterobacter sakazakii*. Considering its affinity to *Enterobacter cloacae*, the newly distinguished species was included into the genus *Enterobacter* [Iversen and Forsythe 2004]. Six genomic groups were identified based on the analyses of 16S rRNA gene sequences of strains belonging to the *E. sakazakii* species. Then, a novel genus called *Cronobacter* had been proposed within the family *Enterobacteriaceae*. Classification of the genus *Cronobacter* was published in 2008. First, it included five species, i.e.: *C. dublinensis*, *C. malonaticus*, *C. muytjensii*, *C. sakazakii*, and *C. turicensis* [Iversen et al. 2008]. Other two *C. condimenti* and *C. universalis* species were identified in 2012 [Joseph et al. 2012, Singh et al. 2015].

#### CHARACTERISTICS OF BACTERIA FROM THE GENUS CRONOBACTER

The *Cronobacter* genus bacteria are Gram(–), motile, flagellated, non-spore forming, facultative anaerobes with the length of  $1.2-3.0 \,\mu\text{m}$  and the width of  $0.1-0.6 \,\mu\text{m}$ . They produce nitrate reductase as well as ornithine and arginine decarboxylases but they do not produce lysine decarboxylase. They are capable of fermenting glucose, mannitol,

arabinose, rhamnose, xylose, trehalose, cellobiose, maltose, melibiose, and lactose, thus producing acid or gas, but they do not ferment sorbitol and do not synthesize urease. Most of the strains turn out positive in the Voges–Proskauer reaction and are incapable of producing indole. They grow in a temperature range of 6–45°C (some strains even up to 47°C) and at pH (optimum) – from 5.0 to 9.0 (minimum pH 3.6) [Iversen et al. 2008, Beuchat et al. 2009].

As *Cronobacter* spp. do not survive a standard pasteurization treatment (72°C/15 s), it is likely that the contamination occurs because the heat temperature regime used by manufacturers is not sufficient to eliminate the pathogen, or more likely, because contamination occurs after the heat treatment [Brandão et al. 2017], like e.g. during the addition of dry ingredients (herbs, spices, etc.), and during post-pasteurization handling and packaging [Hochel et al. 2012]. Although there is discrepancy between reported *D*-values (decimal reduction time; is the time required under given conditions, e.g. temperature, to kill 90% or 1 log of microorganisms) at 58°C, from 0.4 to 2.6 min [Iversen et al. 2004] there is general agreement that *Cronobacter* spp. are a thermotolerant organisms. Edelson-Mammel and Buchanan [2004] determined the ability of *Cronobacter* strains to survive heating in rehydrated PIF at temperature 58°C. Two distinct heat resistance phenotypes were observed and *D*-values at temperature 58°C ranged from 0.51 to 9.86 min. A  $D_{71°C}$ -value for *C. sakazakii* NCTC 11467 in reconstituted PIF was determined as 0.7 s [Iversen et al. 2004].

Previous studies have demonstrated *C. sakazakii* bacteria to be capable of forming biofilms and therefore adhering to abiotic surfaces (silicon, latex, polycarbonate, stainless steel, glass, and polyvinyl chloride), namely to the typical materials used in food producing plants [Singh et al. 2009]. They are contaminants in processing plants, and their presence has been confirmed not only in factories producing PIF but also in cereal and potato powder processing plants [Ogihara et al. 2014].

An important trait of bacteria from this genus, which is significant from the viewpoint of the food production process, is their unusual resistance to dry, osmotic, and acid stress conditions compared to the other *Enterobacteriaceae* species [Mohammed et al. 2015]. *Cronobacter sakazakii* is known to survive for at least two years in PIF at low  $a_w$  (water activity) [Barron and Forsythe 2007]. Beuchat et al. [2009] reported that, over a 12-month storage period, the pathogen survived better in PIF at low  $a_w$  (0.25–0.30) than at high  $a_w$ (0.69–0.82) and at a temperature of 4°C compared to 30°C.

