Cronobacter condimenti sp. nov., isolated from spiced meat, and *Cronobacter universalis* sp. nov., a species designation for *Cronobacter* sp. genomospecies 1, recovered from a leg infection, water and food ingredients

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A re-evaluation of the taxonomic position of five strains, one assigned to *Cronobacter sakazakii* (strain 1330^T, isolated from spiced meat purchased in Slovakia), two previously assigned to *Cronobacter* genomospecies 1 (strains NCTC 9529^T and 731, isolated from water and a leg infection, respectively) and two previously assigned to *Cronobacter turicensis* (strains 96 and 1435, isolated from onion powder and rye flour, respectively) was carried out. The analysis included phenotypic characterization, 16S rRNA gene sequencing and multilocus sequence analysis (MLSA) of seven housekeeping genes (*atpD*, *fusA*, *glnS*, *gltB*, *gyrB*, *infB*, *ppsA*; 3036 bp). 16S rRNA gene sequence analysis and MLSA showed that strain 1330^T formed an independent phylogenetic lineage in the MLSA, with *Cronobacter dublinensis* LMG 23823^T as the closest neighbour. DNA–DNA reassociation and phenotypic analysis revealed that strain 1330^T represented a novel species, for which the name *Cronobacter condimenti* sp. nov. is proposed (type strain 1330^T=CECT 7863^T=LMG 26250^T). Strains NCTC 9529^T, 731, 96 and 1435 clustered together within an independent phylogenetic lineage, with *C. turicensis* LMG 23827^T as the closest neighbour in the MLSA. DNA–DNA reassociation and phenotypic analysis confirmed that these strains represent a novel species, for which the name *Cronobacter universalis* sp. nov. is proposed (type strain NCTC 9529^T=CECT 7864^T=LMG 26249^T).

The genus *Cronobacter* was created by the reclassification of the species *Enterobacter sakazakii* (Iversen *et al.*, 2007) and belongs to the family *Enterobacteriaceae* of the class *Gammaproteobacteria*. It includes facultatively anaerobic, Gram-negative, oxidase-negative, catalase-positive, non-spore-forming rods that, in general, are motile, able to reduce nitrate to nitrite and to produce acetoin (Voges–Proskauer test), and negative for the methyl red test (Iversen *et al.*, 2007). Species of this genus are primarily inhabitants of plant material and are often associated with human diseases, most notably severe neonatal infections (Iversen & Forsythe, 2004; Forsythe, 2005; Osaili & Forsythe, 2009). The genus includes five species, which were differentiated according to the 16 *E. sakazakii* biogroups, each biogroup being defined by their phenotype based on 10 tests (Farmer *et al.*, 1980; Iversen *et al.*, 2006b): *Cronobacter sakazakii* (biogroups 1–4, 7, 8, 11 and 13), *Cronobacter malonaticus* (biogroups 5, 9 and 14), *Cronobacter turicensis* (biogroups 16, 16a and 16b), *Cronobacter muytjensii* (biogroup 15) and *Cronobacter dublinensis* (biogroups 6,

Abbreviations: MLSA, multilocus sequence analysis; MLST, multilocus sequence typing.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 1330^T, NCTC 9529^T, 1435, 731 and 96 are FN539031, EF059877 and JN205049–JN205051, respectively, and for the *atpD*, *fusA*, *glnS*, *gltB*, *gyrB*, *infB* and *ppsA* sequences of *C. sakazakii* ATCC 29544^T, *C. malonaticus* LMG 23826^T, *C. muytjensii* ATCC 51329^T, *C. dublinensis* LMG 23823^T, *C. turicensis* LMG 23827^T, strain NCTC 9529^T, strain 1330^{T}and *Citrobacter koseri* ATCC BAA-895 are JF268258–JF268313; they can also be accessed from the *Cronobacter* multilocus sequence typing website (http://pubmlst.org/cronobacter).

