Tatumella saanichensis sp. nov., isolated from a cystic fibrosis patient

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Polyphasic taxonomic analysis was performed on a clinical isolate (NML 06-3099T) from a cystic fibrosis patient, including whole-genome sequencing, proteomics, phenotypic testing, electron microscopy, chemotaxonomy and a clinical investigation. Comparative whole-genome sequence analysis and multilocus sequence analysis (MLSA) between Tatumella ptyseos ATCC 33301T and clinical isolate NML 06-3099T suggested that the clinical isolate was closely related to, but distinct from, the species T. ptyseos. By 16S rRNA gene sequencing, the clinical isolate shared 98.7 % sequence identity with T. ptyseos ATCC 33301T. A concatenate of six MLSA loci (totalling 4500 bp) revealed <93.9 % identity between T. ptyseos ATCC 33301T, other members of the genus and the clinical isolate. A whole-genome sequence comparison between NML 06-3099T and ATCC 33301T determined that the average nucleotide identity was 76.24 %. The overall DNA G+C content of NML 06-3099T was 51.27 %, consistent with members of the genus Tatumella. By matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS analysis, NML 06-3099T had a genus-level match, but not a species-level match, to T. ptyseos. By shotgun proteomics, T. ptyseos ATCC 33301T and NML 06-3099T were found to have unique proteomes. The two strains had similar morphologies and multiple fimbriae, as observed by transmission electron microscopy, but were distinguishable by phenotypic testing. Cellular fatty acids found were typical for members of the Enterobacteriaceae. NML 06-3099T was susceptible to commonly used antibiotics. Based on these data, NML 06-3099T represents a novel species in the genus Tatumella, for which the name Tatumella saanichensis sp. nov. is proposed (type strain NML 06-3099T =CCUG 55408T =DSM 19846T).

Clinical isolates of Tatumella ptyseos, a member of the family Enterobacteriaceae, are primarily obtained from patient sputa (Berkt et al., 2001; Hollis et al., 1981; Tan et al., 1989), and members of the genus Tatumella have been isolated previously from food and environmental samples (Brady et al., 2010; Garcia-Armisen et al., 2010; Ken et al., 2005; Marin-Cevada et al., 2010; Papalexandratou et al., 2011b). Phylogenetic studies using 16S rRNA gene analysis suggest that T. ptyseos is closely related to bacteria of the genus Erwinia (Drancourt et al., 2001; Spröer et al., 1999), and comparative genetic analyses have demonstrated that T. ptyseos is also related to Morganella morganii and the genus Pantoea (Paradis et al., 2005; Brady et al., 2010).

Abbreviations: ANI, average nucleotide identity; CF, cystic fibrosis; CFA, cellular fatty acid; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MLSA, multilocus sequence analysis; MSP, mass spectral profile; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene, tuf, atpD, sodA, cpr60 and rpoB sequences of strain NML 06-3099T are EU215774, EU215784, EU215775, EU215781, EU215772 and EU567306. The GenBank/EMBL/DDBJ accession numbers for the whole genome shotgun project are ATMJ00000000 (T. ptyseos ATCC 33301T) and ATM10000000 (NML 06-3099T). A supplementary figure and three supplementary tables are available with the online Supplementary Material.

† Retired.
There are few recent reports linking *T. ptyseos* to human infections, although this bacterium has been implicated in causing severe sepsis (Costa et al., 2008). Chronic lung infections in cystic fibrosis (CF) patients are polymicrobial and result in deterioration of lung tissue and eventually respiratory failure, which is the leading cause of death in CF patients (Sibley et al., 2006). Here, we describe the characterization of a clinical isolate (NML 06-3099T) from the sputum of a CF patient. We have applied a polyphasic taxonomic approach in our comprehensive analysis (Kämpfer, 2012; Sutcliffe et al., 2012; Tindall et al., 2010), including whole-genomic, phenotypic and proteomic approaches to demonstrate that isolate NML 06-3099T is closely related to, but genetically distinct from, *T. ptyseos* and other species in this genus. We propose the name *Tatumella saanichensis* sp. nov. for this novel bacterial species.