Treatment of fresh fruits and vegetables artificially contaminated by *C. sakazakii* with such sanitizers as chlorine, chlorine dioxide, and a peroxyacetic acid-based solution caused their counts to decrease by 1.6–5.4 log CFU (colony forming unit) in apple, tomato, and lettuce [Beuchat et al. 2009]. Disinfectants routinely used in hospitals, and food service kitchens are ineffective in killing some cells of *Cronobacter* spp. embedded in organic matrices [Kim et al. 2007]. Strains of *Cronobacter* spp. exhibited various levels of resistance to the disinfectants, depending on the composition of the disinfectants, amount and type of organic matrix surrounding cells, and exposure time. Populations of planktonic cells suspended in water (7.22–7.40 log CFU·ml<sup>-1</sup>) decreased to undetectable levels (< 0.30 log CFU·ml<sup>-1</sup>) within 1–5 min upon treatment with disinfectants (quaternary ammonium compounds, phenolic compounds, peroxyacetic acid/hydrogen peroxide), while numbers of cells in reconstituted PIF were reduced by only 0.02–3.69 log CFU·ml<sup>-1</sup> after the treatment for 10 min [Kim et al. 2007].

The table below provides an overview of results related to the prevalence of *Cronobacter* spp. bacteria in various groups of food products. Due to changes in the taxonomy and nomenclature of the described group of microorganisms, a species name "*Enterobacter sakazakii*" appearing in studies published before 2008–2009 today means "bacteria from the genus *Cronobacter*" without a species name.

Table. Food products from which Cronobacter spp. have been isolated

| Tabela. | Produk | kty spożywcze, | , z których izo | olowano C | <i>Cronobacter</i> spp. |
|---------|--------|----------------|-----------------|-----------|-------------------------|
|         |        |                |                 |           |                         |

| Food products                                 | References   |  |  |  |
|---|--|--|--|--|
|   | Animal origin food   |  |  |  |
| Infant formula                                | Iversen and Forsythe [2004], Chap et al. [2009], Jaradat et al. [2009],<br>Kandhai et al. [2010], Lee et al. [2012], Pan et al. [2014], Singh et al. [2015],<br>Pei et al. [2016], Xin et al. [2018]   |  |  |  |
| Milk<br>and whey powder                       | Iversen and Forsythe [2004], Jaradat et al. [2009], Kandhai et al. [2010],<br>Turcovský et al. [2011], Hochel et al. [2012], Heperkan et al. [2017]  |  |  |  |
| Cheeses                                       | Iversen and Forsythe [2004], Chaves-Lopez et al. [2006],<br>El-Sharoud et al. [2008]   |  |  |  |
| Milk  | Maćkiw et al. [2011], Singh et al. [2015]  |  |  |  |
| Eggs  | Cabassi et al. [2004], Musgrove et al. [2008], Hochel et al. [2012]  |  |  |  |
| Meat<br>and meat products                     | Kandhai et al. [2010], Turcovský et al. [2011], Mohammed et al. [2015]   |  |  |  |
|   | Plant origin food  |  |  |  |
| Biscuits                                      | Hochel et al. [2012]   |  |  |  |
| Cereal products<br>(for adults and infants)   | Restiano et al. [2006], Kandhai et al [2010], Hochel et al. [2012], Lee et al. [2012], Lou et al. [2014]   |  |  |  |
| Dried pasta                                   | Lou et al. [2014], Akineden et al. [2015]  |  |  |  |
| Fruits<br>and fresh cut fruits                | Kim et al. [2011], Althaus et al. [2012], Lee et al. [2012], Singh et al. [2015]   |  |  |  |
| Grains or flours                              | Iversen and Forsythe [2004], Restiano et al. [2006], Shaker et al. [2007],<br>Kim et al. [2011], Hochel et al. [2012], Cetinkaya et al. [2013], Li et al. [2014],<br>Lou et al. [2014], Brandão et al. [2017]  |  |  |  |
| Herbs and spices                              | Restiano et al. [2006], Iversen and Forsythe [2007], Baumgartner et al. [2009],<br>Jaradat et al. [2009], Kandhai et al. [2010], Sospedra et al. [2010], Maćkiw et al.<br>[2011], Turcovský et al. [2011], Hochel et al. [2012], Li et al. [2014], Ogihara et al.<br>[2014], Garbowska et al. [2015], Singh et al. [2015], Brandão et al. [2017] |  |  |  |
| Instant soups                                 | Iversen and Forsythe [2004], Turcovský et al. [2011], Hochel et al. [2012]   |  |  |  |
| Lettuce                                       | Baumgartner et al. [2009], Althaus et al. [2012], Vojkovska et al. [2016],<br>Berthold-Pluta et al. [2017]   |  |  |  |
| Nuts and seeds                                | Freire and Offord [2002], Iversen and Forsythe [2004], Turcovský et al. [2011],<br>Hochel et al. [2012]  |  |  |  |
| Sprouts                                       | Baumgartner et al. [2009], Kim et al. [2011], Althaus et al. [2012], Vojkovska et al. [2016], Berthold-Pluta et al. [2017]   |  |  |  |
| Tea   | Maćkiw et al. [2011], Stojanović et al. [2011], Turcovský et al. [2011]  |  |  |  |
| Vegetables<br>(including dried<br>and frozen) | Kandhai et al. [2010], Kim et al. [2011], Turcovský et al. [2011], Hochel et al.<br>[2012], Lee et al. [2012], Ogihara et al. [2014], Singh et al. [2015], Vojkovska et al.<br>[2016]  |  |  |  |
| Ready-to-eat (RTE) products                   | Baumgartner et al. [2009], Turcovský et al. [2011], Hochel et al. [2012], Lee et al. [2012], Xu et al. [2015], Vojkovska et al. [2016], Vasconcellos et al. [2018]   |  |  |  |
|   |  |  |  |  |

