Description of *Siccibacter colletis* sp. nov., a novel species isolated from plant material, and emended description of *Siccibacter turicensis*

Emily E. Jackson,[1] Naqash Masood,[1] Khaled Ibrahim,[1] Noémie Urvoy,[2] Sumyya Hariri[1] and Stephen J. Forsythe[1]

Correspondence
Stephen J. Forsythe
stephen.forsythe@ntu.ac.uk

[1]School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham NG11 8NS, UK

[2]Université Paris Est Créteil, Département Génie Biologique, 61 Avenue du Général de Gaulle, 94000 Creteil, France

A re-evaluation of the taxonomic position of two strains, 1383^T and 2249, isolated from poppy seeds and tea leaves, which had been identified as *Siccibacter turicensis* (formerly *Cronobacter zurichensis*), was carried out. The analysis included phenotypic characterization, 16S rRNA gene sequencing, multilocus sequence analysis (MLSA) of five housekeeping genes (*atpD*, *fusA*, *glnS*, *gyrB* and *infB*; 2034 bp) and ribosomal MLSA (53 loci; 22 511 bp). 16S rRNA gene sequence analysis and MLSA showed that the strains formed an independent phylogenetic lineage, with *Siccibacter turicensis* LMG 23730^T as the closest neighbour. Average nucleotide identity analysis and phenotypic analysis confirmed that these strains represent a novel species, for which the name *Siccibacter colletis* sp. nov. is proposed. The type strain is 1383^T (=NCTC 14934^T=CECT 8567^T=LMG 28204^T). An emended description of *Siccibacter turicensis* is also provided.

The genera *Siccibacter*, *Cronobacter* and *Franconibacter* are members of the family *Enterobacteriaceae* of the class *Gammaproteobacteria* and are composed of various former species of the genus *Enterobacter*. Although these three genera are closely related, only species of *Cronobacter* have been linked to cases of human illness (FAO/WHO, 2008). Members of the genus *Siccibacter* have been described as facultatively anaerobic, Gram-negative, weakly oxidase-positive, catalase-positive, non-spore-forming rods that are motile, do not produce acetoin (Voges–Proskauer test) and are positive for the methyl red test (Stephan *et al.*, 2014).

Confusions resulting from phenotypic and biochemical identification of members of the family *Enterobacteriaceae* have been described. For example, a number of strains of *Enterobacter cloacae* and *Enterobacter hormaechei* isolated from human infections were mistakenly assigned to the genus *Cronobacter* using phenotyping tests (Caubilla-Barron *et al.*, 2007; Townsend *et al.*, 2008). Consequently, it is difficult to describe novel members of this family solely on the basis of biochemical traits described previously in the literature. Phenotyping tests were not the primary means of species description in the recent naming of *Cronobacter condimenti*, *Cronobacter helveticus*, *Cronobacter pulveris* or *Cronobacter zurichensis* (Joseph *et al.*, 2012b; Brady *et al.*, 2013). Instead, multilocus sequence analysis (MLSA) and 16S rRNA gene sequencing were used to differentiate the species; however, 16S rRNA gene sequencing has limited application to the genus *Cronobacter* because of the high interspecies similarity, ranging from 97.8 to 99.7 %, and microheterogeneities in the gene (Iversen *et al.*, 2008; Joseph *et al.*, 2012a). Additionally, 16S rRNA gene sequencing showed high levels of similarity between *Cronobacter sakazakii* and *Citrobacter koseri* (97.8 %) and *Cronobacter sakazakii* and *Enterobacter cloacae* (97.0 %; Iversen *et al.*, 2004) Therefore, *fusA* sequence analysis is commonly used for identification of species of the genus *Cronobacter*, the phylogeny of which reflects the whole-genome phylogeny of the genus *Cronobacter* (Joseph *et al.*, 2012a, c). MLSA and *fusA* sequence analyses can also be applied to the genus *Siccibacter*, as a result of its close relationship to and previous inclusion in the genus *Cronobacter*.

MLSA based on housekeeping genes, including *fusA*, has proven to be a useful tool for taxonomic analysis of the

Abbreviations: ANI, average nucleotide; MLSA, multilocus sequence analysis; MLST, multilocus sequence typing.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *atpD*, *fusA*, *glnS*, *gltB*, *gyrB*, *infB* and *ppsA* gene sequences of strain 1383^T are LK054215–LK054222, respectively. These sequences can also be accessed from the PubMLST *Cronobacter* multilocus sequence typing database (http://pubmlst.org/cronobacter). The accession number for the genome sequence of strain 1383^T is JMSQ00000000.

Two supplementary tables and a supplementary figure are available with the online Supplementary Material.