The CF patient was an adolescent male from British Columbia, Canada, who has been followed for regular sputum samples. In January 2006, an unusual Gram-negative bacterium was isolated from the patient’s sputum. The Gram-negative isolate (NML 06-3099T) was sent to the British Columbia Public Health Microbiology and Reference Laboratory in Vancouver, Canada, and was then referred to the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC) in Winnipeg, Manitoba, Canada, for identification. In this study, clinical isolate NML 06-3099T and all strains of *T. ptyseos* were grown at 25 and 35 °C on 5 % sheep blood agar (SBA) for 48 h. *T. ptyseos* ATCC 33301T was obtained from the American Type Culture Collection, and reference strains of *T. ptyseos* A8442 (=ATCC 33302), D3004 and D2432, obtained as a gift from the CDC (Atlanta, GA, USA) in 2008, were also studied. Colonial morphology of isolate NML 06-3099T was consistent with that of *T. ptyseos* ATCC 33301T and other strains of *T. ptyseos*. Colonies were smooth, slightly convex and approximately 1.5 mm in diameter after 48 h of growth on blood agar plates at 35 °C (data not shown). For the oxidase test, BBL Dryslides (BD) were used following the manufacturer’s instructions. Other conventional biochemical tests were performed as outlined previously (Hollis et al., 1981; McFadden, 2000; York et al., 2010). *T. ptyseos* ATCC 33301T and NML 06-3099T were also analysed using the Biolog Gen III microplate system, conducted at 33 °C, in order to better compare with results obtained for *Tatumella citrea*, *T. morbirosei*, *T. punctata* and *T. terrea* using the GN2 Microplate (Biolog) done at 28 °C.

Table 1. Selected phenotypic characteristics that distinguish strain NML 06-3099T from *T. ptyseos* and other species of the genus *Tatumella*

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>6</th>
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<tbody>
<tr>
<td>Motility at 25/35 °C</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Aesculin hydrolysis</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Methyl red test*</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Voges–Proskauer reaction (Coblentz)*</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Arginine dihydrolase</td>
<td>-</td>
<td>v</td>
<td>+</td>
<td>( + )</td>
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<td>-</td>
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<td>Utilization of†</td>
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<tr>
<td>Dextrin</td>
<td>BL +</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>Formic acid</td>
<td>BL +</td>
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<td>Gentioseis</td>
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<tr>
<td>Lactose</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ND</td>
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<tr>
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<td>+</td>
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<td>+</td>
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<tr>
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<td>-</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Sucrose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

*In the emended description of the genus *Tatumella*, Brady et al. (2010) stated that species in this genus were positive for both methyl red (MR) and Voges–Proskauer (VP; Coblentz method) tests. This is in contrast to the results of Hollis et al. (1981) and this study, where all strains of *T. ptyseos* were found to be MR-negative. NML 06-3099T was found to be VP-negative.

†Biolog Gen III results shown; identical results were obtained using tube sugars for lactose, maltose, mannitol, salicin, sucrose and trehalose for NML 06-3099T and *T. ptyseos* ATCC 33301T.
as described previously (Brady et al., 2010). Biochemical results for *T. ptyseos* ATCC 33301T and NML 06-3099T were compared with previously described schemes (Abbott, 2011; Brady et al., 2010). Phenotypic results for NML 06-3099T were found to be consistent with characteristics described for the genus *Tatumella* as emended by Brady et al. (2010). Features that distinguish NML 06-3099T from other members of the genus are summarized in Table 1 and in the species description.

Antimicrobial susceptibility testing was done using the broth microdilution method with Trek Sensititre Gram negative panels (Thermo Fisher Scientific) and interpreted using CLSI guidelines for the *Enterobacteriaceae* (CLSI, 2012). Cellularch fatty acids (CFAs) were extracted from cultures after growth on 5 % SBA at 35 °C in 5 % CO2 for 24 h as described previously (Bernard et al., 1991) and composition analysis was performed using the Sherlock system version 4.5 (MIDI). Predominant CFAs found for NML 06-3099T were (% of total) C12:0 (4 %), C14:0 (6 %), unidentified CFA with equivalent chain-length 14.502 (1 %), C14:0 2-OH (1 %), summed feature 2 (C14:0 3-OH and/or iso-C16:1 I; 8 %), summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH; 22 %), C16:0 (35 %), C17:0 cyclo (9 %) and C18:1ω7c (12 %). CFAs were considered as being consistent with those found for the type strain of *T. ptyseos* (data not shown). CFA data were not provided for the species described by Brady et al. (2010) and so could not be compared directly in this study.