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# PREVALENCE OF CRONOBACTER SPP. BACTERIA IN ANIMAL-ORIGIN FOOD PRODUCTS

The microbiological safety of powdered infant formulas is a major concern to regulatory agencies and producers because these products are intended for neonates, including also low-birth-weight pre-term neonates, who have undeveloped immune systems or lack a competing intestinal flora [Chap et al. 2009]. Considering the especially severe risk posed by the bacteria of *Cronobacter* genus to the life of infants and small children, an increasing attention is being paid to the complete health safety of foods intended for this group of consumers. The EU Commission Regulation (EC) 1441/2007 from the 5 December 2007 on microbiological criteria of foodstuffs specifies the necessity of testing powdered infant formulas and dietary foods for special medical purposes intended for infants below six months of age for *C. sakazakii*. Pursuant to the criteria set in this Regulation, the *C. sakazakii* should be absent in 10 g of products placed on the marketing and during their entire shelf-life period.

Iversen and Forsythe [2004] isolated *Cronobacter* spp. from 2.4% of infant formulas and from 10.2% of infant weaning foods. Chap et al. [2009] found *Cronobacter* spp. in 3.0% of the 290 analyzed samples of powdered follow-up formula samples from seven countries, whereas Lee et al. [2012] reported that *Cronobacter* spp. contamination occurred in 11.1% of dried baby foods in Korea. *Cronobacter* spp. strains were isolated from 0.9% of PIF samples and from 1.2% of follow-up powdered formulas in China [Pei et al. 2016]. Other investigators from different countries have reported *Cronobacter* spp. contamination in 1.4–18.2% of powdered infant formulas and/or related products [Jaradat et al. 2009, Kandhai et al. 2010, Pan et al. 2014, Singh et al. 2015, Pei et al. 2016, Xin et al. 2018].

Powdered milk samples were rarely contaminated with target microorganisms. Iversen and Forsythe [2004] isolated *Cronobacter* spp. from 4.1% of the samples of milk powder. Investigations conducted by other authors demonstrate that *Cronobacter* spp. were isolated from 1.4–12.0% of the analyzed samples [Turcovský et al. 2011, Hochel et al. 2012, Heperkan et al. 2017]. In turn, Jaradat et al. [2009] and Baumgartner et al. [2009] reported no positive results in milk powder. Kandhai et al. [2010] found *Cronobacter* spp. in 4.0% of the milk powder samples but not in raw cow milk samples. Hence, the raw milk appeared not to be the source of these microorganisms, and the contamination of milk powder and other milk-originating products was rather derived from the environment.

*Cronobacter* spp. were also isolated from single samples of pasteurized milk [Maćkiw et al. 2011], cheeses [Iversen and Forsythe 2004, Chaves-Lopez et al. 2006, El-Sharoud et al. 2008], and whey powder [Heperkan et al. 2017].

