Systematic and Applied Microbiology 43 (2020) 126055

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: http://www.elsevier.com/locate/syapm

Serratia nevei sp. nov. and *Serratia bockelmannii* sp. nov., isolated from fresh produce in Germany and reclassification of *Serratia marcescens* subsp. *sakuensis* Ajithkumar et al. 2003 as a later heterotypic synonym of *Serratia marcescens* subsp. *marcescens*☆

Gyu-Sung Cho[a], Maria Stein[a], Erik Brinks[a], Jana Rathje[a], Woojung Lee[b], Soo Hwan Suh[b], Charles M.A.P. Franz[a,*]

[a] *Department of Microbiology and Biotechnology, Max Rubner-Institut, Federal Research Institute of Food and Nutrition, Hermann-Weigmann-Straße 1, 24103 Kiel, Germany*

[b] *Division of Microbiology, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, 187 Osongsaengmyeong 2-ro, Osong, Cheongju, Chungbuk, 28159, Republic of Korea*

ARTICLE INFO

Article history:
Received 22 July 2019
Received in revised form 3 January 2020
Accepted 13 January 2020

Keywords:
Serratia
Endospore
Genome
Multi locus sequence analysis

ABSTRACT

Fifteen enterobacterial strains were isolated from fresh produce. The 16S rRNA gene sequences indicated that these belong to *Serratia,* with twelve strains showing 99.57%–99.93% and three strains showing 99.86–100% 16S rRNA gene sequence similarity with *Serratia marcescens* and *Serratia nematodiphila* as nearest neighbors, respectively. Further comparative multi locus sequence analyses, as well as phylogenomic comparisons, revealed that 6 of the 15 strains were well-separated from their nearest neighbors and formed two clearly distinct taxa. Strains S2, S9, S10 and S15^T were urease-positive, while strains S3^T and S13 were urease-negative. Average nucleotide identity and digital DNA–DNA hybridization comparisons of representative strains S3^T and S15^T with type strains of *S. marcescens*, *S. nematodiphila* and *S. ureilytica* indicated that these shared less than 96% and 70% homology, respectively. Major fatty acids of strains S3^T and S15^T included C16:0, C16:1 ω7c/C16:1 ω6c, C17:0 Cyclo and C18:1 ω6c /C18:1 ω7c. The mol% G + C of genomic DNA of strain S15^T was 59.49% and of strain S3^T was 59.04. These results support the description of two novel species, *Serratia nevei* and *Serratia bockelmannii,* with strains S15^T (=LMG 31536^T =DSM 110085^T) and S3^T (=LMG 31535^T =DSM 110152^T) as type strains, respectively. Although *Serratia marcescens* subsp. *sakuensis* was previously described to form spores, spores could not be determined in this study. As spore formation was the only differential characteristic of this subspecies, *S. marcescens* subsp. *sakuensis* is a later heterotypic synonym of *Serratia marcescens*.

Introduction

Members of the family *Enterobacteriaceae* are Gram-negative, catalase-positive and oxidase-negative rods [8], and can be found in diverse environments such as soil, water, plants, animals and humans. Many enterobacteria are phenotypically similar and identification and classification of various strains depends on the use of genotypic methods e.g. DNA–DNA hybridization, phylogenetic analyses of housekeeping genes such as 16S rRNA, elongation factor Tu, F-ATPase β-subunit and *dnaJ* genes [7,8,22,24], or multi-locus sequence analyses (MLSA) [5,6]. Bacteria belonging to the genus *Serratia* have been isolated from plants, waters, food, nematodes, insects, animals and humans. As many *S. marcescens* strains are red-pigmented and were originally assumed to be non-pathogenic, these bacteria were used as a tracer microorganisms in medical experiments or as a biological warfare test agents [19]. *S. marcescens* is today accepted as a clinical pathogen, while other members of the genus can also occasionally cause infections. *S. marcescens* can cause urinary tract, bloodstream and wound infections, endocarditis, meningitis and pneumonia [19]. Taxonomically, *S. marcescens* has been described to be closely related to *S. nematodiphila* and the urea-dissolving species *S. ureilytica* [3,31] and two subspecies, i.e. *S. marcescens* subsp. *marcescens* and *S. marcescens* subsp. *sakuen-*

Abbreviation: ANI, average nucleotide identity; dDDH, digital DNA-DNA hybridization; MLSA, multi-locus sequence analyses.

☆ The draft whole genome sequences of *Serratia* strains for this study have been deposited into DDBJ/EMBL/GenBank under accession number VKLT00000000 for strain S11 and VOUP00000000 to VOUX00000000 for the strains S16, S14, S12, S9, S8, S6, S5 and S3.

* Corresponding author.
E-mail address: Charles.Franz@mri.bund.de (C.M.A.P. Franz).

https://doi.org/10.1016/j.syapm.2020.126055
0723-2020/

sis have been described [1]. The basis for the description of the *S. marcescens* subsp. *sakuensis* was that it showed high DNA:DNA re-association value of 97% with *S. marcescens* subsp. *marcescens*, indicating it to belong to the same species, but on the other hand it was described to produce endospores, while *S. marcescens* subsp. *marcescens* does not [1]. In this study, 15 *Serratia* strains were isolated from fresh produce obtained from the retail market in Germany and were previously studied for their antibiotic resistances (data not shown). These strains were identified using a polyphasic approach involving phenotypic, genotypic as well as genomic data.

Materials and methods

Phenotypic characterization

All isolates were tested for the production of the catalase and oxidase enzymes by removing colony material from LB agar plates (Roth, Karlsruhe, Germany) with a sterile tooth pick, and streaking onto a Rotitest-Oxidase Test strip containing the cytochrome oxidase substrate *N′,N′,N′,N′*-tetramethyl-p-phenylene diamine (Roth). The presence of the oxidase enzymes was indicated by a color change of the test strips from white to blue. The presence of catalase was tested by adding 3% H_2O_2 (v/v) (Merck, Darmstadt, Germany) to the colonies and observing for gas formation. In addition, the Gram reaction of all strains was tested using the 3% KOH (w/v) method on a glass microscope slide with a sterile toothpick [9]. The production of indole from tryptophan was tested by adding a drop of Kovac's reagent (Roth) to a culture grown in Tryptone-Soy Broth (Thermo Fisher Scientific, Dreieich, Germany) at 37 °C for 24 h, and was scored positive when the medium immediately showed a color change to pink/violet. Strains were also characterized using the API ID 32E miniaturized identification test kit according to the manufacturer's instructions (BioMérieux, Nürtingen, Germany). For this, bacteria were first sub-cultured twice in LB broth for 18 h at 37 °C. One ml of overnight culture was centrifuged at 13 000 *g* for 4 min and the optical density was determined from a 1:10 dilution in the same medium. The bacterial density was adjusted to a McFarland density of 0.5 using 0.85% NaCl and this suspension was used to inoculate the API 32E test strips. The strips were incubated according to the manufacturer's instructions.

Spore formation

A spore staining was done for *S. marces*cens subsp. *sakuensis* DSM 17174^T after growing it for 10 days in LB broth at 25 °C, using malachite green and safranin as stains for spores and cells, respectively, and with *Bacillus cereus* DSM 4312^T as a positive control [31]. Cells were investigated for presence of spores using bright field microscopy at 640× magnification after spore staining [18]. The *S. marcescens* subsp. *sakuensis* DSM 17174^T type strain was also prepared for transmission electron microscopy after 18 h growth at 37 °C. Samples were essentially prepared as described by Soerensen et al. [27], with the modification that 2% (v/v) glutaraldehyde was used for fixation and 1% (w/v) uranyl acetate for negative staining. Electron micrographs were taken using a Tecnai 10 transmission electron microscope (FEI Thermo Fisher scientific, Eindhoven, Netherlands), at an accelerating voltage of 80 kV. Digital micrographs were taken with a Megaview G2 CCD camera (Emsis, Muenster, Germany).