When observed by light microscopy, NML 06-3099T was a Gram-negative rod, similar in size to *T. ptyseos* ATCC 33301T. Strains of *T. ptyseos* as originally outlined by Hollis et al. (1981) were described as being motile or weakly motile at 25 °C and observed to have polar or lateral flagella by light microscopy. In contrast, both *T. ptyseos* ATCC 33301T and NML 06-3099T were found to be non-motile at 25 and 35 °C using the tube motility method in our study; therefore, we attempted to detect flagella using transmission electron microscopy (TEM). Bacterial cultures of NML 06-3099T and *T. ptyseos* ATCC 33301T were grown at 25 and 35 °C on 5 % SBA for both 24 h and 5 days. Cells were negatively stained for TEM as described previously (Garver et al., 2007). Using TEM, both strains had a bacilliform appearance. Cells of NML 06-3099T had a mean length and width of 1396 ± 290 and 477 ± 73 nm (*n* = 157), whereas cells of *T. ptyseos* ATCC 33301T had a mean length and width of 1149 ± 260 and 490 ± 62 nm (*n* = 157). At 24 h post-inoculation, both strains were covered with sparse rod-like, fimbriab structures radiating from the cell surface. Image processing of the fimbriae was performed with the SPIDER and EMAN software, applying the iterative helical real-space reconstruction method (Frank et al., 1996; Ludtke et al., 1999; Egelman, 2000; Pomfret et al., 2007). Analysis of the fimbriae indicated that the two strains had the same overall structure, with a helix of repeating subunits 7 nm in diameter. At 24 h of growth, there was no observable difference in the cells or number of fimbriae for either sample at either incubation temperature (25 or 35 °C). Notably, at 5 days post-inoculation at 25 °C, cells of NML 06-3099T were observed to have more abundant and longer fimbriae than *T. ptyseos* ATCC 33301T at 5 days post-inoculation at 25 °C, or for both strains at 5 days post-inoculation at 35 °C or at 24 h post-inoculation at either 25 or 35 °C (Fig. S1, available in the online Supplementary Material).

In contrast to previous reports, TEM done in this study indicated that neither *T. ptyseos* ATCC 33301T nor NML 06-3099T revealed the presence of any flagellar structures, although it had been noted previously that flagella were difficult to demonstrate and that *T. ptyseos* had weak motility (Hollis et al., 1981).

Proteome analysis of isolate NML 06-3099T was conducted by both matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS and shotgun proteomics by tandem MS (LC-MS/MS). MS-grade reagents were obtained from Sigma-Aldrich, unless otherwise noted. MALDI-TOF MS is an increasingly popular technology for the identification of bacteria in clinical diagnostic laboratories (Doern, 2013; Sauer & Kliem, 2010), and methods were performed on a Bruker Daltonics Autoflex

### Table 2. Results of MALDI-TOF MS bacterial identification with Biotyper software

<table>
<thead>
<tr>
<th>Strain</th>
<th>Biotyper MSP database</th>
<th>1</th>
<th>2</th>
<th>1</th>
<th>2</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Bruker Daltonics</td>
<td></td>
<td></td>
<td>Local</td>
<td></td>
</tr>
<tr>
<td>1. <em>T. ptyseos</em> ATCC 33301T</td>
<td>2.214 ± 0.025 *</td>
<td>NA</td>
<td>NA</td>
<td>2.809 ± 0.008 *</td>
<td>1.637 ± 0.158</td>
</tr>
<tr>
<td>2. NML 06-3099T</td>
<td>1.749 ± 0.082</td>
<td>NA</td>
<td>NA</td>
<td>1.684 ± 0.085</td>
<td>2.771 ± 0.024 *</td>
</tr>
</tbody>
</table>