Enterobacteriaceae and was found to be more effective than phenotyping for speciation of members of the genus *Cronobacter* (Jolley *et al.*, 2004; Jolley & Maiden, 2010; Joseph & Forsythe, 2012). Joseph *et al.* (2012b) used a seven-locus MLSA (*atpD*, *fusA*, *glnS*, *gltB*, *gyrB*, *infB* and *ppsA*; 3036 bp concatenated sequence length) for the definition of two novel species of the genus *Cronobacter*: *Cronobacter universalis* and *Cronobacter condimenti*. In contrast, Brady *et al.* (2013) only used four loci (*atpD*, *gyrB*, *infB* and *rpoB*) to support their reclassification of *Enterobacter helveticus*, *Enterobacter pulveris* and *Enterobacter turicensis* as *Cronobacter helveticus*, *Cronobacter pulveris* and *Cronobacter zurichensis*, respectively. This reclassification is disputed by Stephan *et al.* (2014), who proposed two new genera containing the species *Franconibacter helveticus*, *Franconibacter pulveris* and *Siccibacter turicensis* for the same former species of the genus *Enterobacter*, based on single-nucleotide polymorphism analysis of whole-genome sequences.

The present investigation determined the taxonomic position of two strains (1383^T and 2249) that had previously been assigned to the species *Cronobacter zurichensis* and *Siccibacter turicensis* (Brady *et al.*, 2013; Stephan *et al.*, 2014). Both strains were isolated while screening food products for the presence of members of the genus *Cronobacter*. Strain 1383^T was isolated in 2011 from poppy seeds and strain 2249 was isolated from pear and vanilla herbal tea bags, both purchased in the UK (Jackson *et al.*, 2014). The strains were isolated at 37 °C using *Enterobacteriaceae* enrichment broth (CM1115; Oxoid Thermoscientific) and then plating onto Druggan–Forsythe–Iversen *Enterobacter sakazakii* chromogenic agar (CM1055; Oxoid Thermoscientific). Typical blue–green colonies, indicating α-glucosidase activity, were selected for identification. Following the taxonomic revisions of *Enterobacter turicensis*, both strains were classified as *Cronobacter zurichensis* (Brady *et al.*, 2013) and then *Siccibacter turicensis* (Stephan *et al.*, 2014). This study re-evaluated this identification using a wider range of physiological, phenotyping and DNA-based techniques, including whole-genome sequence analysis.

Phenotypic analysis using API 20 E (bioMérieux) provisionally identified strain 1383^T as *Klebsiella pneumoniae* subsp. *ozaenae*. ID 32E (bioMérieux) provisionally identified the strain as *Enterobacter sakazakii*, prior to the taxonomic recognition of the genus *Cronobacter* (Iversen *et al.*, 2007). However, 16S rRNA gene sequence comparisons showed that the nearest match was to *Enterobacter turicensis*, which was later reclassified as *Cronobacter zurichensis*, then *Siccibacter turicensis* (Brady *et al.*, 2013; Stephan *et al.*, 2007, 2014).

Phenotypic analysis using API 20 E (bioMérieux) provisionally identified strain 2249 as a strain of *Erwinia*, whereas ID 32E (bioMérieux) provisionally identified the strain as *Escherichia vulneris*. However, *fusA* gene sequence analysis (438 bp) showed the nearest match was to *Siccibacter turicensis* strain 1383 in the *Cronobacter* PubMLST database, with 1 nucleotide difference at position 270, and 22 differences from the *fusA* sequence of *Siccibacter turicensis* LMG 23730^T.

The original description of *Siccibacter turicensis* (then called *Enterobacter turicensis*) utilized Biotype 100 test strips, which are no longer available (Stephan *et al.*, 2007). Therefore, the phenotypic tests used to re-evaluate strains 1383^T and 2249 were selected from Iversen *et al.* (2006a, b, 2007, 2008), Stephan *et al.* (2007, 2014), Joseph *et al.* (2012b) and Brady *et al.* (2013). These tests included catalase and oxidase activity, nitrate reduction, acid production from sugars, malonate and sialic acid utilization, production of indole from tryptophan, motility, gas from D-glucose, Voges–Proskauer test, α-glucosidase, pigment production on tryptone soy agar (TSA) (CM0131; Oxoid Thermoscience) at 25 and 37 °C, aerobic and anaerobic growth on TSA (37 °C) and colony morphology on MacConkey agar. Acid production from carbohydrates was determined in nutrient broth supplemented with phenol red and the following substrates (1 %, w/v): *myo*-inositol, putrescine, lactulose, 4-aminobutyrate, maltitol and *trans*-aconitate. Sialic acid utilization, motility, gas from glucose and growth on TSA and MacConkey agar were assessed using conventional methods. The remaining tests (production of indole and hydrogen sulfide, Voges–Proskauer test, α-glucosidase, β-galactosidase, ornithine decarboxylase, hydrolysis of gelatin and urea and acid production from D-mannitol, D-sorbitol, L-rhamnose, 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. The activities of various enzymes were determined by using the API ZYM system (bioMérieux). Appropriate positive and negative controls were included. All tests were performed at 37 °C and incubated for 24 h. Motility was assessed in motility medium (10 g tryptose, 5 g NaCl and 5 g agar l^{-1}, pH 7.2 $\pm$ 0.2) (Iversen *et al.*, 2007). Type strains of all species of the genera *Siccibacter*, *Franconibacter* and *Cronobacter* were evaluated under identical conditions for the selected differential tests included in Table 1.