Fatty acid profile analysis

For cellular fatty acid analyses, the selected strains S2, S3^T, S13, S10 and S15^T were grown in LB broth (Roth) and sent to the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) for commercial identification. For cellular fatty acid analysis, 30 mg of freeze dried cells were prepared. Cellular fatty acids were analyzed after conversion into fatty acid methyl esters (FAMEs) by saponification, methylation and extraction using minor modifications of the method of Miller [21] and Kuykendall et al. [17]. The fatty acid methyl esters mixtures were separated by gas chromatography and detected by a flame ionization detector using Sherlock Microbial Identification System MIS MIDI, Microbial ID, Newark, DE 19711 USA. Peaks were automatically integrated and fatty acid names and percentages calculated by the MIS Standard Software Microbial ID.

16S rRNA gene sequencing

The total genomic DNA of all strains was isolated according to the methods of Pitcher et al. [25] after growth in LB broth for 18 h at 37 °C. The DNA was used as template to amplify the almost complete (ca. 1450 bp) 16S rRNA gene, using the primers 27 F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1540R (5′-TAC GGY YAC CTT GTT ACG ACT-3′) as described previously [10]. The PCR product was cleaned using a Peqgold cycle pure kit (Peqlab, Erlangen, Germany) and sent for sequencing at GATC Biotech (Cologne, Germany). The 16S rRNA gene sequences similarities were determined using the EzTaxon Identification Service (http://www.ezbiocloud.net/identify) [23].

Multi locus sequence analysis (MLSA) and whole genome sequencing

The genomic DNA of all *Serratia* strains was also extracted using the ZR Fungal/Bacterial DNA MiniPrep^{TM} kit (Zymo Research, Freiburg, Germany) for MLSA and for whole genome sequencing, and DNA concentration was measured with Qubit 3.0 fluorometer (Thermo Fisher Scientific). The genome sequencing library was prepared with an Illumina Nextera XT library prep kit (Illumina, San Diego, USA) and was done on an Illumina MiSeq platform (paired-end, 2 × 250 bp reads). Raw sequence data which contained adapters were trimmed using Trimmomatic (v. 0.32) [4], and *de-novo* genome assembly was performed with SPAdes 3.10.1. [2]. The quality of the obtained genome contigs was evaluated using the QUAST tool [13] and all contigs that were longer than 500 bp were used for genome annotation by the PATRIC genome annotation server [29].

In order to identify all strains, a multi locus sequencing analysis (MLSA) was first applied in this study. All housekeeping gene sequences were extracted from whole genome sequence data, and those sequences were aligned using the MegAlign tool of the DNASTAR program (v.13). All sequences were concatenated and edited to the same length as indicated in Suppl. Table 2. The sequences were clustered and then a phylogenetic tree was re-constructed using the maximum likelihood method with 1000 bootstrap resamplings and the kimura-2 parameter for nucleotide substitutions MEGA7 [28] (Fig. 2).

In order to construct a phylogenetic tree based on genome sequences, the conserved core genome sequences were extracted and concatenated by the PATRIC server. A homologous group filtering and a group alignment were performed and an estimated phylogenetic tree from concatenated alignment sequences was calculated with the outgroup type strain *Xenorhabdus nematodiphila* ATCC 19061^T, using a FastTree method with all shared proteins parameters [26].

Overall genome related indexes

The digital DNA–DNA hybridization (dDDH) values and average nucleotide identity (ANI) values were determined using the

whole genome sequence of strains from this study as well as reference genome sequences for appropriate reference strains obtained from the NCBI databank. The dDDH and Ortho ANI analyses were done using the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) genome-genome distance calculator (GGDC) version 2.1 using formula 2 (https://ggdc.dsmz.de/ggdc.php#) and the OrthoANI was also determined using a calculator with USEARCH [30]. dDDH values of >70% or ANI values >96% indicated the same species, lower values indicated a different species [30,11].

Results and discussion

Phenotypic characterization

The strains were Gram-negative, catalase-positive and oxidase-negative rods, which is typical for enterobacterial group strains. Unlike the closely-related *S. marcescens* and *S. nematodipila* (see below) strains, four of the 15 strains (S2, S9, S10 and S15) were urease-positive. Six of the 15 strains (S5, S8, S11, S12, S14 and S16) were red pigmented when they grew on LB agar plate, which is typical for *S. marcescens,* after 24 h incubation at 30 °C, while the others showed a milky white colony. Other phenotypic characteristics were determined using the API 32E miniaturized test kit (BioMerieux, Nürtingen, Germany) and are shown in Suppl. Table 1. The fatty acid profiles of five selected strains are shown in Table 1. The major fatty acids determined for these strains (S2, S10 and S15^T) were C16:0, C16:1 ω7c/C16:1 ω6c and C18:1 ω6c /C18:1 ω7c and the major fatty acid profiles were similar to those of other *Serratia* type strains [15] (Table 1). Strain S3^T and S13 showed that the major fatty acids profiles were C16:0, C16:1 ω7c/C16:1 ω6c, C18:1 ω6c /C18:1 ω7c and C17:0 Cyclo (Table 1). Cells of the strain S15^T (=DSM 110085^T), which did not form red colonies and were urease-positive, could be observed to be rod-shaped by bright light and transmission electron microscopy, respectively, possessing numerous peritrichous flagella and no endospores (Fig. 1a). Cells of the strain S3^T (=DSM 110152^T) were urease-negative, non-red pigmented and did also not produce endospores under transmission electron microscopy (Fig. 1b). This strain showed a difference in one major fatty acid when compared to strain S15^T, as it produced the fatty acid C17:0 Cyclo. Endospores could not be detected in the cells of *S. marcescens* subsp. *sakuensis* DSM 17174^T by either spore staining and bright field microscopy, or by transmission electron microscopy (Fig. 1).

Phylogenetic analyses and genome features of Serratia *spp. from fresh produce*

The 16S rRNA gene sequence analyses by EzTaxon showed that 12 of the 15 strains (S2, S3^T, S5, S6, S8, S9, S10, S13, S14, S15^T, S16 and S18) were tentatively identified as *S. marcescens,* with 99.50–99.93% sequence similarity, while 3 of the 15 strains (S11, S12 and S17) were tentatively identified as *S. nematodiphila* strains with 99.86–100.00% sequence similarity (Suppl. Table 1).

The MLSA showed that the 15 strains all grouped relatively closely with *S. marcescens* subsp. *marcescens* ATCC 13880^T, *S. marcescens* subsp. *sakuensis* DSM 17174^T, *S. nematodiphila* DSM 21420^T, and *S. ureilytica* LMG 28860^T type strains in a cluster, indicating a close relationship between the isolates and *S. marcescens, S. nematodiphila* and *S. ureilytica*. This cluster could be subdivided into 5 groups, of which group I contained the type strains of both the *S. marcescens* subspecies and five of the strains (S5, S11, S12, S14 and S16), group II contained the *S. nematodiphila* type strain DSM 21420^T and strain S8, group III consisted of the strains S6, S17, S18 and *S. ureilytica* LMG 28860^T type strain, group IV consisted of the strains S3^T and S13, while group V consisted of the strains S2, S9, S10 and S15^T (Fig. 2).