Vineeth et al. [2014] emphasized that *Cronobacter* spp. may contaminate cattle environment and by this means also their meat. Minced meat can be contaminated at any stage starting from the slaughtering and cutting of carcasses, further processing, storage, and handling. Although, heat treatment kills these pathogens, it is most likely that contamination happens after this process, for example from food handlers, the environment, spices or during storage [Mohammed et al. 2015]. Kandhai et al. [2010] detected *Cronobacter* spp. in 3.2% of the analyzed meat samples, whereas Mohammed et al. [2015] – in 15.6% of the tested samples of ground beef. In turn, Wang et al. [2012] demonstrated the presence of *Cronobacter* spp. in 5.4% of the analyzed pork samples, but not in the samples of chicken

meat and sausages. Some knowledge on the sources of meat and meat products contamination was brought by the study of Turcovský et al. [2011], who detected *C. sakazakii* in only one sample of raw meat (i.e. in 1.6% of the 64 samples examined), but in as many as 17 samples of spiced meat (i.e. in 35.4% of the 48 examined samples). Apart from *C. sakazakii*, they isolated *C. dublinensis* and *C. malonaticus* strains.

*Cronobacter* spp. were isolated from egg shells collected at every of the three processing plants in a study carried out by Musgrove et al. [2008], but also from the surface of hen eggs examined by Hochel et al. [2012], and from ostrich eggs analyzed by Cabassi et al. [2004].

#### PREVALENCE OF CRONOBACTER SPP. IN PLANT-ORIGIN FOOD PRODUCTS

Fresh plant-origin products may be a vehicle for the transmission of human pathogens, for example Escherichia coli, Listeria monocytogenes, and Salmonella spp. In recent years, the percentage of food poisonings induced by the consumption of contaminated vegetables and other products thereof in the EU increased from 2.1% in 2009 to 7.1% in 2014 [EFSA 2011, 2015]. Likewise of many other representatives of the family Entero*bacteriaceae*, the plant environment appears to be the natural habitat of *Cronobacter* spp. This hypothesis is confirmed by results obtained by Baumgartner et al. [2009], Kim et al. [2011], Althaus et al. [2012], Hochel et al. [2012], Vojkovska et al. [2016], and Berthold--Pluta et al. [2017] as well as by the fact of *Cronobacter* spp. isolation from samples of leafy vegetables (lettuce) and sprouts. In Poland, Berthold-Pluta et al. [2017] detected Cronobacter spp. in 30.0% of the analyzed samples of leafy vegetables (rucola, lamb's lettuce, endive escarole, and mixes) and in 75.0 % of the samples of sprouts (alfalfa, broccoli, lentil, sunflower, small radish, leek, and mixes); the isolated species included: C. sakazakii (61.9% of the strains), C. muytjensii (19.0%), C. turicensis (9.5%), C. condimenti (4.8%) and C. malonaticus (4.8%). In Czech Republic, the percentage of positive samples of leafy greens accounted for 1.1%, that of whole vegetables - for about 13.0%, that of frozen vegetables – for 33.3%, and that of sprouts – for 40.0% [Vojkovska et al. 2016]. Contamination of vegetable samples in other countries ranged from 4.0 to 41.7% [Turcovský et al. 2011, Lee et al. 2012, Ogihara et al. 2014, Singh et al. 2015]. In turn, Kim et al. [2011] reported Cronobacter spp. in as many as 70.0% of the analyzed samples of root vegetables, but only in 5.1% of the soil samples from root-crop farms.

Fruits can be contaminated with pathogens from different sources (human, animal or environmental) during growth, harvesting, processing, distribution or handling, and the contaminated fruits have been implicated in a number of documented food-borne outbreaks [Santo et al. 2018]. Althaus et al. [2012] detected *C. sakazakii* in fresh-cut fruits (in two samples out of 64 analyzed), whereas in studies conducted by Lee et al. [2012] and by Singh et al. [2015], the percentage of positive fruit samples accounted for 7.3 and 26.7%, respectively.

The prevalence of *Cronobacter* spp. was particularly high in samples of grains and flours. Brandão et al. [2017] detected them in 66.7% of the analyzed samples of flours made of different grains and isolated three species: *C. sakazakii* (detected in all positive samples), *C. malonaticus*, and *C. dublinensis*. The presence of *Cronobacter* spp. in grains and flours has been reported also in other studies [Iversen and Forsythe 2004, Restiano et

al. 2006, Shaker et al. 2007, Kim et al. 2011, Hochel et al. 2012, Centinkaya et al. 2013, Li et al. 2014]. Lou et al. [2014] detected *Cronobacter* spp. in all analyzed samples of wheat flour; with their MPN (most probable number) ranging from 0.36 to 110 per 100 g of sample. So high prevalence of *Cronobacter* spp. in cereal materials may be due to the intrinsic contamination of grains and flours, since plant roots may be natural hosts of *Cronobacter* spp. [Brandão et al. 2017].