Strains 1383^T and 2249 were found to differ from strains of their closest relative, *Siccibacter turicensis*, in gas production from D-glucose, in the utilization of sialic acid and in two enzyme activities, acid phosphatase and *N*-acetyl-β-glucosaminidase (Table 1).

The susceptibility of strains 1383^T and 2249 to 12 antibiotics was assessed according to the standards and procedures of the British Society for Antimicrobial Chemotherapy (BSAC, 2014), using *Escherichia coli* NCTC 10418 as the control organism. The strains were classified as susceptible, intermediate or resistant according to BSAC criteria. The following antibiotic-containing discs obtained from Mast Diagnostics were tested: amikacin (AK30), ampicillin (AP10), amoxicillin plus clavulanic acid (AUC30), cefotaxime (CTX30), cefuroxime (CXM30), ceftazidime (CAZ30), chloramphenicol (C30), ciprofloxacin (CIP1), doxycycline

Table 1. Phenotypic characters that differentiate *Siccibacter colletis* sp. nov. and other members of the genera *Siccibacter*, *Franconibacter* and *Cronobacter*

Taxa: 1, *Siccibacter colletis* sp. nov. strains 1383^T and 2249; 2, *Siccibacter turicensis* (*n*=2); 3, *Franconibacter helveticus* (*n*=2); 4, *Franconibacter pulveris* (*n*=6); 5, *Cronobacter sakazakii* (*n*=163); 6, *Cronobacter malonaticus* (*n*=22); 7, *Cronobacter turicensis* (*n*=8); 8, *Cronobacter universalis* (*n*=4); 9, *Cronobacter muytjensii* (*n*=7); 10, *Cronobacter dublinensis* (*n*=8); 11, *Cronobacter condimenti* 1330^T. Data for reference taxa were from Iversen *et al.* (2007, 2008), Stephan *et al.* (2007, 2008, 2014), Joseph *et al.* (2012b), Brady *et al.* (2013) and the current study. +, Positive; V, variable (25–75 % positive); −, negative; ND, no data available. Key traits for differentiation of species of the genus *Siccibacter* are shown in bold. Reactions of type strains are shown in parentheses. All strains are positive for acid production from D-glucose and negative for H_2S production.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Acid phosphatase	− (−)	+ (+)	V (−)	+ (+)	−	+	+	+	−	−	−
***N*-Acetyl-*β*-glucosaminidase**	+ (+)	− (−)	(−)	+ (+)	+	+	+	+	+	+	+
Motility	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	V (+)	+ (+)	V (−)	+ (+)	+ (+)	−
Voges–Proskauer test	− (−)	− (−)	− (−)	− (−)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+
Indole production	− (−)	− (−)	− (−)	− (−)	− (−)	+ (+)	− (−)	− (−)	+ (+)	+ (+)	+
Carbon source utilization											
Sialic acid	− (−)	+ (+)	− (−)	+ (+)	+ (+)	− (−)	− (−)	− (−)	− (−)	− (−)	−
D-Glucose, gas production	− (−)	+ (+)	ND	ND	ND	ND	ND	V (−)	ND	ND	−
Sucrose, acid production	− (−)	− (−)	− (−)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+
Dulcitol	+ (+)	+ (+)	+	−	− (−)	− (−)	+ (+)	+ (+)	+ (+)	− (−)	−
Malonate	− (−)	− (−)	+ (−)	− (−)	− (−)	+ (+)	V (+)	+ (+)	+ (+)	+ (+)	+
Melezitose	− (−)	− (−)	−	−	− (−)	+ (+)	+ (+)	+ (+)	− (−)	+ (+)	−
Inositol	− (−)	− (−)	− (−)	− (−)	V (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	−
trans-Aconitate	− (−)	(−)	+ (+)	+ (+)	− (−)	+ (+)	− (−)	− (−)	+ (+)	+ (+)	−
Maltitol	− (−)	− (−)	− (−)	V	+ (+)	+ (+)	+ (+)	+ (+)	− (−)	+ (+)	−
D-Arabitol	− (−)	− (−)	− (−)	+ (+)	− (−)	− (−)	− (−)	− (−)	− (−)	− (−)	−
Lactulose	− (−)	− (−)	− (−)	V	+ (+)	V (+)	+ (+)	+ (+)	+ (+)	+ (+)	−
Putrescine	− (−)	− (−)	+ (+)	V	+ (+)	+ (+)	+ (+)	− (−)	+ (+)	+ (+)	−