10 and 12) (Iversen *et al.*, 2007, 2008). However, not all *E. sakazakii* strains were accommodated in the genus *Cronobacter*. Strain NCTC 9529^T, the sole member of biogroup 16c, was suspected to represent another species of the genus *Cronobacter*, but insufficient strains and biochemical tests were available to define the species and consequently strain NCTC 9529^T was assigned to *Cronobacter* genomospecies 1 (Iversen *et al.*, 2007).

The taxonomy of the genus *Cronobacter* is complex due to the high interspecies similarity of the 16S rRNA gene sequences, which ranges from 97.8 to 99.7 %, the overlap of biochemical profiles and a poor correlation between genotypic and phenotypic analyses (Duaga & Breuwer, 2008; Kucerova *et al.*, 2010). Furthermore, confusions with other members of the *Enterobacteriaceae* have been described: a number of *Enterobacter cloacae* and *Enterobacter hormaechei* strains isolated from human infections were assigned to the genus *Cronobacter* using phenotypic tests (Caubilla-Barron *et al.*, 2007; Townsend *et al.*, 2008). The existence of microheterogeneities in the 16S rRNA gene sequence is another factor that can generate misidentifications, as has been reported for certain strains of *C. sakazakii* and *C. malonaticus* that could not be differentiated (Iversen *et al.*, 2007). Multilocus sequence analysis (MLSA) based on the sequences of housekeeping genes has proven to be a useful tool for the *Enterobacteriaceae* (Lacher *et al.*, 2007; Ibarz Pavón & Maiden, 2009). Baldwin *et al.* (2009) applied MLSA based on seven housekeeping genes (*atpD*, *fusA*, *glnS*, *gltB*, *gyrB*, *infB* and *ppsA*) to *C. sakazakii* and *C. malonaticus* and demonstrated a robust phylogenetic analysis that separated the two species. The latter study also showed that some previous confusion between the two species may have been due to incorrect species identification of some biotype index strains (Baldwin *et al.*, 2009). Further MLSA revealed a clear differentiation of all members of the genus *Cronobacter* and the association of *C. sakazakii* sequence type 4 with neonatal meningitis (Joseph & Forsythe, 2011; Kucerova *et al.*, 2011). The scheme has open access at the *Cronobacter* multilocus sequence typing (MLST) website (http://pubmlst.org/cronobacter). The present investigation determined the taxonomic position of five *Cronobacter* strains recovered from a leg infection, spiced meat, water and two food ingredients (onion powder and rye flour).

Strain 1330^T (also known as strain 040407/32^T) was previously isolated from spiced meat purchased in Slovakia (Turcovský *et al.*, 2011). Phenotypic analysis placed it in biogroup 1 and therefore as a strain of *C. sakazakii* (Iversen *et al.*, 2007). However, partial 16S rRNA gene sequence analysis (657 bp) showed the nearest match was *C. dublinensis* (Turcovský *et al.*, 2011). Strain 1330^T was isolated at 45 °C using selective enrichment in a modified lauryl sulfate tryptose broth containing 0.5 mol NaCl l^{-1} and 10 mg vancomycin l^{-1} and then plating onto *Cronobacter* chromogenic agar and selecting colonies, as described by Turcovský *et al.* (2011). Using an API 20 E kit (bioMérieux) and additional recommended tests (Farmer *et al.*, 1980; Iversen *et al.*, 2006b), strain 1330^T was phenotypically verified as a member of the genus *Cronobacter* and biogroup 1.

NCTC 9529^T was previously assigned to *E. sakazakii* biogroup 16c as defined by Iversen *et al.* (2006b) on the basis of non-motility, acid production from inositol and dulcitol and utilization of malonate. The strain was later assigned to *Cronobacter* genomospecies 1 (Iversen *et al.*, 2007). The remaining strains within biogroup 16 were defined as *C. turicensis* based on phenotyping, DNA–DNA hybridization and amplified fragment length polymorphism (Iversen *et al.*, 2007).

Strain 731 was isolated in 2005 from a post-operative mixed infection of a 9-year-old boy with humeral fracture treated by osteosynthesis using intra-medullar nailing. The strain was phenotypically identified as *E. sakazakii* and not further characterized. *Staphylococcus aureus* was also isolated from the infected site (M.-F. Prère, personal communication).