Significant contamination (from 41.2 to 100.0% of positive samples) with bacteria from the genus *Cronobacter* was also reported in sparse studies addressing the microbiological quality of nuts and seeds. Species isolated from their samples included: *C. malonaticus*, *C. sakazakii*, and *C. turicensis* [Freire and Offord 2002, Iversen and Forsythe 2004, Turcovský et al. 2011, Hochel et al. 2012].

The prevalence of *Cronobacter* spp. in breakfast cereals was reported to range from 4.9 to 16.0% [Restiano et al. 2006, Kandhai et al. 2010, Hochel et al. 2012, Lee et al. 2012, Lou et al. 2014]. Labels of the majority of various cereal products recommend mixing a portion of the cereals with liquid milk or water before consumption. Even if the number of *Cronobacter* spp. is very low in infant cereals, it may sharply increase after the addition of warm water or milk, especially if the prepared meal is kept at room temperature which may promote pathogen growth [Richards et al. 2005, Beuchat et al. 2009]. Investigations carried out by Beuchat et al. [2009] demonstrate that *C. sakazakii* can survive for up to one year in infant cereals with  $a_w$  in the range from 0.30 to 0.69, when cereals are stored under conditions simulating those to which they may be exposed during distribution, and at home.

The presence of *Cronobacter* spp. was demonstrated in cereal products with a low water activity, like e.g. dried pasta and biscuits, and the percentage of their positive samples reached 10.6–100.0% [Lou et al. 2014, Akineden et al. 2015] and 100.0% [Hochel et al. 2012], respectively.

The high prevalence of the *Cronobacter* genus bacteria was also reported in dried products, i.e. spices and herbs. Turcovský et al. [2011] demonstrated the presence of C. sakazakii in 11 samples (mainly ginger, black and red pepper), C. malonaticus in one sample of black pepper, and C. muytjensii in one sample of caraway. In turn, Iversen and Forsythe [2007] reported that Cronobacter spp. were isolated from 40 (33.0%) of the 122 analyzed samples of spices and herbs. The Cronobacter spp. bacteria were also isolated from 39.0% of the samples of Jordanian herbs and seasonings analyzed by Jaradat et al. [2009] and in the Spanish ones examined by Sospedra et al. [2010]. The Cronobacter genus bacteria were also detected by Ogihara et al. [2014] in 19.0% of the samples of spices and in 47.0% of the analyzed samples of herbs; the isolated strains included: C. dublinensis, C. muytjensii, C. sakazakii, and C. turicensis. The presence of *Cronobacter* spp. in herbs and spices has also been reported by other authors, with the prevalence ranging from 3.6 to 36.7% [Restiano et al. 2006, Baumgartner et al. 2009, Kandhai et al. 2010, Hochel et al. 2012, Li et al. 2014, Garbowska et al. 2015, Singh et al. 2015, Brandão et al. 2017]. The Cronobacter genus bacteria were also isolated from tea samples [Maćkiw et al. 2011, Turcovský et al. 2011]. The study conducted by Stojanović et al. [2011] showed high prevalence (from 11 to 75%) of C. sakazakii in all kinds of herbal teas, including those intended for infants and children (for example tea from *Matricaria recutita*).

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Two pathogenic *Cronobacter* species *C. sakazakii* and *C. malonaticus* dominated among these bacteria isolated from plant- and animal-based dried foods like instant soups [Iversen and Forsythe 2004, Turcovský et al. 2011, Hochel et al. 2012].

#### PREVALENCE OF CRONOBACTER SPP. IN RTE PRODUCTS

The presence of *Cronobacter* spp. in RTE products may pose risk to the health of consumers, particularly in the case of the elderly and immunocompromised persons, as these products are consumed without any heat treatment. The contamination of the RTE products (including RTE salads) may result directly from the contamination of plant raw-materials, since *Cronobacter* spp. bacteria had already been reported in materials of this type [Baumgartner et al. 2009, Althaus et al. 2012, Vojkovska et al. 2016, Berthold-Pluta et al. 2017]. The direct sources of contamination may also include herbs and spices [Baumgartner et al. 2009, Sospedra et al. 2010, Turcovský et al. 2011, Garbowska et al. 2015, Singh et al. 2015, Brandão et al. 2017], as well as rice and pasta [Akineden et al. 2015], since *Cronobacter* had already been isolated from samples of these products. Furthermore, *Cronobacter* contamination may be due to inappropriate, non-hygienic practices of dishes preparation. *Cronobacter* spp. have also been isolated from kitchen utensils, such as cutlery, blenders, sinks, countertops, dishcloths, and sponges [Molloy et al. 2009, Kilonzo-Nthenge et al. 2012].

