# IJSEM Papers in Press. Published August 4, 2009 as doi:10.1099/ijs.0.012070-0

1	Transfer of Pantoea citrea, Pantoea punctata and Pantoea terrea to the genus						
2	Tatumella emend. as Tatumella citrea comb. nov., Tatumella punctata comb. nov.,						
3	and Tatumella terrea comb. nov. (Kageyama et al., 1992) and description of						
4	Tatumella morbirosei sp. nov.						
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6	Carrie L. Brady <sup>1</sup> , Stephanus N. Venter <sup>1</sup> , Ilse Cleenwerck <sup>2</sup> , Katrien						
7	Vandemeulebroecke <sup>2</sup> , Paul de Vos <sup>2</sup> and Teresa A. Coutinho <sup>1</sup>						
8							
9	<sup>1</sup> Department of Microbiology and Plant Pathology, Forestry and Agricultural						
10	Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa						
11	<sup>2</sup> BCCM/LMG Bacteria Collection, Ghent University, K.L. Ledeganckstraat 35, B-						
12	9000, Ghent, Belgium						
13							
14	Correspondence:						
15	email: <u>teresa.coutinho@fabi.up.ac.za</u>						
16	tel: +2712 420 3934 fax: +2712 420 3960						
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18	Running title: New Tatumella species						
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20	Subject category: New taxa, Proteobacteria						
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22	Footnote: The GenBank/EMBL accession numbers for the 16S rRNA gene sequences						
23	for Tatumella ptyseos LMG 7888 <sup>T</sup> , Tatumella punctata LMG 22050 <sup>T</sup> , Tatumella						
24	terrea LMG 22051 <sup>T</sup> , <i>Tatumella citrea</i> LMG 22049 <sup>T</sup> and <i>Tatumella morbirosei</i> LMG						

25 23360<sup>T</sup> and LMG 23359 are EU344770, EF688006-EF688008, EU344769 and
26 FJ617235, respectively.

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#### 28 Summary

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30 Pantoea citrea, Pantoea punctata and Pantoea terrea were described for strains 31 isolated from fruit and soil originating in Japan. These three "Japanese" species have 32 been shown to be phylogenetically distant from the remaining species of the genus 33 Pantoea. It has previously been observed using multilocus sequence analysis (MLSA) 34 that the "Japanese" species consistently form a distinct clade with an extended branch 35 length, casting doubt on the inclusion of these species within the genus *Pantoea*. 36 Furthermore, the "Japanese" species are closely related to Tatumella ptyseos, strains 37 of which originate from human clinical specimens. DNA-DNA hybridization and 38 phenotypic tests confirmed the observed phylogenetic distance of P. citrea, 39 P. punctata and P. terrea from the genus Pantoea and the affiliation of these species 40 with *Tatumella*. In addition, strains causing pink disease of pineapple, previously 41 identified as *P. citrea*, were shown to belong to a separate species by 16S rRNA gene 42 sequence analysis, MLSA and DNA-DNA hybridization data. The name *Tatumella morbirosei* sp. nov. with the type strain as LMG  $23360^{T}$  (= BD  $878^{T}$  = NCPPB  $4036^{T}$ 43 = CMC6<sup>T</sup>) is proposed for these strains. The new combinations *Tatumella citrea* 44 (Kageyama *et al.* 1992) comb. nov. with the type strain LMG  $22049^{T}$  (= BD  $875^{T}$  = 45 ATCC  $31623^{T} = SHS 2003^{T}$ ), *Tatumella punctata* (Kageyama *et al.* 1992) comb. nov. 46 with the type strain LMG  $22050^{T}$  (= BD  $876^{T}$  = ATCC  $31626^{T}$  = SHS  $2006^{T}$ ) and 47 48 Tatumella terrea (Kageyama et al. 1992) comb. nov. with the type strain LMG

49 22051<sup>T</sup> (= BD 877<sup>T</sup> = ATCC 31628<sup>T</sup> = SHS 2008<sup>T</sup>) are proposed for *P. citrea*, *P. punctata* and *P. terrea*, respectively.