Cultures were analysed in triplicate and were compared to both a proprietary spectral database (Bruker Daltonics) and a locally produced mass spectral database. Score results are defined by Biotyper software (Bruker Daltonics) as follows: highly probable species identification (≥ 2.3), secure genus identification/probable species identification (≥ 2.0), probable genus identification (≥ 1.7), and not reliable identification (<1.699). Bacterial cultures identified to the species level are designated by an asterisk (*); other cultures were identified only to probable genus level or gave unreliable identification. NA, Not available in the Biotyper database.
III Smartbeam mass spectrometer as described previously (Tracz et al., 2013a), with analysis by Biotyper 3.0 software using the Bruker Daltonics database (which contained 4613 spectra at the time of writing). In addition, reference mass spectral profiles (MSPs) of isolate NML 06-3099T and T. ptyseos ATCC 33301T were constructed from triplicate extractions of pure bacterial culture, spotted eightfold, for a total of 24 individual spectra. Isolate NML 06-3099T had a primary match to T. ptyseos ATCC 33301T (= DSM 5000T) with a probable genus-level identification, while the T. ptyseos type strain culture matched ATCC 33301T at a probable species-level identification (Table 2). Previous studies have recommended using a Biotyper database supplemented with locally produced MSPs to improve MALDI-TOF MS bacterial identification (Christensen et al., 2012; Sogawa et al., 2012; Tracz et al., 2013a). Samples of NML 06-3099T had a highly probable species identification match to the locally produced MSP for NML 06-3099T (Table 2), but only matched T. ptyseos ATCC 33301T with a genus-level identification. These results suggest NML 06-3099T is distinct from T. ptyseos ATCC 33301T, based on MALDI-TOF MS.

Tandem MS (LC-MS/MS) has previously been applied as a proteomic approach to bacterial species identification (Dworzanski et al., 2004, 2006; Dworzanski & Snyder, 2005; Tracz et al., 2013b). A major advantage of LC-MS/MS is the ability to analyse the total protein content in a sample through the sequencing and matching of peptides to a protein database, allowing the identification of the protein and bacterial source, as well as a measure of taxonomic relatedness (Dworzanski & Snyder, 2005). Bacterial culture processing for shotgun proteomics with LC-MS/MS was performed as described previously by Tracz et al. (2013). Samples of NML 06-3099T were extracted of pure bacterial culture, spotted eightfold, for a total of 24 individual spectra. LC-MS/MS analysis was performed with BLAST (http://www.ncbi.nlm.nih.gov/blast/) and, wherever possible, sequences of type strains or strains held in major culture collections are available in Table S1) and, wherever possible, sequences of type strains or strains held in major culture collections were used. Searches for similar DNA sequences in GenBank (accession numbers are given in parentheses). GenBank accession numbers are given in parentheses.

Initial genetic analysis focused on a comparison of core genes in NML 06-3099T and T. ptyseos ATCC 33301T. Total cellular DNA was isolated from a loopful of bacterial culture using a DNeasy tissue kit (Qiagen). For 16S rRNA gene sequencing, PCR was performed using 16S rRNA-specific primers (Edwards et al., 1989) with a Taq DNA polymerase kit (Roche) on a GeneAmp 9700 thermocycler (Applied Biosystems). PCR was setup in 100 µl reaction volumes with the following components: dNTPs (0.2 mM each); MgSO4 (2 mM); primers (1 µM each). All PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced using the Big Dye Terminator kit version 3.1 (Applied Biosystems) on an ABI 3730 instrument (Applied Biosystems). For comparative sequence analysis, DNA sequences for T. ptyseos and other selected bacterial species were obtained from GenBank (accession numbers are available in Table S1) and, wherever possible, sequences of type strains or strains held in major culture collections were used. Searches for similar DNA sequences in GenBank were performed with BLAST (http://www.ncbi.nlm.nih.gov/blast/). Raw DNA sequences produced in this study were