Xu et al. [2015] reported *Cronobacter* spp. presence in 18.6% of the samples of Chinese RTE products. In Korea, *C. sakazakii* was isolated from RTE fresh cut salads [Lee et al. 2012], whereas in the Czech Republic the percentage of positive retail food samples ranged from 6.9 to 13.3% [Hochel et al. 2012, Vojkovska et al. 2016]. Three *Cronobacter* species (*C. sakazakii, C. dublinensis* and *C. malonaticus*) were isolated from 45% of the samples of RTE salads and foods from Japanese cuisine in Brazil [Vasconcellos et al. 2018]. Turcovsky et al. [2011] detected these pathogens (*C. sakazakii* and *C. malonaticus*) in 11.1% of the samples of RTE meat products. Bacteria from *Cronobacter* genus were also isolated from 29.7% of the samples of chocolate products (isolates of *C. sakazakii* and *C. malonaticus*) [Turcovský et al. 2011] and from 7.1% of the samples of confectionery products [Baumgartner et al. 2009].

#### CONCLUSIONS

Plant-origin foods appear to be one of the most possible natural "reservoirs" of *Cro-nobacter* spp. Their low prevalence in products of animal origin indicates that products of this type do not represent inherent sources of these bacteria and so these foods are contaminated during some stages of the manufacturing process.

The ability of *Cronobacter* spp. to produce biofilms coupled with their resistance to sanitizers and disinfectants points to the importance of following the principles for cleaning and sanitizing food preparation areas, containers and utensils while preparing and serving formulas for neonates and foods for adults under hospital and household conditions.

Members of the Cronobacter genus...

Controlling the microbiological load in food products and understanding both the optimal growth conditions and conditions of the technological treatment of raw materials would contribute positively to the reduction of the health risk posed to individuals. The health risk associated with *Cronobacter* spp. prevalence in food products should also be minimized by knowing their epidemiology and pathogenicity factors.

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# GATUNKI Z RODZAJU CRONOBACTER – CHARAKTERYSTYKA I WYSTĘPOWANIE W ŻYWNOŚCI

Streszczenie. Bakterie z rodzaju *Cronobacter* są Gram(–), ruchliwymi, nietworzącymi przetrwalników pałeczkami z rodziny *Enterobacteriaceae*. Rodzaj *Cronobacter* obejmuje jedenaście gatunków, z których trzy – *C. malonaticus, C. sakazakii* i *C. turicensis* – to patogeny dla człowieka. Zostały wyizolowane od chorych noworodków i dorosłych z obniżoną odpornością. W badaniach nad ekologią bakterii z rodzaju *Cronobacter* stwierdzono ich obecność w różnych środowiskach, w tym gospodarstwach domowych, zakładach hodowli zwierząt i zakładach przemysłu spożywczego. Do niedawna uważano, że bakterie z rodzaju *Cronobacter* występują tylko w sproszkowanych preparatach do żywienia niemowląt i małych dzieci. W ostatnich latach pojawiło się wiele publikacji dotyczących występowania tych bakterii w żywności pochodzenia roślinnego. Niemniej jednak główny "rezerwuar" i drogi zanieczyszczenia żywności przez bakterie *Cronobacter* spp. nadal nie zostały definitywnie ustalone. Bakterie z rodzaju *Cronobacter* spp. nadal nie zostały definitywnie ustalone. Bakterie z rodzaju *Cronobacter* izolowano z różnych źródeł, począwszy od produktów spożywczych pochodzenia zwierzęcego, takich jak: preparaty w proszku do żywienia niemowląt i małych dzieci, mleko i produkty mleczne, jaja, mięso i produkty mięsne, ryby i owoce morza, a także z produktów spożywczych pochodzenia

Members of the Cronobacter genus...

roślinnego, takich jak: nasiona zbóż, kiełki, orzechy, warzywa, zioła i przyprawy, oraz z produktów gotowych do spożycia (RTE).

Słowa kluczowe: gatunki z rodzaju *Cronobacter*, *Enterobacter sakazakii*, występowanie, zanieczyszczenie żywności