(DXT30), gentamicin (GM10), imipenem (IMI10) and trimethoprim plus sulfamethoxazole (TS25). Both strains 1383^T and 2249 were found to be resistant to doxycycline. Additionally, strain 1383^T was resistant to ciprofloxacin and showed intermediate resistance to cefotaxime. Both strains were sensitive to all other antibiotics tested.

It is recognized that the former reliance on phenotyping to define members of the genus *Cronobacter* and closely related species and incorrect speciation of biotype strains has led to contradictions in the biochemical descriptions of species of the genus *Cronobacter* (Baldwin *et al.*, 2009; Joseph *et al.*, 2013). Hence, the DNA-sequence-based techniques multilocus sequence typing (MLST), ribosomal MLST (rMLST), average nucleotide identity (ANI) and whole-genome analysis were used in this study as more reliable means of defining the novel species of the genus *Siccibacter*.

For phylogenetic studies of the 16S rRNA gene and for MLSA of five housekeeping genes, strains 1383^T and 2249 were cultured on TSA (CM0131; Oxoid Thermoscience) at 37 °C. DNA was extracted from a single colony by using a GenElute Bacterial Genomic DNA kit (Sigma-Aldrich) following the manufacturer's instructions. Primers and conditions for amplification and sequencing of the 16S rRNA (1361 bp), *atpD* (390 bp), *fusA* (438 bp), *glnS* (363 bp), *gyrB* (402 bp) and *infB* (441 bp) genes have been described previously (Iversen *et al.*, 2007; Baldwin *et al.*, 2009). Concatenated sequences (*atpD*, *fusA*, *glnS*, *gyrB* and *infB*; 2034 bp) were aligned in MEGA software version 5.2 (Tamura *et al.*, 2011) using the CLUSTAL W algorithm. Genetic distances and clustering were determined using Kimura's two-parameter model (Kimura, 1980) and evolutionary trees were reconstructed by the neighbour-joining method (Saitou & Nei, 1987). Trees were also reconstructed using the maximum-likelihood method (Tamura *et al.*, 2011) to ensure the robustness of the analysis. Stability of relationships was assessed by the bootstrap method (1000 replicates). The phylogenetic tree of the full 16S rRNA gene sequence (1451 bp; Fig. S1, available in the online Supplementary Material) was reconstructed using new and previously available 16S rRNA gene sequences (trimmed length 1361 bp) available in GenBank for all species of the genera *Siccibacter*, *Cronobacter* and *Franconibacter*. Phylogenetic trees for the five-locus MLSA (Fig. 1) used the existing curated sequences at the *Cronobacter* PubMLST database (http://pubmlst.org/cronobacter) initially created by Baldwin *et al.* (2009) and the new sequences obtained in this study.

The 16S rRNA gene tree (Fig. S1) showed that strain 1383^T clustered with *Siccibacter turicensis* LMG 23730^T and formed a cluster separate from the genera *Cronobacter* and *Franconibacter*. This supports the proposal of Stephan