Strain 96 was isolated from onion powder purchased in the UK using *Enterobacteriaceae* enrichment broth and *E. sakazakii* chromogenic agar, as described by Iversen & Forsythe (2004). The strain was phenotypically identified as a member of *E. sakazakii* and assigned to biogroup 16 on the basis of motility, acid production from inositol and dulcitol, and utilization of malonate and ornithine (Iversen *et al.*, 2006b). It was assigned to *E. sakazakii* cluster 2 according to its partial 16S rRNA gene sequence (528 bp) (accession no. AY579172) and *hsp60* sequence (accession no. AY579197) by Iversen *et al.* (2004). Cluster 2 was later renamed without further analysis of strain 96 as *C. turicensis* in the taxonomic revision of *E. sakazakii* (Iversen *et al.*, 2007).

Strain 1435 was isolated from rye flour purchased in Turkey using *Enterobacteriaceae* enrichment broth and *Cronobacter* chromogenic agar. The strain was phenotypically identified as a member of the genus *Cronobacter* in our laboratory.

The phenotypic tests used to evaluate strains 1330^T, NCTC 9529^T, 731, 96 and 1435 in the present study were selected from Iversen *et al.* (2006a, b, 2007, 2008): catalase and oxidase activity, nitrate reduction, acid production from sugars, malonate utilization, production of indole from tryptophan, motility, gas from D-glucose, Voges–Proskauer, methyl red, α-glucosidase activity, pigment production on tryptone soy agar (TSA; 21 and 37 °C), aerobic and anaerobic growth on TSA (37 °C), growth on MacConkey agar and hydrolysis of DNA. Acid production from carbohydrates was determined in nutrient broth supplemented with phenol red and the following substrates (1 %, w/v): sucrose, L-arabinose, cellobiose, lactose, raffinose, L-rhamnose, inositol, D-mannitol, D-sorbitol, *N*-acetylglucosamine and salicin. These tests were performed at least twice using conventional methods and, additionally, some tests (production of indole and hydrogen sulphide, Voges–Proskauer test, α-glucosidase and β-galactosidase, ornithine

decarboxylase, hydrolysis of gelatin and urea and acid production from D-mannitol, D-sorbitol, L-rhamnose, *myo*-inositol, sucrose and L-arabinose) were performed in parallel using the API 20 E and ID 32 E systems (bioMérieux). Fermentation/oxidation of 49 carbohydrates was tested using the API 50 CH system (bioMérieux), according to the manufacturer's instructions. Appropriate positive and negative controls were included. All tests were performed at 37 °C and evaluated for 48 h. Type strains belonging to all species of the genus *Cronobacter* were evaluated under identical conditions for the selected differential tests included in Table 1. Between three and 12 of these tests were able to distinguish the test strains from other members of the genus *Cronobacter*.

Strains 1330^T, NCTC 9529^T, 731, 96 and 1435 were Gram-negative, oxidase-negative, catalase-positive, facultatively anaerobic rods that were positive for acetoin production (Voges–Proskauer) and yellow pigmentation on TSA at 21 °C after 48 h and negative for the methyl red test. They fermented glucose, sucrose, cellobiose, arabinose, mannitol, amygdalin and galacturonic acid, reduced nitrate, utilized citrate, malonate and ornithine, and exhibited delayed DNase activity. They did not hydrolyse urea or produce acid from sorbitol, 5-ketogluconate or adonitol. These traits are common in the genus *Cronobacter* (Iversen *et al.*, 2007, 2008).

Strain 1330^T was found to be biochemically different from all other members of the genus *Cronobacter* by at least six different characters (Table 1). This strain was classified as a member of *C. sakazakii* (biogroup 1) using criteria of Farmer *et al.* (1980), but on the basis of the present results it could be differentiated from this biogroup because it was not motile, was able to produce indole from tryptophan and to utilize malonate, and was not able to produce acid from turanose, inositol, lactulose, putrescine, *cis*-aconitate, 4-aminobutyrate, maltitol or palatinose. Strain 1330^T was relatively similar to strains NCTC 9529^T, 731, 96 and 1435 but could be differentiated from them by several characters, i.e. indole production and no acid production from dulcitol, melezitose, inositol, lactulose and maltitol (Table 1).