**Fig. 1.** 16S rRNA gene sequence alignment done using CLUSTAL W and relationships inferred using the maximum-likelihood algorithm found in MEGA 6.06. Bootstrap values at nodes are expressed as percentages of 1000 replications. Bar, 0.005 substitutions per nucleotide position. Pantoea agglomerans NCTC 9381T and Escherichia coli ATCC 11775T were used as an outgroup. Results were essentially identical to those found using the neighbour-joining algorithm in MEGA 6.06 (not shown). GenBank accession numbers are given in parentheses.
analysed with Lasergene SeqMan software (DNASTAR). Phylogenetic and molecular evolutionary analyses, including multiple sequence alignments with CLUSTAL W and reconstruction of phylogenetic trees (maximum-likelihood or neighbour-joining methods), were conducted using MEGA 6.06. Analyses generated by the maximum-likelihood and neighbour-joining algorithms using 16S rRNA gene sequences were found to provide essentially identical results, so the maximum-likelihood algorithm was selected to illustrate relationships among species (Fig. 1). This analysis revealed that NML 06-3099T occupied a branch separate from *T. ptyseos* ATCC 33301T and other members of the genus *Tatumella* in a pairwise alignment (based on 1347 nt; GenBank accession numbers listed in Table S1). Pairwise sequence alignments (shown in Table S2) were made between NML 06-3099T and species reassigned to the genus *Tatumella* in 2010 (Brady et al., 2010), including (identity to the type strain): *T. citreus* (98.3 %), *T. morbirosei* (97.9 %), *T. punctata* (98.6 %) and *T. terreus* (98.7 %). Notably, the 16S rRNA gene sequence for NML 06-3099T has been detected in separate sources of fruit in independent studies, including cocoa beans from Ecuador (Papalexandratou et al., 2011a) and Malaysia (Papalexandratou et al., 2013), pineapples from Mexico (Marin-Cevada et al., 2010) and a garden microbiome in Panama (GenBank accession no. HM556908). 16S rRNA gene sequence results were suggestive that the clinical isolate was related to the genus *Tatumella* but distinct from *T. ptyseos*, since 98.7 % identity is on the cusp for assignment as a separate taxon group (Stackebrandt & Ebers, 2006). Multilocus sequence alignment (MLSA) of housekeeping genes has been proposed as a method to delineate species in the absence of DNA–DNA hybridization data (Kuhnert & Korczak, 2006; Martens et al., 2007), and has previously been used to validate the transfer of species of the genus *Pantoea* into the genus *Tatumella* (Brady et al., 2010). Therefore, we selected five additional genes (*atpD, cpn60, rpoB, sodA* and *tuf*) that have been used previously for taxonomic comparisons of *T. ptyseos* and other proteobacteria (Hill et al., 2004; Ken et al., 2005; Khamis et al., 2003; Kuhnert & Korczak, 2006; Paradis et al., 2005). PCR was performed with Platinum High Fidelity Taq (Invitrogen) following the manufacturer’s directions on a GeneAmp 9700 thermocycler (Applied Biosystems). The following primers were used in PCR: T1 and T2 for *tuf* (Paradis et al., 2005); A2 and A3 for *atpD* (Paradis et al., 2005); Mn-SOD 5’ and 3’ for *soda* (Ken et al., 2005); and H729 and H730 for *cpn60* (Brousseau et al., 2001). For *rpoB* PCR, *rpoB-F1* (5’-ATCCGTAACGTTAAGGGCGAAG-3’), *rpoB-F2* (5’-TCCGCAACGCTGATCCGTTC-3’), *rpoB-R3* (5’-TGACGTTGATTTGCGACC-CAT-3’) and *rpoB-R4* (5’-AACGGCAACAGCGTTGTCATAC-3’) were selected by selection of conserved sites within *rpoB* encoded by other proteobacteria (primer pairs were F1–R4 and F2–R3 to amplify an overlapping region of *rpoB*) (Brady et al., 2008). PCR conditions were: initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s and extension at 68 °C for 1 min, with a final extension at 68 °C for 5 min. PCR for *cpn60* used similar conditions, with the exception of an annealing temperature of 55 °C and a total of 40 PCR cycles. Global pairwise sequence alignments were performed with EMBoss (http://www.ebi.ac.uk/emboss/align/). Sequence analysis of these genetic loci demonstrated that NML 06-3099T was genetically distinct from *T. ptyseos* and other species in the genus, where data were available (Table S2; GenBank accession numbers in Table S1). A pairwise global alignment of a six-locus concatenate (16S rRNA gene and the five protein-encoding loci, totalling 4500 bp) was 93.9 % identical between *T. ptyseos* ATCC 33301T and isolate NML 06-3099T.