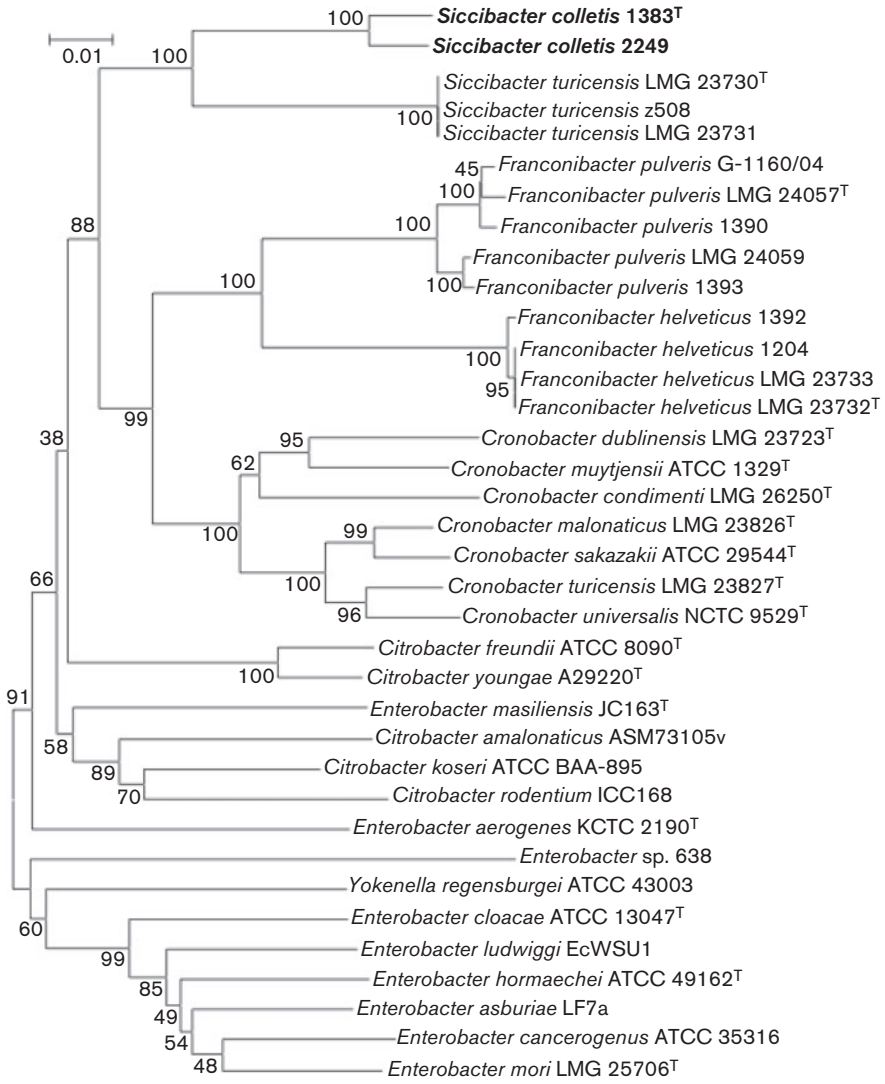

Fig. 1. Neighbour-joining phylogenetic tree based on concatenated *atpD*, *fusA*, *glnS*, *gyrB* and *infB* sequences (2034 bp), showing the position of strains 1383^T and 2249 within the genera *Siccibacter*, *Franconibacter*, *Cronobacter*, *Citrobacter* and *Enterobacter*. Sequences were accessed via the database at http://pubmlst.org/cronobacter. Bootstrap percentages based on 1000 replications are shown at branch nodes. Bar, 1 substitution per 100 nucleotide positions.

et al. (2014) that the species *Cronobacter zurichensis* (former *Enterobacter turicensis*) should be recognized as a member of a new genus; the genus *Siccibacter*, containing one species, *Siccibacter turicensis*. Additionally, *Siccibacter turicensis* and the novel species represented by strain 1383^T appear on two separate branches of the cluster, and strong bootstrap support (81 %) suggests that they should be classified as two separate species. The *Siccibacter* cluster was close to *Enterobacter cloacae* and the genera *Citrobacter* and *Kosakonia*. *Franconibacter helveticus* and *Franconibacter pulveris* clustered with the genus *Cronobacter*. Thus, based on the 16S rRNA gene sequence analysis, it is unclear whether the latter two species (formerly *Enterobacter helveticus* and *Enterobacter pulveris*, respectively) belong to the genus *Cronobacter* or not (Brady *et al.*, 2013; Stephan *et al.*, 2014). It should be noted that 16S rRNA gene sequencing has been shown to be unreliable for species differentiation of *Cronobacter*; therefore, the five-locus MLSA was considered to be more reliable (Jolley *et al.*, 2004; Jolley & Maiden, 2010; Joseph & Forsythe, 2012; Joseph *et al.*, 2012a).

This apparent contradiction was further investigated using MLSA. The five-locus MLSA tree showed that the genera *Siccibacter*, *Franconibacter* and *Cronobacter* formed discrete clusters separate from the genera *Citrobacter* and *Enterobacter* (Fig. 1). Strains 1383^T and 2249 clustered near *Siccibacter turicensis* LMG 23730^T, but on their own branch with strong bootstrap support (100 %), again suggesting that strains 1383^T and 2249 belong to a novel

species and not to *Siccibacter turicensis*. This analysis also supported the recognition of the two genera *Siccibacter* and *Franconibacter*, as proposed by Stephan *et al.* (2014), and not the inclusion of the former species *Enterobacter helveticus*, *Enterobacter turicensis* and *Enterobacter pulveris* within the genus *Cronobacter*, as proposed by Brady *et al.* (2013).