Strains NCTC 9529^T, 731, 96, and 1435 were found to be biochemically similar and different from all other members of the genus *Cronobacter* by at least three characters (Table 1). They could be differentiated from *C. turicensis* (biogroups 16, 16a and 16b) because they did not produce acid from turanose, putrescine or 4-aminobutyrate.

The susceptibility of strains 1330^T, NCTC 9529^T, 731, 96 and 1435 to 17 antibiotics was assessed according to the standards and procedures of the British Society for Antimicrobial Chemotherapy (2010). The strains were classified as susceptible, intermediate or resistant. Discs (Mast Diagnostics) containing the following antibiotics were used (μg per disc): amikacin (30), ampicillin (10), amoxicillin/clavulanic acid (30), cefotaxime (30), cefuroxime (30), cefpodoxime (10), ceftazidime (30), chloramphenicol (30), ciprofloxacin (1), doxycycline (30), gentamicin (10), imipenem (10), piperacillin/tazobactam (75/10), trimethoprim (2.5), ceftazidime/clavulanic acid (30/10), cefotaxime/clavulanic acid (30/10) and cefpodoxime/clavulanic acid (10/1) were tested.

Table 1. Phenotypic characters differentiating *Cronobacter condimenti* sp. nov., *Cronobacter universalis* sp. nov. and other members of the genus *Cronobacter*

Taxa: 1, *Cronobacter condimenti* sp. nov. 1330^T; 2, *Cronobacter universalis* sp. nov. (n=4); 3, *C. sakazakii* ATCC 29544^T; 4, *C. malonaticus* LMG 23826^T; 5, *C. turicensis* LMG 23827^T; 6, *C. muytjensii* ATCC 51329^T; 7, *C. dublinensis* subsp. *dublinensis* LMG 23823^T; 8, *C. dublinensis* subsp. *lactaridi*; 9, *C. dublinensis* subsp. *lausannensis*. Data in columns 1 and 2 and in parentheses (i.e. results for type strains) were obtained in this study and data in columns 3–9 were from Iversen *et al.* (2007, 2008). +, Positive; v, 20–80 % variable; −, negative.

Characteristic	1	2	3	4	5	6	7	8	9
Motility	−	v (−)	+ (+)	v (+)	+ (+)	+ (+)	+ (+)	+	+
Carbon utilization:									
Dulcitol	−	+ (+)	− (−)	− (−)	+ (+)	+ (+)	− (−)	−	−
Indole	+	− (−)	− (−)	− (−)	− (−)	+ (+)	+ (+)	+	v
Malonate	+	+ (+)	− (−)	+ (+)	v (+)	+ (+)	+ (+)	−	−
10-Methyl α-D-glucopyranoside	+	+ (+)	+ (+)	+ (+)	+ (+)	− (−)	+ (+)	+	+
Melezitose	−	+ (+)	− (−)	− (−)	+ (+)	− (−)	+ (+)	−	−
Turanose	−	− (−)	+ (+)	+ (+)	+ (+)	v (+)	+ (+)	v	−
Inositol	−	+ (+)	v (+)	v (−)	+ (+)	+ (+)	+ (+)	+	−
Lactulose	−	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+	−
Putrescine	−	− (−)	+ (+)	v (+)	+ (+)	+ (+)	+ (+)	+	v
cis-Aconitate	−	v (−)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+	+
trans-Aconitate	−	− (−)	− (−)	+ (+)	− (−)	+ (+)	+ (+)	+	+
4-Aminobutyrate	−	− (−)	+ (+)	+ (+)	+ (+)	v (+)	+ (+)	+	+
Maltitol	−	+ (+)	+ (+)	+ (+)	+ (+)	− (−)	+ (+)	+	−
Palatinose	−	v (−)	+ (+)	+ (+)	+ (+)	v (+)	+ (+)	+	+