As the MLSA with four housekeeping genes showed all 15 strains to be closely related to *S. marcescens*, *S. nematodiphila* and *S. ureilytica*, the genomes of all isolates were sequenced for a phylogenomic study (Fig. 3). In addition, *in silico* digital DNA–DNA hybridization (dDDH) and ANI values were determined using the Genome-to-Genome Distance calculator version 2.1 formula 2 and using the OrthoANI a calculator with USEARCH, respectively [30,20].

Based on whole genome sequence (WGS) data, all 15 strains again clustered closely with the *S. marcescens* subsp. *marcescens*, *S. marcescens* subsp. *sakuensis*, *S. nematodiphila* and *S. ureilytica* type and reference strains (Fig. 3). All the strains again clustered within the same groups as for MLSA. Strain S8 (MLSA group V, Fig. 2), which clustered together with the *S. nematodiphila* DSM 21420^T type strain, showed 84.6% and 98.5% similarity values in dDDH and ANI comparisons, respectively (Tables 2 and 3), indicating that this strain could be identified as *S. nematodiphila*. The MLSA group IV strains S6, S17 and S18 (Fig. 2) showed 90.1, 89.8 and 91.6% similarity in dDDH, and 98.79, 98.67 and 99.02 ANI with the *S. ureilytica* Lr5/4 reference strain, respectively, indicating that these strains could be identified as *S. ureilytica* strains. The MLSA group I strains S5, S11, S12, S14 and S16 (Fig. 2) showed highest dDDH values (88.5–94.7%) and also highest ANI values (96.0–98.99%) when compared to the *S. marcescens* subspecies type strains, indicating that these could be identified as *S. marcescens* (Tables 2 and 3).

The strains S2, S9, S10 and S15^T clustered together very closely in MLSA group V (Fig. 2) and clearly separate to the clusters containing the *S. marcescens* subsp. *marcescens*, *S. marcescens* subsp. *sakuensis*, *S. nematodiphila* and *S. ureilytica* type and reference strains in the phylogenomic tree (Fig. 3). *In silico* hybridization of the genomes of these four strains with the type strains *S. marcescens* subsp. *marcescens* ATCC13880^T, *S. marcescens* subsp. *sakuensis* DSM 17174^T, *S. nematodiphila* DSM 21420^T and the reference *S. ureilytica* Lr5/4, respectively, yielded values of well less than 70 % similarity in all cases (Table 2), indicating that these strains represent a novel taxon. The ANI data showed that the four strains exhibited ANI values of less than 96% when compared to *S. marcescens* subsp. *marcescens* ATCC 13880^T, *S. marcescens* subsp. *sakuensis* DSM 17174^T, *S. nematodiphila* DSM 21420^T and *S. ureilytica* Lr5/4, again indicating that these strains form a novel taxon (Suppl. Table 4). The strains S2, S9, S10 and S15^T on the other hand, showed ANI values above 98.8% similarity, indicating the strains to belong to the same species (result not shown).

The strains S3^T and S13 of MLSA group III clustered apart and clearly separate from other strains in both the MLSA and the phylogenomic trees (Figs. 2 and 3). These strains all showed dDDH hybridization values of <70% when compared to all the *Serratia* type strains (Table 2). The highest dDDH value of S3^T strain with type strain *S. marcescens* subsp. *marcescens* was 66.8 % similarity (Table 2). Also, all ANI values of strains S3^T and S13 when compared to the type and reference strains of *Serratia* species were <96% (Suppl. Table 4), while the ANI value between strain S3^T and S13 was 98.8% similarity (result not shown). These results clearly indicated that the strains S3^T and S13 also form a novel taxon.

Our study shows that 16S rRNA gene sequence analyses are not always sufficient to accurately identify the *Serratia* strains. For an accurate identification, MLSA and phylogenomic analyses, especially when done in combination may suffice. However, the strains often group so closely in MLSA or phylogenomic analyses, that the use of dDDH and ANI values are necessary to confirm the relationships of isolates to species. Taken together, the 16S rRNA gene sequence and multi locus sequence analysis, the phylogenomic analyses, as well as physiological and biochemical tests, revealed that the four strains S2, S9, S10 and S15^T formed a taxon well-separated from its nearest neighbors and other established *Serratia* species. Thus, the representative S15^T indicates a new *Serratia* species, for which the name *Serratia nevei* sp. nov. is proposed, with

Table 1
Cellular fatty acid profiles of representative *Serratia* isolates and closely related *Serratia* type strains. Strain A, *S. fonticola* CCUG 37824^T; B, *S. liquefaciens* CCUG 9285^T; C, *S. marcescens* CCUG 1647^T; D, *S. ureilytica* CCUG 50595^T; E, *S. entomophila* CCUG 55496^T; F, *S. marcescens* subsp. *sakuensis* DSM 17174^T. All fatty acid data were obtained from the previous studies [1,15]. Values are percentage of total fatty acids.

	S2	S10	S15^T	S3^T	S13	A	B	C	D	E	F
C10:0	0.91	0.79	1.01	0.17	0.18	0	0	0	0	0	0
C10:0 3-OH	0.00	0.00	0.06	0.24	0.23	0	0	0	0	0	0
C11:0	0.06	0.05	0.07	0.00	0	0	0	0	0	0	0
C12:0	6.59	5.94	6.99	1.17	1.90	2.90	0	1.90	1.40	3.50	1.25
C12:2	0	0	0	0	0	0	0	0	0	0	2.94
C12:0 2-OH	1.08	1.11	1.02	0.58	1.12	0	0.30	0	0.40	0.60	0
C12:0 3-OH	0.14	0.12	0.14	0	0	0	0	0	0	0	0
C14:0	4.40	4.43	4.28	5.59	4.34	7.50	6.60	7.90	6.30	6.00	0
C14:0 2-OH	2.94	3.06	3.00	2.72	2.39	0	0.80	2.20	2.50	1.30	2.83
C14:0 3-OH/C16:1 Iso I	7.78	7.68	7.80	8.20	8.42	6.30	8.80	8.80	8.30	6.40	0
C16:1 ω7c/C16:1 ω6c	11.48	11.23	10.82	15.23	15.83	22.4	23.10	27.00	19.70	24.40	0
C16:1 ω5c	0.11	0.11	0.11	0	0	0	0	0	0	0	0
C16:0	30.5	30.79	30.23	31.94	31.29	24.9	32.50	31.80	30.90	29.50	33.2
C17:0 Cyclo	14.51	16.26	15.48	15.55	14.41	16.5	12.00	2.70	10.20	10.50	0
C17:0	0.21	0.19	0.24	0.59	0.63	2.20	0.40	0.00	1.10	1.70	0.66
C17:1	0	0	0	0	0	0	0	0	0	0	28.31
C16:0 3-OH	0.26	0.29	0.27	0.14	0.17	0	0	0	0	0	0.14
C18:0	0.35	0.34	0.37	0.35	0.39	0.50	1.40	0	0.60	0.60	1.93
C19:0 Cyclo ω8c	3.86	2.64	4.47	2.53	2.71	0	0.60	0	1.20	0.30	0
C19:1	0	0	0	0	0	0	0	0	0	0	19.94
C18:1 ω6c /C18:1 ω7c	11.82	13.03	11.16	14.68	15.44	5.70	12.50	15.10	13.50	11.90	0
Unknown 10.928	0	0	0	8.52	8.72	0	0	0	0	0	0
Unknown 11.799	2.00	3.62	4.45	0	0	0	0	0	0	0	0
Unknown 13.957	0.34	0.71	0.69	0	0	0	0	0	0	0	0
Unknown 14.502	0.31	0.53	0.71	0	0	0	0.60	1.10	0.70	0.70	0
C15:0	0.10	0.22	0.27	0	0	9.40	0.40	0.00	1.40	1.90	0.58