Analysis of the sequences of genes encoding ribosomal proteins (rMLST) has been proposed as a means of integrating microbial genealogy and typing (Jolley *et al.*, 2012). For rMLST analysis, 32 whole-genome sequences from members of the genera *Siccibacter*, *Cronobacter* and *Franconibacter* were analysed using the Analysis/Genome Comparator option with default settings in the *Cronobacter* PubMLST database (http://pubmlst.org/cronobacter). This tool extracts the ribosomal gene sequences from the selected genomes, and these sequences can then be used for phylogenetic analysis. The total concatenated length of the 53 loci was 22 511 bp.

Phylogenetic analysis of the rMLST sequences showed that strain 1383^T clustered with *Siccibacter turicensis* LMG 23730^T, but on a unique branch of the tree (Fig. S2). As with the other phylogenetic analyses, strong bootstrap support (100 %) indicates that strain 1383^T does not belong to the species *Siccibacter turicensis* and should be assigned to a distinct species. Additionally, the genera *Siccibacter*, *Franconibacter* and *Cronobacter* formed discrete clusters that were separate from the genera *Citrobacter* and *Enterobacter* (Fig. S2). This analysis also supported the recognition of *Siccibacter* and *Franconibacter* as discrete genera separate from *Cronobacter* (Stephan *et al.*, 2014), as also shown using the five-locus MLSA.

ANI analysis compares whole-genome sequences *in silico* and has been proposed as a replacement for DNA–DNA hybridization as a measure of the degree of relatedness between two different genomes (Konstantinidis & Tiedje, 2005; Chun & Rainey, 2014), the threshold for species differentiation being 95–96 % (Richter & Rosselló-Móra, 2009). There is, however, no accepted ANI value for genus demarcation (Kim *et al.*, 2014; Qin *et al.*, 2014). The ANI values for 11 whole-genome sequences of strains of the genera *Siccibacter*, *Cronobacter* and *Franconibacter*, including *Siccibacter turicensis* LMG 23730^T and strain 1383^T, were determined using a web-based service (http://enve-omics.ce.gatech.edu/ani/) and are given in Table S1. The ANI value between strain 1383^T and *Siccibacter turicensis* LMG 23730^T was 87.2 %, which is below the threshold for species demarcation. Although there is no accepted ANI demarcation for the genus boundary, it was notable that the ANI was 84–86 % between members of the genera *Siccibacter*, *Franconibacter* and *Cronobacter*, as shown in Table S1.

16S rRNA gene sequencing, MLSA, ANI analysis and phenotypic characterization clearly differentiated strains 1383^T and 2249 from existing species of the genera *Siccibacter*, *Cronobacter* and *Franconibacter* and indicated that these strains constitute an independent lineage within the genus *Siccibacter*. Therefore, the novel species *Siccibacter colletis* sp. nov. is proposed to accommodate these strains.

Description of *Siccibacter colletis* sp. nov.

Siccibacter colletis (col.le′tis. L. gen. n. *colletis* of a kind of vervain, referring to the isolation of the type strain).

Cells are straight, Gram-negative, non-spore-forming, motile rods, approximately 2 × 1 μm. Facultatively anaerobic. Colonies on TSA incubated at 37 °C for 24 h are 2–3 mm in diameter, opaque, circular and yellow (strain 2249) or cream-coloured (strain 1383^T). Colonies are pale yellow to yellow and glossy on TSA incubated at 25 °C. Grows on MacConkey agar, producing pink–purple colonies with large halos. In TSB, grows at 42 °C (optimum 37 °C), but not at 5 °C. Produces catalase, α-glucosidase and β-galactosidase, and is weakly positive for oxidase. Does not produce gas from D-glucose. Does not produce indole from tryptophan or hydrogen sulfide. Does not produce acetoin (Voges–Proskauer negative). Does not hydrolyse gelatin or urea. Negative for lysine decarboxylase and ornithine decarboxylase. Positive for methyl red test. Reduces nitrate. Utilizes dulcitol, aesculin, melibiose and L-rhamnose. Does not utilize malonate, melezitose, turanose, inositol, lactulose, *trans*-aconitate, putrescine, 4-aminobutyrate, maltitol or sialic acid. Produces acid from glucose, dulcitol, L-arabinose, cellobiose, lactose, L-rhamnose, D-mannitol, *N*-acetylglucosamine, salicin and 2-ketogluconate, but not from inositol, melezitose, sucrose, D- or L-fucose, adonitol, turanose, D-sorbitol or 5-ketogluconate. Does not produce acid phosphatase. Produces *N*-acetyl-β-glucosaminidase. Capable of growth in modified lauryl sulfate broth containing 1 M NaCl at 37 °C, but not at 42 °C. Resistant to doxycycline. The API 20 E and ID 32 E profiles of the type strain are 3204153 and 04077563310, respectively.