Since initial MLSA suggested that NML 06-3099T represents a distinct bacterial species, we proceeded to perform comparative whole-genome sequencing to produce a global analysis of genetic differences. Bacterial cultures of NML 06-3099T and *T. ptyseos* ATCC 33301T were grown at 35 °C for 48 h on SBA and subcultured with a single-colony pick. Total cellular genomic DNA was isolated using a QiaGen DNEasy kit (according to the manufacturer’s instructions) and quantified using a Qubit version 2.0 reader (Invitrogen). Genomic libraries were prepared using a Nextera kit (Illumina), according to the manufacturer’s instructions. A Bioanalyzer (Agilent Technologies) was used to confirm labelled genome DNA quality and concentration. Whole-genome sequencing was performed on an Illumina MiSeq (version 2), according to the manufacturer’s specifications, producing 50 × sequence coverage. Genome assemblies were generated with Velvetoptimizerversion 2.1.7 with a k-mer value of 99 (Zerbino & Birney, 2008; Zerbino, 2010). The analysed genomic sequence of NML 06-3099T consisted of 56 contigs with an assembly N50 value of 14585. The genome of NML 06-3099T is approximately 3.3 Mbp with a G+C content of 51.27 %, which was consistent with the range of 49.8 to 53 mol% (done by HPLC) described for other members of this genus (Brady et al., 2010). The DNA G+C content for the *T. ptyseos* type strain (3.5 Mbp) was found here by whole-genome sequencing to be 51.6 %.

Average nucleotide identity (ANI) scores using MUMMER (ANIm) and BLASTN (AN Ib) were calculated using jSpecies (Richter & Rosselló-Móra, 2009); these were employed here as a substitute for DNA–DNA hybridization experiments (Goris et al., 2007; Konstantinidis & Tiedje, 2007). Comparative genome analysis was performed with the following bacterial genomes: *Pantoea vagans* C9-1, *Pantoea ananatis* AJ13355, *Erwinia billingiae* Eb661, *Erwinia amylovora* ATCC 49946 and *Erwinia pyrifoliae* DSM 12163T. Goris et al. (2007) proposed that a novel species could be delineated genotypically based on a whole-genome sequence comparison yielding an ANI value which was lower than 95 % (Goris et al., 2007; Konstantinidis & Tiedje, 2007); this value is thought to be complementary to species-level delineation by the gold-standard DNA–DNA hybridization method (Goris et al., 2007; Konstantinidis & Tiedje, 2007;
Richter & Rosselló-Móra, 2009; Vandamme & Peeters, 2014). Whole-genome sequence comparison between NML 06-3099T and T. ptysos ATCC 33301T was found to give an ANIb value of 76.24 % (Table S3) over 3.3 Mbp total sequence, suggesting that a novel species designation was appropriate (Goris et al., 2007; Konstantinidis & Tiedje, 2007). NML 06-3099T had ANIb values of 71–72 % when compared with members of the closely related genera Pantoea and Erwinia, which, when considered with the genome comparison to T. ptysos ATCC 33301T, also supported a distinct species designation within the genus Tatumella. An ANIm value of 84 % was obtained using the MUMMER algorithm after a comparison of NML 06-3099T and T. ptysos ATCC 33301T, corroborating significant genetic divergence between these taxa (data not shown).

This comprehensive polyphasic taxonomic analysis determined isolate NML 06-3099T to be phenotypically and genetically distinct compared with all known bacterial species, but was most similar to T. ptysos, and is deemed to represent a novel species in the genus Tatumella. We propose the species name, *Tatumella saanichensis* sp. nov., after the Saanich region of Vancouver Island, British Columbia, Canada. The prevalence and clinical importance of this novel species of the genus *Tatumella* is currently unknown, although notably, sequence for this bacterium had been detected by 16S rRNA gene sequencing in separate studies from fruit (Marín-Cevada et al., 2010; Papalexandratou et al., 2011a, 2013). Papalexandratou et al. (2013) suggested that this species (*T. saanichensis* sp. nov.) may in fact be responsible for the degradation of citric acid in cocoa bean fermentation (Papalexandratou et al., 2013). In utilizing multiple approaches, this polyphasic study answers recent proposals for the integration of molecular and phenotypic methodology in bacterial systematics (Kämpfer, 2012; Sutcliffe et al., 2012; Tindall et al., 2010).