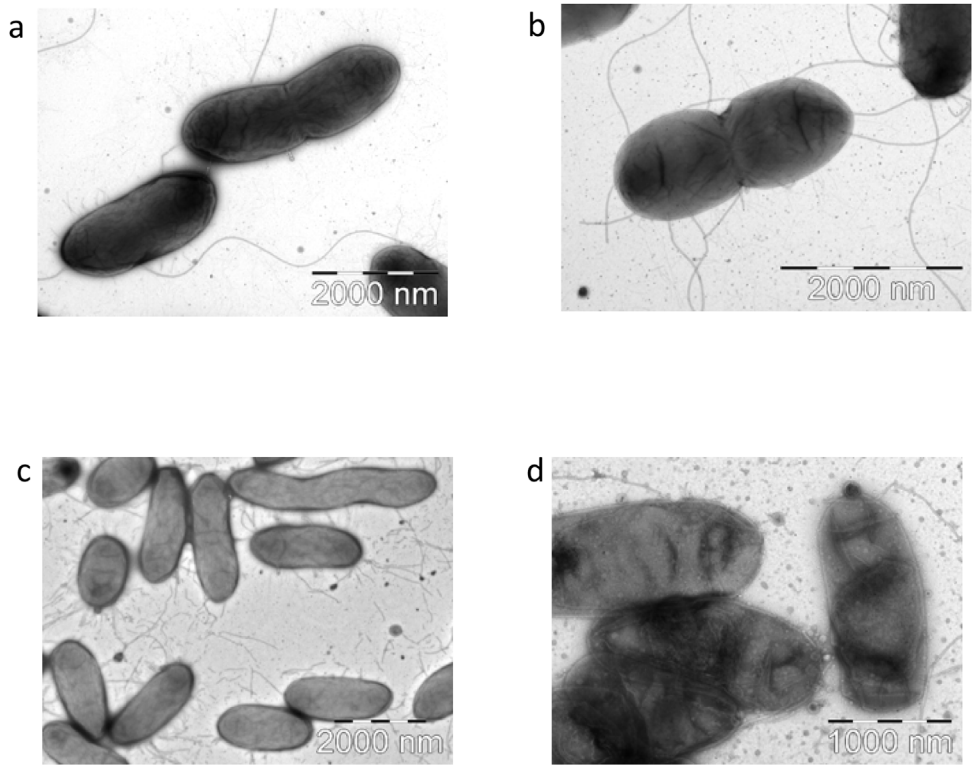

Fig. 1. Transmission electron micrographs of new type strains S3^T and S15^T after 18 h at 35 °C (a and b) showing flagella in different magnification and strain *S. marcescens* subsp. *sakuensis* DSM 17174^T after 18 h (c) and 30 d (d) incubation at 25 °C. Bars, 1 μm.

strain S15^T (=DSM 110085^T, =LMG 31536^T) as type strain. These are phenotypically different from the closely related *S. marcescens,* as they are urease-positive and do not produce a red pigment on LB medium. The novel species can also be distinguished from *S. ureilytica* as the strains are methyl red negative (Table 3). Similarly, these tests and analyses indicated that the two strains S3^T and S13 also represent a novel species, for which the name *Serratia bockelmannii* is proposed, with strain S3^T (=DSM 110152^T, = LMG 31535^T) as type strain. The strains of this novel species can be phenotypically distinguished from the closely related *S. marcescens* in that they do not produce red pigment on LB medium and from *S. ureilytica* in that they are urease-negative (Table 3).

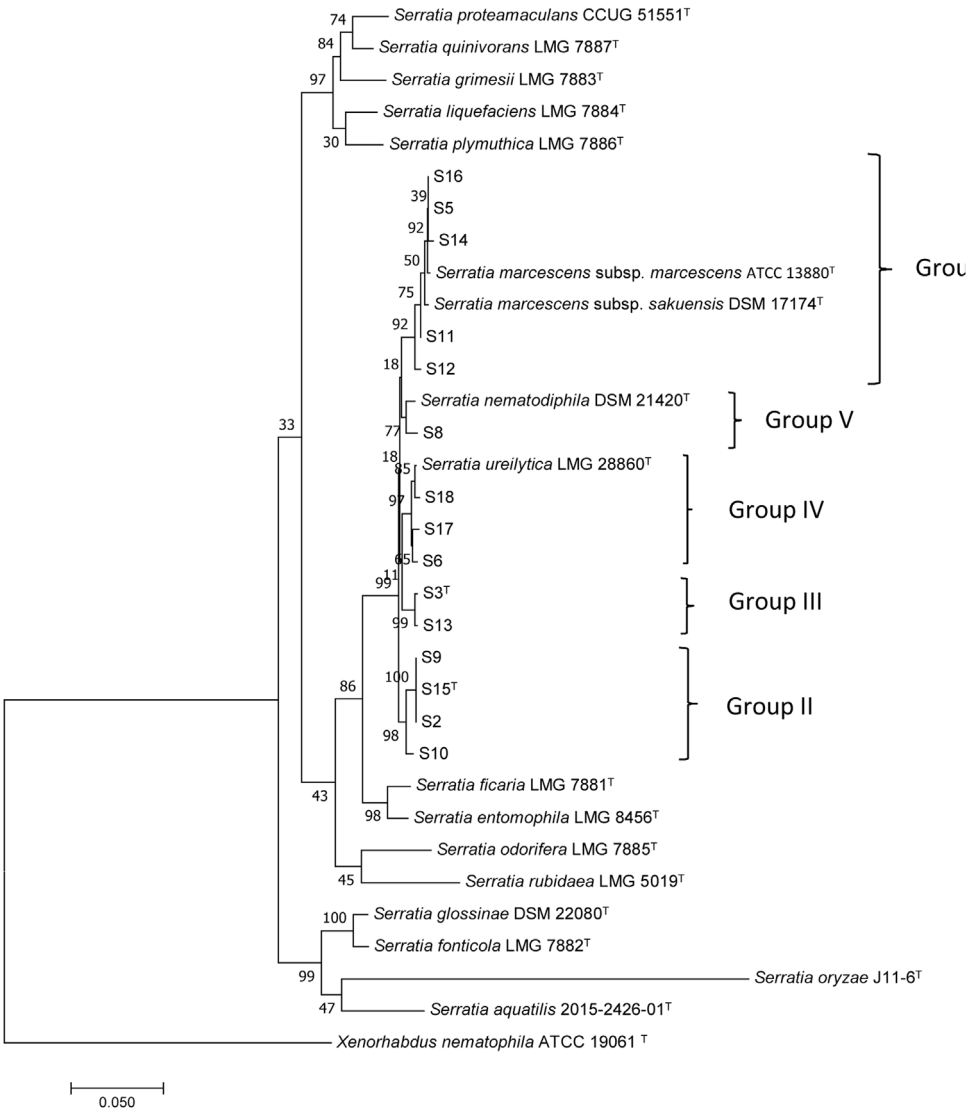

Fig. 2. Phylogenetic analysis of two novel strain groups (i.e. *S. nevei* S2, S9, S10 and S15^T and *S. bockelmannii* S3^T and S13) with closely related type and reference strains of the genus *Serratia* based on concatenated partial *atpD*, *gyrB*, *infB* and *rpoB* sequences (MLSA). *Xenorhabdus nematophila* ATCC 19061^T was used as an out-group and the phylogenetic tree was calculated using maximum-likelihood method with 1000 replications (bootstraps analysis) using Kimura-2 parameter. Bar indicated 0.05 substitutions per nucleotide position. Bar, 0.05 substitutions per nucleotide position.