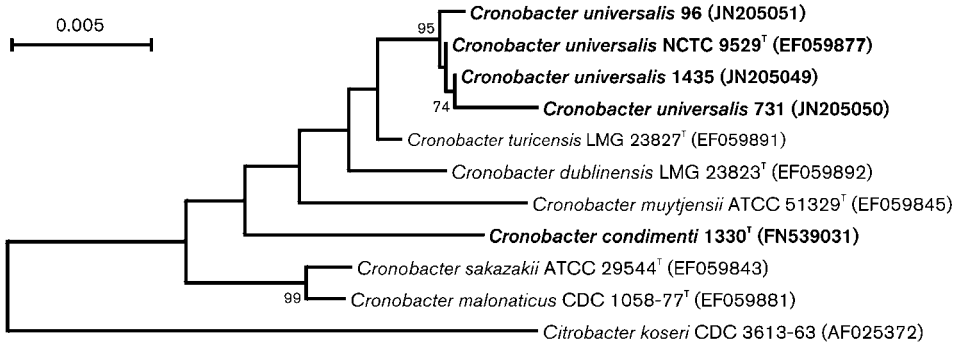

Fig. 1. Neighbour-joining phylogenetic tree derived from 16S rRNA gene sequences (1361 bp), showing positions of strains 1330^T, NCTC 9529^T, 96, 731 and 1435 in the genus *Cronobacter*. Bootstrap values (>70 %) based on 1000 replications are shown at branch nodes. Bar, 5 substitutions per 1000 nucleotide positions.

For phylogenetic studies of the 16S rRNA gene and seven housekeeping genes (MLSA), strains were cultured on TSA at 37 °C. DNA was extracted from a single colony by using a GenElute Bacterial Genomic DNA kit (Sigma-Aldrich), according to the manufacturer's instructions. The primers and conditions for amplification and sequencing of the 16S rRNA gene (1361 bp), *atpD* (390 bp), *fusA* (438 bp), *glnS* (363 bp), *gltB* (507 bp), *gyrB* (402 bp), *infB* (441 bp) and *ppsA* (495 bp) genes have been described elsewhere (Iversen *et al.*, 2007; Baldwin *et al.*, 2009). Amplification products were sequenced with an ABI sequencer (Applied Biosystems). 16S rRNA gene sequences (1361 bp) for the type strains of all members of the genus *Cronobacter* were obtained from GenBank and available housekeeping gene sequences (Baldwin *et al.*, 2009) were obtained from the MLST database. Sequences were independently aligned using CLUSTAL W version 2 (Larkin *et al.*, 2007) and MEGA version 4 (Tamura *et al.*, 2007). Genetic distances and clustering were determined using Kimura's two-parameter model (Kimura, 1980) and evolutionary trees were reconstructed using the neighbour-joining method (Saitou & Nei, 1987). The stability of the relationships was assessed using the bootstrap method with 1000 replicates. The 16S rRNA gene sequence similarities (1361 bp) were determined using the EzTaxon server (Chun *et al.*, 2007).

The 16S rRNA gene phylogenetic tree of the genus *Cronobacter* (Fig. 1.) showed that strain 1330^T formed one independent lineage and that strains NCTC 9529^T, 731, 96 and 1435^T formed another independent lineage within the cluster containing *C. dublinensis* DES187^T and *C. turicensis* z3032^T. 16S rRNA gene sequence similarities between strains 1330^T and NCTC 9529^T and the other recognized members of the genus *Cronobacter* were 99.7–98.2 %, which corresponds to 4–24 bp difference. The highest sequence similarities were obtained between strain NCTC 9529^T and *C. turicensis* z3032^T (99.7 %; 4 bp difference) and between strain 1330^T and *C. turicensis* z3032^T (98.6 %; 19 bp difference). Sequence similarities between strain NCTC 9529^T and strains 731, 96 and 1435 were 99.4, 99.6 and 99.7 % (8, 5 and 4 bp difference), respectively.