The type strain is 1383^T (=NCTC 14934^T=CECT 8567^T=LMG 28204^T), isolated from poppy seeds. The whole genome sequence of strain 1383^T has been deposited in the *Cronobacter* PubMLST database and in GenBank under accession number JMSQ00000000 (Masood *et al.*, 2014).

Emended description of *Siccibacter turicensis* Stephan *et al.* 2014

Produces gas from D-glucose. Grows on sialic acid as a sole carbon source. Produces acid phosphatase. Does not produce *N*-acetyl-β-glucosaminidase.

Acknowledgements

We are grateful to Nottingham Trent University for their financial support, M. Carmen Macián for assistance with 16S rRNA gene sequence analysis and Maria Jose Figueras for helpful criticism of the manuscript. The development of the *Cronobacter* PubMLST database (http://pubmlst.org/cronobacter) was funded by the Wellcome Trust.

References

Baldwin, A., Loughlin, M., Caubilla-Barron, J., Kucerova, E., Manning, G., Dowson, C. & Forsythe, S. (2009). Multilocus sequence typing of *Cronobacter sakazakii* and *Cronobacter malonaticus* reveals stable clonal structures with clinical significance which do not correlate with biotypes. *BMC Microbiol* **9**, 223.

Brady, C., Cleenwerck, I., Venter, S., Coutinho, T. & De Vos, P. (2013). Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA): proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii*, *E. radicincitans*, *E. oryzae* and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radicincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. *Syst Appl Microbiol* **36**, 309–319.

BSAC (2014). *BSAC Methods for Antimicrobial Susceptibility Testing*, version 13. http://bsac.org.uk/wp-content/uploads/2014/06/BSAC-disc-susceptibility-testing-method-June-2014.pdf

Caubilla-Barron, J., Hurrell, E., Townsend, S., Cheetham, P., Loc-Carrillo, C., Fayet, O., Prère, M.-F. & Forsythe, S. J. (2007). Genotypic and phenotypic analysis of *Enterobacter sakazakii* strains from an outbreak resulting in fatalities in a neonatal intensive care unit in France. *J Clin Microbiol* **45**, 3979–3985.

Chun, J. & Rainey, F. A. (2014). Integrating genomics into the taxonomy and systematics of the *Bacteria* and *Archaea*. *Int J Syst Evol Microbiol* **64**, 316–324.

FAO/WHO (2008). *Enterobacter sakazakii* (*Cronobacter* spp.) in powdered follow-up formula. *Microbiological Risk Assessment Series*, no. 15. Geneva: World Health Organization. http://www.who.int/foodsafety/publications/micro/mra_followup/en/

Iversen, C., Waddington, M., On, S. L. W. & Forsythe, S. (2004). Identification and phylogeny of *Enterobacter sakazakii* relative to *Enterobacter* and *Citrobacter* species. *J Clin Microbiol* **42**, 5368–5370.

Iversen, C., Lancashire, L., Waddington, M., Forsythe, S. & Ball, G. (2006a). Identification of *Enterobacter sakazakii* from closely related species: the use of artificial neural networks in the analysis of biochemical and 16S rDNA data. *BMC Microbiol* **6**, 28.

Iversen, C., Waddington, M., Farmer, J. J., III & Forsythe, S. J. (2006b). The biochemical differentiation of *Enterobacter sakazakii* genotypes. *BMC Microbiol* **6**, 94.

Iversen, C., Lehner, A., Mullane, N., Bidlas, E., Cleenwerck, I., Marugg, J., Fanning, S., Stephan, R. & Joosten, H. (2007). The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov. *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter* genomospecies 1. *BMC Evol Biol* **7**, 64.

Iversen, C., Mullane, N., McCardell, B., Tall, B. D., Lehner, A., Fanning, S., Stephan, R. & Joosten, H. (2008). *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter* genomospecies 1, and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *lausannensis* subsp. nov. and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. *Int J Syst Evol Microbiol* **58**, 1442–1447.

Jackson, E. E., Sonbol, H., Masood, N. & Forsythe, S. J. (2014). Genotypic and phenotypic characteristics of *Cronobacter* species, with particular attention to the newly reclassified species *Cronobacter helveticus*, *Cronobacter pulveris*, and *Cronobacter zurichensis*. *Food Microbiol* **44**, 226–235.