Table 2
In silico DNA-DNA hybridization performed with whole genome sequence data of representative *Serratia* genomes, using Genome-to-Genome Distance calculator (GGDC) version 2.1 formula 2 [20]. The values indicated were obtained using formula 2 recommended by the GGDC.

	S2	S3^T	S5	S6	S8	S9	S10	S11	S12	S13	S14	S15^T	S16	S17	S18
*S. grimesii*_NBRC 13537^T	24.6	24.5	24.7	24.8	24.6	24.6	24.6	24.6	24.6	24.5	24.7	24.6	24.7	24.8	24.7
S. ficaria NCTC 12148^T	35	34.2	34.9	34.5	34.8	35	35.1	34.6	34.6	34.2	34.8	35	34.9	34.6	34.5
S. fonticola DSM4576^T	24.8	24.8	24.6	24.7	24.7	24.7	24.7	24.7	24.7	24.7	24.6	24.7	24.6	24.8	24.8
S. liquefaciens ATCC 27592^T	27.1	26.9	27.1	27.1	26.9	27.1	27.2	27.1	27.1	26.9	27.1	27.1	27.1	27.1	27
S. marcescens subsp. *marcescens* ATCC13880^T	62.2	66.8	94.6	62	73.4	62.2	62	88.5	88.5	66.8	94.7	62.3	94.6	62.7	62.1
S. marcescens subsp. *sakuensis* DSM 17174^T	62.6	66.5	90.8	62.9	74	62.6	62.5	92.4	92.4	66.7	90.4	62.6	90.8	63.6	63.3
S. nematodiphila DSM 21420^T	61.1	63.4	73.9	62.4	84.6	61.1	61.3	74.9	74.9	63.7	73.9	61.1	73.9	62.5	62.4
S. odorifera NCTC 11214^T	25.3	25.1	25.3	25.4	25.3	25.3	25.3	25.1	25.1	25.1	25.2	25.3	25.3	25.4	25.4
S. plymutica NCTC 12961^T	28.3	28.1	28.3	28.2	28.1	28.2	28.3	28.2	28.2	28.1	28.3	28.3	28.3	28.3	28.2
S. quinivorans NCTC 11544^T	27.2	26.9	27	27.2	27.1	27.2	27.2	27	27	27	27	27.2	27	27.1	27.2
S. rubidaea CIP 103234^T	26.6	26.4	26.5	26.6	26.5	26.6	26.6	26.5	26.5	26.3	26.5	26.6	26.5	26.6	26.5
S. symbiotica DSM 23270^T	26.8	26.7	26.8	26.7	26.8	26.5	26.8	26.6	26.6	26.8	26.6	26.6	26.8	26.7	26.7
S. ureilytica Lr5/4	57.7	62.5	62.8	90.1	62.2	57.6	58.7	62.6	62.6	63.3	62.6	57.8	62.8	89.8	916

Description of S. nevei *sp. nov*

Serratia nevei sp. nov. (ne've.i. N.L. gen. masc. n. *nevei*, of Neve, in honor of Dr. Horst Neve in recognition of his outstanding work in the area of phage biology). Cells are Gram-negative, catalase-positive, oxidase-negative and urease-positive. The strains can grow in LB medium containing 1% to 7% NaCl. The optimal growth temperature ranged from 32 °C to 35 °C, but growth was also

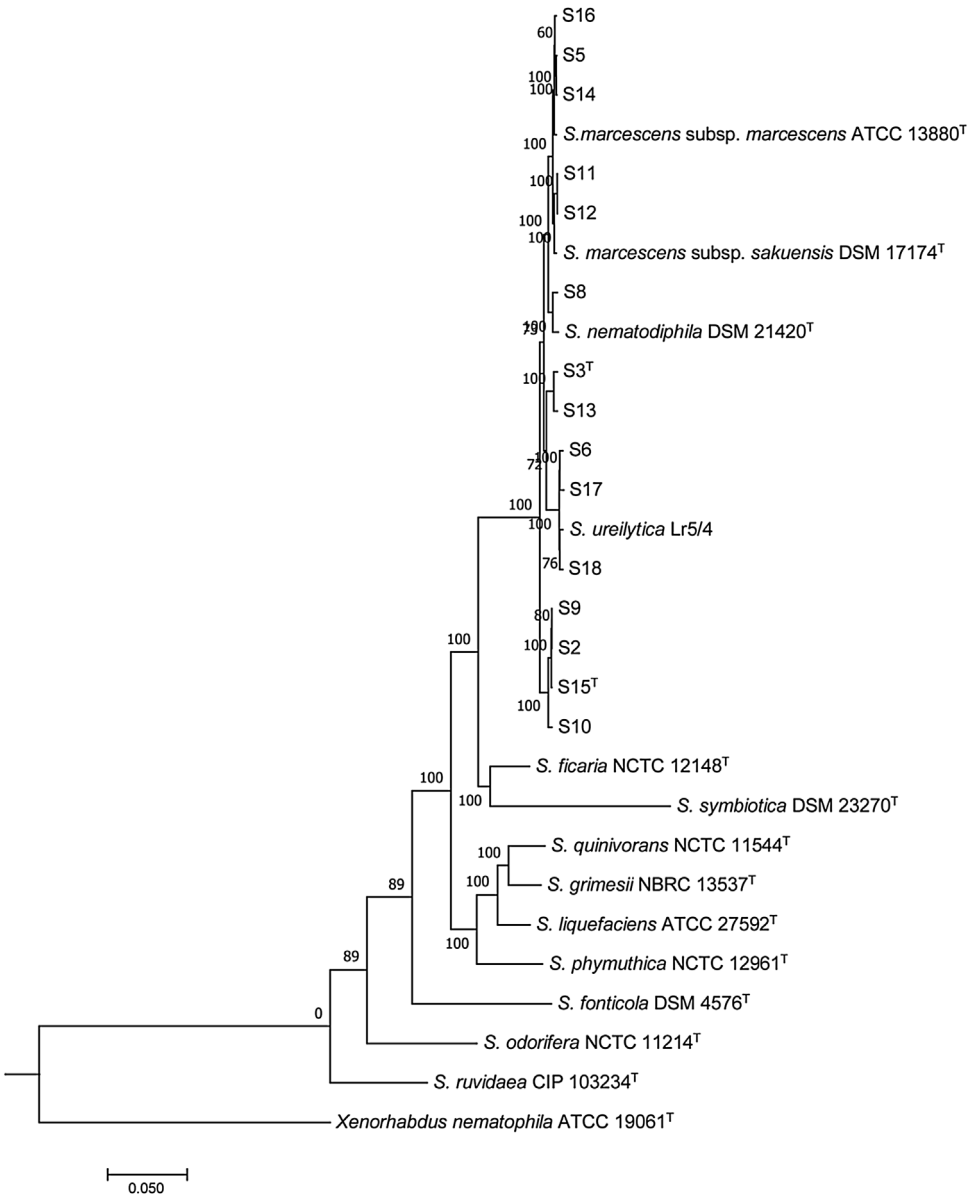

Fig. 3. Phylogenomic analysis of two novel strain groups (i.e. *S. nevei* S2, S9, S10 and S15^T and *S. bockelmannii* S3^T and S13 with closely related *Serratia* type and reference strains. A homologous group filtering and concatenated sequence alignment with the outgroup type strain *Xenorhabdus nematodiphila* ATCC 19061^T were performed using a FastTree pipeline in PATRIC server. Bar, 0.05 substitutions per nucleotide position.

Table 3
Biochemical characteristics of *Serratia* isolates and related species. +, postitive; -, negative; nd, not detected.