MLSA showed that strains 1330^T, NCTC 9529^T, 731, 96 and 1435 belonged to the genus *Cronobacter*, but represented two independent branches (Fig. 2). The MLSA phylogenetic tree revealed that the closest phylogenetic neighbour to strain 1330^T was *C. dublinensis* LMG 23823^T, despite strain 1330^T having highest 16S rRNA gene sequence similarity with *C. turicensis* z3032^T. However, *C. turicensis* LMG 23827^T was the closest neighbour of strains NCTC 9529^T, 731, 96 and 1435, as also shown by 16S rRNA gene sequence analysis.

DNA–DNA hybridization (direct and reciprocal) experiments were performed between strains 1330^T and NCTC 9529^T and between these two strains and the type strains of the currently accepted species of the genus *Cronobacter*. DNA was extracted according to Marmur (1961) and DNA–DNA hybridization was conducted according to Urdiain *et al.* (2008) under optimal conditions at 68 °C. Single- and double-stranded DNA was separated with hydroxyapatite. Colour development was measured at 405 nm using a Bio Whittaker Kinetic-QCL microplate reader. DNA–DNA reassociation values were determined at least three times. All results were below the 70 % limit for species definition (Table 2) (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Although DNA–DNA relatedness is considered to give information on the similarity of entire bacterial genomes, it has been criticized because of the high number of experimental errors, the lack of reproducibility and the failure to generate collective databases (Rosselló-Mora, 2006). Moreover, DNA–DNA relatedness does not provide any information concerning phylogenetic relationships (Harayama & Kasai, 2006), in contrast to the phylogenetic reconstruction with MLSA (Baldwin *et al.*, 2009).

16S rRNA gene sequencing, MLSA, DNA–DNA relatedness and phenotypic characterization clearly differentiated strains 1330^T, NCTC 9529^T, 731, 96 and 1435 from existing species of the genus *Cronobacter* and showed that they constituted

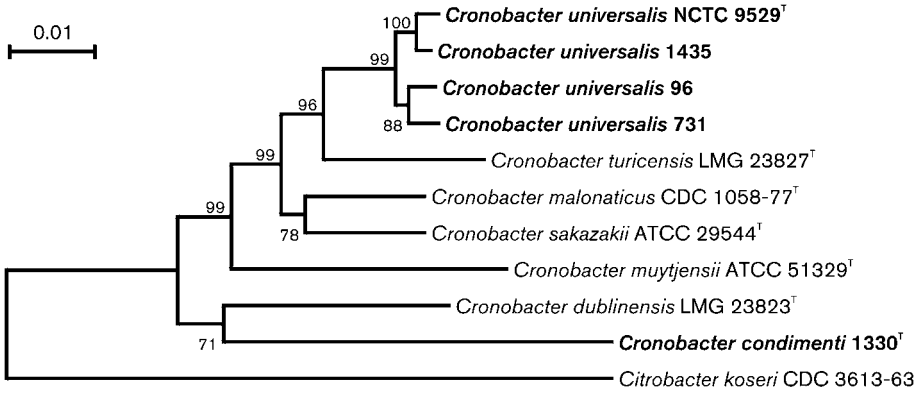

Fig. 2. Neighbour-joining phylogenetic tree based on concatenated *atpD*, *fusA*, *glnS*, *gltB*, *gyrB*, *infB* and *ppsA* sequences (http://pubmlst.org/cronobacter), showing the position of strains 1330^T, NCTC 9529^T, 96, 731 and 1435 within the genus *Cronobacter*. Bootstrap values (>70 %) based on 1000 replications are shown at branch nodes. Bar, 1 substitution per 100 nucleotide positions.

two independent lineages within the genus. Therefore, two novel species are proposed to accommodate these strains: *Cronobacter condimenti* sp. nov. (strain 1330^T) and *Cronobacter universalis* sp. nov. (strains NCTC 9529^T, 731, 96 and 1435).

Description of *Cronobacter condimenti* sp. nov.

Cronobacter condimenti (con.di.men′ti. L. gen. n. *condimenti* of spice, seasoning).