Jolley, K. A. & Maiden, M. C. J. (2010). BIGSdb: scalable analysis of bacterial genome variation at the population level. *BMC Bioinformatics* **11**, 595.

Jolley, K. A., Chan, M.-S. & Maiden, M. C. J. (2004). mlstdbNet – distributed multi-locus sequence typing (MLST) databases. *BMC Bioinformatics* **5**, 86.

Jolley, K. A., Bliss, C. M., Bennett, J. S., Bratcher, H. B., Brehony, C., Colles, F. M., Wimalarathna, H., Harrison, O. B., Sheppard, S. K. & other authors (2012). Ribosomal multilocus sequence typing: universal characterization of bacteria from domain to strain. *Microbiology* **158**, 1005–1015.

Joseph, S. & Forsythe, S. J. (2012). Insights into the emergent bacterial pathogen *Cronobacter* spp., generated by multilocus sequence typing and analysis. *Front Microbiol* **3**, 397.

Joseph, S., Sonbol, H., Hariri, S., Desai, P., McClelland, M. & Forsythe, S. J. (2012a). Diversity of the *Cronobacter* genus as revealed by multilocus sequence typing. *J Clin Microbiol* **50**, 3031–3039.

Joseph, S., Cetinkaya, E., Drahovska, H., Levican, A., Figueras, M. J. & Forsythe, S. J. (2012b). *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. *Int J Syst Evol Microbiol* **62**, 1277–1283.

Joseph, S., Desai, P., Ji, Y., Cummings, C. A., Shih, R., Degoricija, L., Rico, A., Brzoska, P., Hamby, S. E. & other authors (2012c). Comparative analysis of genome sequences covering the seven *Cronobacter* species. *PLoS ONE* **7**, e49455.

Joseph, S., Hariri, S. & Forsythe, S. J. (2013). Lack of continuity between *Cronobacter* biotypes and species as determined using multilocus sequence typing. *Mol Cell Probes* **27**, 137–139.

Kim, M., Oh, H.-S., Park, S. C. & Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* **64**, 346–351.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Konstantinidis, K. T. & Tiedje, J. M. (2005). Towards a genome-based taxonomy for prokaryotes. *J Bacteriol* **187**, 6258–6264.

Masood, N., Jackson, E., Moore, K., Farbos, A., Paszkiewicz, K., Dickins, B., McNally, A. & Forsythe, S. (2014). Draft genome sequence of "*Candidatus* Cronobacter colletis" NCTC 14934^{T}, a new species in the genus *Cronobacter*. *Genome Announc* **2**, e00585-14.

Qin, Q.-L., Xie, B.-B., Zhang, X.-Y., Chen, X.-L., Zhou, B.-C., Zhou, J., Oren, A. & Zhang, Y.-Z. (2014). A proposed genus boundary for the prokaryotes based on genomic insights. *J Bacteriol* **196**, 2210–2215.

Richter, M. & Rosselló-Móra, R. (2009). Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* **106**, 19126–19131.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Stephan, R., Van Trappen, S., Cleenwerck, I., Vancanneyt, M., De Vos, P. & Lehner, A. (2007). *Enterobacter turicensis* sp. nov. and

Enterobacter helveticus sp. nov., isolated from fruit powder. *Int J Syst Evol Microbiol* **57**, 820–826.

Stephan, R., Van Trappen, S., Cleenwerck, I., Iversen, C., Joosten, H., De Vos, P. & Lehner, A. (2008). *Enterobacter pulveris* sp. nov., isolated from fruit powder, infant formula and an infant formula production environment. *Int J Syst Evol Microbiol* **58**, 237–241.

Stephan, R., Grim, C. J., Gopinath, G. R., Mammel, M. K., Sathyamoorthy, V., Trach, L. H., Chase, H. R., Fanning, S. & Tall, B. D. (2014). Re-examination of the taxonomic status of *Enterobacter helveticus*, *Enterobacter pulveris* and *Enterobacter turicensis* as members of the genus *Cronobacter* and their reclassification in the genera *Franconibacter* gen. nov. and *Siccibacter* gen. nov. as *Franconibacter helveticus* comb. nov., *Franconibacter pulveris* comb. nov. and *Siccibacter turicensis* comb. nov., respectively. *Int J Syst Evol Microbiol* **64**, 3402–3410.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Townsend, S. M., Hurrell, E., Caubilla-Barron, J., Loc-Carrillo, C. & Forsythe, S. J. (2008). Characterization of an extended-spectrum beta-lactamase *Enterobacter hormaechei* nosocomial outbreak, and other *Enterobacter hormaechei* misidentified as *Cronobacter* (*Enterobacter*) *sakazakii*. *Microbiology* **154**, 3659–3667.