Characteristic	*S. marcescens* subsp. *marcescens* ATCC 13880^T	*S. marcescens* subsp. *sakuensis* DSM 17174^T	*S. ureilytica* LMG 22860^T	*S. nematodiphila* DSM 21420^T	S2	S3^T	S9	S10	S13	S15^T
Urease	–	–	+	–	+	–	+	+	–	+
Catalase	+	+	+	+	+	+	+	+	+	+
Arginine dihydrolase	–	–	+	+	+	–	+	+	+	+
Lipase	+	+	+	+	+	+	+	+	+	+
Methyl red test	–	–	+	–	–	–	–	–	–	–
Carbohydrate utilisation										
L-Arabinose	+	–	–	+	–	–	–	–	–	–
Cellobiose	–	nd	+	–	–	–	–	–	–	–
Adonitol	–	+	+	+	+	+	+	+	+	+
L-Rhamnose	–	–	–	nd	–	–	–	–	–	–
D-Sorbitol	+	+	+	nd	+	+	+	+	+	+
Sucrose	+	+	+	nd	+	+	+	+	+	+
D-Maltose	+	nd	+	nd	+	–	+	+	+	+

Table 4
Description of *Serratia nevei* sp. nov.

Species name	*Serratia nevei*
Genus name	*Serratia*
Specific epithet	*nevei*
Species status	sp. nov.
Species etymology	ne've.i., N.L. masc. n. *nevei* in honor of Dr. Horst Neve in recognition of his outstanding work in the area of phage biology
Authors	Gyu-Sung Cho, Maria Stein, Erik Brinks, Jana Rathje, Woojung Lee, Soo Hwan Suh and Charles M.A.P. Franz
Title	*Serratia nevei* sp. nov. isolated from cucumber in Germany, reclassification of *Serratia marcescens* subsp. *sakuensis* Ajithkumar et al. 2003 as a later heterotypic synonym of *Serratia marcescens* subsp. *marcescens*.
Corresponding author	Charles M.A.P. Franz
E-mail of the corresponding author	Charles.Franz@mri.bund.de
Submitter	Gyu-Sung Cho
E-mail of the submitter	Gyusung.cho@mri.bund.de
Designation of the type strain	S15
Strain collection numbers	DSM 110085 = LMG 31536
Genome accession number [RefSeq]	VTUT01000000
Genome status	Draft genome sequence
Genome size	5.25Mbp
GC mol%	59.49
Country of origin	Germany
Region of origin	Kiel
Date of isolation	02.05.2015
Source of isolation	Cucumber
Sampling date	02.05.2015
Geographic location	Kiel / Germany
Latitude	54°32′33"N
Longitude	10°13′94"E
Altitude	5
Number of strains in study	4
Source of isolation of non-type strains	Vegetable (cucumber and basil)
Growth medium, incubation conditions [temperature, pH, and further information] used for standard cultivation	Luria-Bertani medium [35 °C, pH 7.0]
Is a defined medium available	LB [Bertani, G. (1951) *J. Bacteriol.* 62(3): 293-300
Alternative medium 1	Müller-Hinton [Müller J.H. (1941) Proceedings of the Society for Experimental Biology and Medicine 48:330]
Alternative medium 2	Trypticase Soy Broth [Doyle, et al., (1968) Appl. Microbiol. 16:1742-1744]
Conditions of preservation	Liquid medium (LB 20%) mixed with 80% (v/v) glycerol and stored at −70 °C
Gram stain	Negative
Cell shape	Rod
Cell size (length or diameter)	2∼3 μm
Motility	Motile
If motile	Pili
Colony morphology	Colonies were milky, approximately 1 mm in diameter, irregular and raised with an undulate margin
Temperature optimum	35
pH optimum	7.5
pH category	Neutrophile
Salinity optimum	0.5
Salinity category	Hyposaline
Relationship to O_2	Aerobe
O_2 conditions for strain testing	Aerobiosis
Carbon source used [class of compounds]	Sugars, amino acids
Carbon source used [specific compounds]	D-glucose, Lactose, sucrose
Carbon source not used [specific compounds]	D-arabinol, L-arabinose, L-aspartate, cellobiose, malonate, palatinose, rhamnose
Carbon source variable [specific compounds]	L-arginine, L-arabinol, adonitol, inositol, D-mannitol, L-lysine, maltose, mannitol, ornithine, trehalose,
Energy metabolism	Chemoorganotroph
Oxidase	negative
Urease	positive
Catalase	Positive
Positive tests	Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase
Negative tests	Anaerobic growth with arginine or DMSO, hydrolysis of Tween 20, casein hydrolysis, Voges-Proskauer test, production of H_2S, gas formation with nitrate
Major fatty acids	$C_{16:0}$; $C_{16:1\ \omega7c}$ /$C_{16:1\ \omega6c}$; $C_{18:1\ \omega6c}$ /$C_{18:1\ \omega7c}$
Biosafety level	2
Habitat	Fresh produce
Biotic relationship	Free-living
Known pathogenicity	None

observed at 10 °C but not at 45 °C. It was tested positive for arbutine and esculine iron citrate, while negative for production of indole from tryptophane. β-galactosidase, ornithine decarboxylase, lysine decarboxylase, lipase and arginine dihydrolase are present. Acid is produced from L-arabinol, D-mannitol, adonitol, glucose, sucrose, *N*-acetyl-glucosamine, maltose, D-trehalose, inositol and sorbitol. α−galactosidase, β glucuronidase, L-aspartate-arylamidase, α-maltosidase are absent. The major cellular fatty acids produced are

Table 5
Description of *Serratia bockelmanniii* sp. nov.

Species name	*Serratia bockelmannii*
Genus name	*Serratia*
Specific epithet	*nevei*
Species status	sp. nov.
Species etymology	bo.ckel.mann'i.i. N.L. masc. n. *bockelmannii* in honor of Dr. Wilhelm Bockelmann in recognition of his outstanding work in the area of Biotechnology
Authors	Gyu-Sung Cho, Maria Stein, Erik Brinks, Jana Rathje, Woojung Lee, Soo Hwan Suh and Charles M.A.P. Franz
Title	*Serratia nevei* sp. nov. isolated from cucumber in Germany, reclassification of *Serratia marcescens* subsp. *sakuensis* Ajithkumar et al. 2003 as a later heterotypic synonym of *Serratia marcescens* subsp. *marcescens*.
Corresponding author	Charles M.A.P. Franz
E-mail of the corresponding author	Charles.Franz@mri.bund.de
Submitter	Gyu-Sung Cho
E-mail of the submitter	Gyusung.cho@mri.bund.de
Designation of the type strain	S3
Strain collection numbers	DSM 110152 = LMG 31535
Genome accession number [RefSeq]	VOUX00000000
Genome status	Draft genome sequence
Genome size	5.29Mbp
GC mol%	59.04
Country of origin	Germany
Region of origin	Kiel
Date of isolation	02.05.2015
Source of isolation	Mixed salad
Sampling date	02.05.2015
Geographic location	Kiel / Germany
Latitude	54°32′33"N
Longitude	10°13′94"E
Altitude	5
Number of strains in study	2
Source of isolation of non-type strains	Fresh produce (mixed salad and basil)
Growth medium, incubation conditions [temperature, pH, and further information] used for standard cultivation	Luria-Bertani medium [35 °C, pH 7.0]
Is a defined medium available	LB [Bertani, G. (1951) *J. Bacteriol.* 62(3): 293-300
Alternative medium 1	Müller-Hinton [Müller J.H. (1941) Proceedings of the Society for Experimental Biology and Medicine 48:330]
Alternative medium 2	Trypticase Soy Broth [Doyle, et al., (1968) Appl. Microbiol. 16:1742-1744]
Conditions of preservation	Liquid medium (LB 20%) mixed with 80% (v/v) glycerol and stored at −70 °C
Gram stain	Negative
Cell shape	Rod
Cell size (length or diameter)	2–3 μm
Motility	Motile
If motile	Pili
Colony morphology	Colonies were milky, approximately 1 mm in diameter, irregular and raised with an undulate margin
Temperature optimum	35
pH optimum	7.5
pH category	Neutrophile
Salinity optimum	0.5
Salinity category	Hyposaline
Relationship to O_2	Aerobe
O_2 conditions for strain testing	Aerobiosis
Carbon source used [class of compounds]	Sugars, amino acids
Carbon source used [specific compounds]	D-glucose, lactose, sucrose
Carbon source not used [specific compounds]	D-arabinol, L-arabinose, L-arginine, L-aspartate, cellobiose, malonate, palatinose, rhamnose,
Carbon source variable [specific compounds]	Adonitol, L-arabinol, inositol, L-lysine, maltose, D-mannitol, ornithine, trehalose
Energy metabolism	Chemoorganotroph
Oxidase	negative
Urease	negative
Catalase	Positive
Positive tests	Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase
Negative tests	Anaerobic growth with arginine or DMSO, hydrolysis of Tween 20, casein hydrolysis, Voges-Proskauer test, production of H_2S, gas formation with nitrate
Major fatty acids	$C_{16:0}$; $C_{17:0\ Cyclo}$; $C_{16:1\ \omega7c}$ /$C_{16:1\ \omega6c}$; $C_{18:1\ \omega6c}$ /$C_{18:1\ \omega7c}$
Biosafety level	2
Habitat	Fresh produce
Biotic relationship	Free-living
Known pathogenicity	None