Cells are straight, Gram-negative, non-motile, non-spore-forming rods. Colonies on TSA incubated at 37 °C for 24 h are 2–3 mm in diameter, opaque, circular and yellow. Grows on MacConkey agar. In TSB, grows at 45 °C (optimum 37 °C), but not at 5 °C. No haemolysis is observed on sheep blood agar at 37 °C. Produces catalase, α-glucosidase, β-galactosidase and DNase, but not oxidase. Produces acetoin (Voges–Proskauer positive) and indole from tryptophan, but not hydrogen sulphide. Hydrolyses gelatin, but not urea. Reduces nitrate. Does not produce gas from glucose. Utilizes ornithine, citrate and malonate. Produces acid from 10-methyl α-D-glucopyranoside, glycerol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, *N*-acetylglucosamine, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, *myo*-inositol, raffinose, β-gentiobiose, D-fucose, L-fucose and galacturonic acid, but not from dulcitol, inositol, melezitose, turanose, lactulose, putrescine, *cis*- or *trans*-aconitate, 4-aminobutyrate, maltitol, palatinose, D-erythritol, D-arabinose, adonitol, methyl β-D-xyloside, L-sorbose, dulcitol, sorbitol, methyl α-D-glucoside, inulin, glycogen, xylitol, D-lyxose, D-tagatose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. Resistant to doxycycline; susceptible to the other antimicrobials tested. The API 20 E and ID 32 E profiles of the type strain are 3367373 and 342137610030, respectively.

The type strain is 1330^T (=CECT 7863^T=LMG 26250^T), isolated from spiced meat purchased in Slovakia.

Description of *Cronobacter universalis* sp. nov.

Cronobacter universalis (u.ni.ver.sa′lis. L. masc. adj. *universalis* of or belonging to all, universal).

Table 2. DNA–DNA relatedness between *Cronobacter condimenti* sp. nov. and *Cronobacter universalis* sp. nov. with other members of the genus *Cronobacter*

Data in column 1 were taken from this study and in column 2 from Iversen *et al.* (2008).

Strain	Hybridization (mean ± SD; %) with labelled DNA from:	
	C. condimenti 1330^T	*C. universalis* NCTC 9529^T
C. sakazakii ATCC 29544^T	40.3 ± 7.7	55.5 ± 1.0
C. malonaticus CDC 1058-77^T	53.0 ± 14.4	60.1 ± 1.3
C. muytjensii ATCC 51329^T	42.0 ± 9.3	53.1 ± 6.6
C. dublinensis LMG 23823^T	54.2 ± 8.7	45.9 ± 2.0
C. turicensis LMG 23827^T	47.9 ± 5.9	55.0 ± 3.3
C. universalis NCTC 9529^T	50.7 ± 7.6	–

The species description is based on four strains. Cells are straight, Gram-negative, non-spore-forming rods with variable motility (the type strain is non-motile). Colonies on TSA incubated at 37 °C for 24 h are 2–3 mm in diameter, opaque, circular and yellow. Grows on MacConkey agar. In TSB, grows at 45 °C (optimum 37 °C), but not at 5 °C. No haemolysis is observed on sheep blood agar at 37 °C. Produces catalase, α-glucosidase, β-galactosidase and DNase, but not oxidase. Produces acetoin (Voges–Proskauer positive), but not indole from tryptophan or hydrogen sulphide. Does not hydrolyse gelatin or urea. Reduces nitrate. Utilizes malonate, ornithine and citrate. Produces acid from glucose, 10-methyl α-D-glucopyranoside, dulcitol, inositol, melezitose, lactulose, sucrose, L-arabinose, cellobiose, lactose, *myo*-inositol, L-rhamnose, D-mannitol, *N*-acetylglucosamine, salicin, maltitol, D-fucose, amygdalin and galacturonic acid, but not from turanose, D-sorbitol, putrescine, *trans*-aconitate, L-fucose, adonitol, 5-ketogluconate or 4-aminobutyrate. Variable results are obtained for acid production from *cis*-aconitate and palatinose and production of gas from glucose (the type strain is negative). Resistant to doxycycline; susceptible to the other antimicrobials tested. The API 20 E and ID 32 E profiles of the type strain are 3205373 and 24276777051, respectively.

The type strain is NCTC 9529^T (=CECT 7864^T=LMG 26249^T), isolated from fresh water and deposited at the NCTC (London) in 1954.

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