**Description of Tatumella saanichensis sp. nov.**

*Tatumella saanichensis* (sa.aa.ni.chen’sis. N.L. fem. adj. saanichensis pertaining to the Saanich municipality of Vancouver Island, British Columbia, Canada, the source of the type strain).

Phenotypic characteristics correspond to those found in the emended description of the genus *Tatumella* (Brady et al., 2010), except for reactions to the methyl red and Voges–Proskauer tests and utilization of arabinose, mannitol and trehalose. Gram-negative, non-spore-forming, straight rods, 1396 ± 290 nm long and 477 ± 73 nm wide by TEM. Grows on 5 % SBA, producing colonies that are 0.5–1 mm in diameter after 24 h of growth and 1.5 mm after 48 h at 25 and 35 °C. Colonies are circular, white, smooth and glossy. Non-motile at both 25 and 35 °C using tube motility medium, and flagella cannot be detected by TEM, although many fimbriae can be observed. Facultatively anaerobic, fermentative, catalase-positive, oxidase-negative and reduces nitrate to nitrite. Positive for utilization of phenylalanine, citrate (Simmons’) and sodium acetate; fermenters glucose without gas, lactose, arabinose, xylose, mannose, melibiose and sodium acetate. Negative for oxidase, indole, urease, Voges–Proskauer reaction (Coblentz method), methyl red test, H₂S production (TSI agar), lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, aesculin hydrolysis, malonate, succrose, mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, raffinose, rhamnose, maltose, trehalose, cellobiose, methyl α-glucoside, arabitol, glycerol, D-glucose 6-phosphate, glycyrl-1-proline, L-aspartic acid, L-glutamic acid, D-gluconic acid, D-citreric acid and malic acid; borderline results are observed for the utilization of dextrin, D-glucose, D-fucose, pectin, glucuronamide, mucic acid, quinic acid, α-ketoglutaric acid, bromosuccinic acid, γ-aminobutyric acid, acetocacetic acid and formic acid. The following are not utilized: maltose, trehalose, cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, melibiose, methyl β-D-glucoside, salicin, N-acetyl-D-glucosamine, N-acetyl-β-mannosamine, N-acetyl-D-galactosamine, N-acetylenuraminic acid, 3-methyl glucose, L-fucose, L-rhamnose, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, D-fructose 6-phosphate, d-ascartic acid, D-serine, L-serine, gelatin, L-alanine, L-arginine, L-histidine, L-xyloglutaric acid, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, D-saccaric acid, p-hydroxyphenylacetic acid, methylpyruvate, D-lactic acid methyl ester, L-lactic acid, D-malic acid, Tween 40, α-hydroxybutyric acid, β-hydroxy-DL-butyric acid, α-ketobutyric acid, propionic acid and acetic acid. In sensitivity tests, tetrazolium redox dye is reduced at pH 5 and 6; dye is reduced in the presence of 1 % NaCl, sodium lactate, fusidic acid, D-serine, treleandomycin, rifamycin SV, lincomycin, niaproleurin, vancomycin, tetrazolium violet, tetrazolium blue, nalidixic acid, potassium tellurite, aztreonam and sodium butyrate, not in the presence of 4 or 8 % NaCl, minocycline, guanidine hydrochloride, lithium chloride or sodium bromate. Predominant CFAs are C₁₂:0, C₁₄:0 summed feature 2 (C₁₄:0 3-oh and/or iso-C₁₅:0 1 I), summed feature 3 (C₁₆:1 Δ9c and/or iso-C₁₅:0 2-oh), C₁₆:0, C₁₇:0 cyclo and C₁₈:1ω7c. Susceptible to the following antibiotics: ampicillin, cephalothin, chloramphenicol, gentamicin, nalidixic acid, tetracycline and trimethoprim-sulfamethoxazole.

The type strain, NML 06-3099T (= DSM 19846T = CCUG 55408T), was isolated from the sputum of a CF patient. The type strain has a genome G+C content of 51.2 % based on 3.3 Mbp of total sequence.

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References