C16:0, C16:1 ω7c/C16:1 ω6c and C18:1 ω6c /C18:1 ω7c. The overall fatty acid profile of S15^T strain is shown in Table 1. The type strain S15^T (= DSM 110085^T, =LMG 31536^T) was isolated from cucumber in Germany in 2015 and has a DNA mol% G + C content of 59.49 (Table 4).

Description of S. bockelmannii *sp. nov*

Serratia bockelmannii sp. nov. (bo.ckel.mann'i.i. N.L. gen. masc. n. *bockelmannii*, of Bockelmann, in honor of Dr. Wilhelm Bockelmann in recognition of his outstanding work in

the area of biotechnology and microbiology). Cells are Gram-negative, catalase-positive, oxidase-negative and urease-negative. The strains can grow in LB medium containing 1% to 7% NaCl. The optimal growth temperature ranged from 32 °C to 35 °C. Growth was also observed at 10 °C, but not at 45 °C. It tested positive for esculine iron citrate, while negative for arbutine and production of indole from tryptophane. β-galactosidase, ornithine decarboxylase, lysine decarboxylase and lipase present, while arginine dihydrolase is absent. Acid is produced from L-arabinol, D-mannitol, adonitol, glucose, sucrose, *N*-acetyl-glucosamine, maltose, D-trehalose, inositol 5 ketogluconate, galacturonate and sorbitol. α−galactosidase, β glucuronisase, L-aspartate-arylamidase, α-maltosidase are absent. The major cellular fatty acids produced are C16:0, C17:0 Cyclo, C16:1 ω7c/C 16:1 ω6c, C18:1 ω6c /C 18:1 ω7c and unknown 10.9. The overall fatty acid profile of S3^T strain is shown in Table 1. The strain differed from type strain S15^T as it produced the fatty acid C17:0 cyclo and because it was urease-negative. The type strain S3^T = DSM 110152^T, =LMG 31535^T) was isolated from mixed salad in Germany in 2015 and has a DNA mol % G + C content of 59.04 (Table 5).

Serratia marcescens subsp. sakuensis Ajithkumar et al. 2003 is a later heterotypic synonym of Serratia marcescens subsp. marcescens

Ajithkumar et al. [1] described *S. marcescens* subsp. *sakuensis* strain KRED^T = DSM 17174^T) subsp. nov. on the basis that this subspecies forms spores and that the DNA–DNA hybridization similarity value, determined by wet chemistry, was more than 70%. The *S. marcescens* subsp. *sakuensis* type strain DSM 17174^T was obtained from the DSMZ culture collection (Braunschweig, Germany), and the cells were investigated for spore formation by bright field and transmission electron microscopy. With both methods, endospores could not be detected. The genome of *S. marcescens* subsp. *sakuensis* was obtained from the NCBI databank and revealed only two genes that showed a similarity with a spore coat protein domain. In comparison, a *Bacillus subtilis* genome shows >50 genes associated with spore formation and maturation [16] related to spore formation. In a meeting of the Subcommittee on the taxonomy of *Enterobacteriaceae* of the International Committee on Systematics of Prokaryotes in Istanbul Turkey in 2009, the Subcommittee noted that a *Serratia* species was reported to be able to produce spores and the Subcommittee decided that this issue should be addressed to confirm or deny the observation [14]. According to the minutes of the meeting, one of the Subcommittee members had already done a preliminary investigation but failed to demonstrate the presence of spores [14], which can be confirmed by the results of our study. Recently, Diojad and Chakraborty sequenced the genome of the *S. marcescens* subsp. *sakuensis* type strain DSM 17174^T, carried out the analyses recommended to define a subspecies [12] and also concluded that DSM 17174^T does not satisfy the genomic criteria for defining a subspecies. They therefore proposed "...the removal of the *S. marcescens* subsp. *sakuensis* designation". Thus, based on our investigation regarding spore formation and the genomic analyses by Doijad and Chakraborty [12] we conclude that *S. marcescens* subsp. *sakuensis* is a later heterotypic synonym of *S. marcescens* subsp. *marcescens*.

Acknowledgements

The authors would like to thank Giovanna Felis for her taxonomical advice and Jessica Obermeyer for excellent technical assistance.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.syapm.2020.126055.

References

[1] Ajithkumar, B., Ajithkumar, V.P., Iriye, R., Doi, Y., Sakai, T. (2003) Spore-forming *Serratia marcescens* subsp. *sakuensis* subsp. nov., isolated from a domestic wastewater treatment tank. Int. J. Syst. Evol. Microbiol. 53, 253–258.
[2] Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477.
[3] Bhadra, B., Roy, P., Chakraborty, R. (2005) *Serratia ureilytica* sp. nov., a novel urea-utilizing species. Int. J. Syst. Evol. Microbiol. 55, 2155–2158.
[4] Bolger, A.M., Lohse, M., Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.
[5] Brady, C., Cleenwerck, I., Venter, S., Vancanneyt, M., Swings, J., Coutinho, T. (2008) Phylogeny and identification of Pantoea species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). Syst. Appl. Microbiol. 31, 447–460.
[6] 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.
[7] Brenner, D.J. (1978) Characterization and clinical identification of Enterobacteriaceae by DNA hybridization. Prog. Clin. Pathol. 7, 71–117.
[8] D.J. Brenner, Family I. Enterobacteriaceae Rahn 1937, Nom. Fam. Cons. Opin. 15, Jud. Comm. 1958, 73; Ewing, Farmer and Brenner 1980, 674; Judicial Commission 1981, 104. Williams & Wilkins, Baltimore MD, 1984.
[9] Buck, J.D. (1982) Nonstaining (KOH) method for determination of gram reactions of marine bacteria. Appl. Environ. Microbiol. 44, 992–993.
[10] Cho, G.S., Li, B., Rostalsky, A., Fiedler, G., Rosch, N., Igbinosa, E., Kabisch, J., Bockelmann, W., Hammer, P., Huys, G., Franz, C. (2018) Diversity and antibiotic susceptibility of Acinetobacter strains from milk powder produced in Germany. Front. Microbiol. 9, 536.
[11] Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., da Costa, M.S., Rooney, A.P., Yi, H., Xu, X.W., De Meyer, S., Trujillo, M.E. (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int. J. Syst. Evol. Microbiol. 68, 461–466.
[12] Doijad, S., Chakraborty, T. (2019) Genome-based analyses indicate that *Serratia marcescens* subsp. *marcescens* and *Serratia marcescens* subsp. *sakuensis* do not merit separation to subspecies status. Int. J. Syst. Evol. Microbiol. 69, 3924–3926.
[13] Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G. (2013) QUAST: quality assessment tool for genome assemblies. Bioinformatics 29, 1072–1075.
[14] Holmes, B., Farmer, J.J. (2009) International Committee on Systematics of Prokaryotes; Subcommittee on the taxonomy of *Enterobacteriaceae*. Int. J. Syst. Evol. Microbiol. 59, 2643–2645.
[15] Kampfer, P., Glaeser, S.P. (2016) *Serratia aquatilis* sp. nov., isolated from drinking water systems. Int. J. Syst. Evol. Microbiol. 66, 407–413.
[16] Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., Bertero, M.G., Bessieres, P., Bolotin, A., Borchert, S., Borriss, R., Boursier, L., Brans, A., Braun, M., Brignell, S.C., Bron, S., Brouillet, S., Bruschi, C.V., Caldwell, B., Capuano, V., Carter, N.M., Choi, S.K., Cordani, J.J., Connerton, I.F., Cummings, N.J., Daniel, R.A., Denziot, F., Devine, K.M., Dusterhoft, A., Ehrlich, S.D., Emmerson, P.T., Entian, K.D., Errington, J., Fabret, C., Ferrari, E., Foulger, D., Fritz, C., Fujita, M., Fujita, Y., Fuma, S., Galizzi, A., Galleron, N., Ghim, S.Y., Glaser, P., Goffeau, A., Golightly, E.J., Grandi, G., Guiseppi, G., Guy, B.J., Haga, K., Haiech, J., Harwood, C.R., Henaut, A., Hilbert, H., Holsappel, S., Hosono, S., Hullo, M.F., Itaya, M., Jones, L., Joris, B., Karamata, D., Kasahara, Y., Klaerr-Blanchard, M., Klein, C., Kobayashi, Y., Koetter, P., Koningstein, G., Krogh, S., Kumano, M., Kurita, K., Lapidus, A., Lardinois, S., Lauber, J., Lazarevic, V., Lee, S.M., Levine, A., Liu, H., Masuda, S., Mauel, C., Medigue, C., Medina, N., Mellado, R.P., Mizuno, M., Moestl, D., Nakai, S., Noback, M., Noone, D., O'Reilly, M., Ogawa, K., Ogiwara, A., Oudega, B., Park, S.H., Parro, V., Pohl, T.M., Portelle, D., Porwollik, S., Prescott, A.M., Presecan, E., Pujic, P., Purnelle, B., Rapoport, G., Rey, M., Reynolds, S., Rieger, M., Rivolta, C., Rocha, E., Roche, B., Rose, M., Sadaie, Y., Sato, T., Scanlan, E., Schleich, S., Schroeter, R., Scoffone, F., Sekiguchi, J., Sekowska, A., Seror, S.J., Serror, P., Shin, B.S., Soldo, B., Sorokin, A., Tacconi, E., Takagi, T., Takahashi, H., Takemaru, K., Takeuchi, M., Tamakoshi, A., Tanaka, T., Terpstra, P., Togoni, A., Tosato, V., Uchiyama, S., Vandebol, M., Vannier, F., Vassarotti, A., Viari, A., Wambutt, R.,

Wedler, H., Weitzenegger, T., Winters, P., Wipat, A., Yamamoto, H., Yamane, K., Yasumoto, K., Yata, K., Yoshida, K., Yoshikawa, H.F., Zumstein, E., Yoshikawa, H., Danchin, A. (1997) The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. Nature 390, 249–256.

[17] Kuykendall, L.D., Roy, M.A., Neill, J.J., Devine, T.E. (1988) Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. Int. J. Syst. Evol. Microbiol. 38, 358–361.

[18] Lechtman, M.D., Bartholomew, J.W., Phillips, A., Russo, M. (1965) Rapid methods of staining bacterial spores at room temperature. J. Bacteriol. 89, 848–854.

[19] Mahlen, S.D. (2011) Serratia infections: from military experiments to current practice. Clin. Microbiol. Rev. 24, 755–791.

[20] Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.P., Goker, M. (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinf. 14, 60.

[21] Miller, L.T. (1982) Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. J. Clin. Microbiol. 16, 584–586.

[22] Paradis, S., Boissinot, M., Paquette, N., Belanger, S.D., Martel, E.A., Boudreau, D.K., Picard, F.J., Ouellette, M., Roy, P.H., Bergeron, M.G. (2005) Phylogeny of the *Enterobacteriaceae* based on genes encoding elongation factor Tu and F-ATPase beta-subunit. Int. J. Syst. Evol. Microbiol. 55, 2013–2025.

[23] Park, K.S., Ki, C.S., Kang, C.I., Kim, Y.J., Chung, D.R., Peck, K.R., Song, J.H., Lee, N.Y. (2012) Evaluation of the GenBank, EzTaxon, and BIBI services for molecular identification of clinical blood culture isolates that were unidentifiable or misidentified by conventional methods. J. Clin. Microbiol. 50, 1792–1795.

[24] Pham, H.N., Ohkusu, K., Mishima, N., Noda, M., Monir Shah, M., Sun, X., Hayashi, M., Ezaki, T. (2007) Phylogeny and species identification of the family *Enterobacteriaceae* based on dnaJ sequences. Diagn. Microbiol. Infect. Dis. 58, 153–161.

[25] Pitcher, D.G., Saunders, N.A., Owen, R.J. (1989) Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. 8, 151–156.

[26] Price, M.N., Dehal, P.S., Arkin, A.P. (2010) FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One 5, e9490.

[27] Sørensen, M.C.H., Gencay, Y.E., Birk, T., Baldvinsson, S.B., Jäckel, C., Hammerl, J.A., Vegge, C.S., Neve, H., Brøndsted, L. (2015) Primary isolation strain determines both phage type and receptors recognised by *Campylobacter jejuni* bacteriophages. PLoS One 10, e0116287.

[28] Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

[29] Wattam, A.R., Davis, J.J., Assaf, R., Boisvert, S., Brettin, T., Bun, C., Conrad, N., Dietrich, E.M., Disz, T., Gabbard, J.L., Gerdes, S., Henry, C.S., Kenyon, R.W., Machi, D., Mao, C., Nordberg, E.K., Olsen, G.J., Murphy-Olson, D.E., Olson, R., Overbeek, R., Parrello, B., Pusch, G.D., Shukla, M., Vonstein, V., Warren, A., Xia, F., Yoo, H., Stevens, R.L. (2017) Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. Nucleic Acids Res. 45, D535–D542.

[30] Yoon, S.H., Ha, S.M., Lim, J., Kwon, S., Chun, J. (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110, 1281–1286.

[31] Zhang, C.X., Yang, S.Y., Xu, M.X., Sun, J., Liu, H., Liu, J.R., Liu, H., Kan, F., Sun, J., Lai, R., Zhang, K.Y. (2009) *Serratia nematodiphila* sp. nov., associated symbiotically with the entomopathogenic nematode *Heterorhabditidoides chongmingensis* (Rhabditida: Rhabditidae). Int. J. Syst. Evol. Microbiol. 59, 1603–1608.