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#### 52 Introduction

53 Following the proposal of the novel genus *Pantoea* in 1989 (Gavini *et al.*, 1989), but 54 preceding the transfer of Erwinia ananas and Erwinia stewartii to Pantoea (Mergaert 55 et al., 1993), three novel Pantoea species that produce 2,5-diketo-D-gluconic acid 56 (DKGA) from D-glucose were described from fruit and soil samples in Japan 57 (Kagevama et al., 1992). Pantoea citrea, Pantoea punctata and Pantoea terrea were 58 included in the genus Pantoea based on general phenotypic data and DNA-DNA 59 hybridization values, despite the inability of the original *Pantoea* species to produce 60 DKGA. Until recently, no phylogenetic study had been performed on all validly 61 published Pantoea species, giving no reason to doubt the inclusion of P. citrea, 62 P. punctata and P. terrea in the genus Pantoea. However, the most recent edition of 63 Bergey's Manual of Systematic Bacteriology (Grimont & Grimont, 2005) states that 64 more taxonomic work is required to justify the assignment of these three species to 65 the genus Pantoea. A recent phylogenetic study of the Enterobacteriaceae revealed 66 an *atpD* sequence indel which is specific to *Pantoea* and *Tatumella* (Paradis *et al.*, 67 2005), indicating a close phylogenetic relationship between these two genera. This 68 was in agreement with the initial suggestion of P. Grimont that the "Japanese" species 69 may be more similar to *Tatumella ptyseos* than *Pantoea* in their nutritional patterns 70 (Kageyama et al., 1992). The single species genus Tatumella was created for clinical 71 strains isolated in North and South America between 1960 and 1980 (Hollis et al., 72 1981). A MLSA scheme based on gyrB, rpoB, atpD, and infB genes was recently 73 performed on 102 Pantoea strains including the "Japanese" species and Tatumella

74 ptyseos (Brady et al., 2008). A concatenated tree constructed from the sequences of 75 the four genes was found to be the most robust approach for revealing phylogenetic 76 relationships amongst Pantoea strains. The MLSA study indicated a clear 77 phylogenetic divergence between P. citrea, P. punctata and P. terrea and the 78 remaining *Pantoea* species, and confirmed *Tatumella ptyseos* as a close phylogenetic 79 relative of the "Japanese" species. It was concluded that the "Japanese" species 80 should be transferred to the genus Tatumella. A similar study based on multilocus 81 typing of *Pantoea* species using six protein-coding genes, was in agreement regarding 82 the taxonomic position of the "Japanese" species and also suggested the 83 reclassification of these three species as *Tatumella* (Delétoile et al., 2008).

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#### 85 **Isolation of strains**

Strains used in this study were obtained from the BCCM/LMG Bacteria Collection
(http://www.belspo.be/bccm) and the Centres for Disease Control, Atlanta, Georgia,
U.S.A., and are listed in Table 1. An alkali extraction method (Niemann *et al.*, 1997)
was used to isolate genomic DNA from the strains which was stored at -20 °C.

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## 91 16S rRNA gene sequencing

The almost complete 16S rRNA gene was sequenced for the type strains of *P. citrea* (LMG 22049<sup>T</sup>), *P. punctata* (LMG 22050<sup>T</sup>) and *P. terrea* (LMG 22051<sup>T</sup>), and additional *P. citrea* strains (LMG 23359 and LMG 23360), which were found to cause pink disease of pineapple (Cha *et al.*, 1997), using the primers and conditions determined by Coenye *et al.* (1999). The sequences were aligned using ClustalX (Thompson *et al.*, 1997) and the overhangs were trimmed. The Modeltest 3.7 programme (Posada & Crandall, 1998) was then applied to determine the best-fit evolutionary model. Maximum likelihood and neighbour joining analyses were
performed using Phyml (Guindon & Gascuel, 2003) and PAUP 4.0b10 (Swofford,
2000) respectively, by applying the models and parameters determined by Modeltest
(only Maximum likelihood phylogenetic trees are shown). Bootstrap analysis with
1000 replicates was performed to assess the support for these clusters.

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105 In the 16S rRNA gene sequence tree (Fig. 1) the genus *Pantoea* is phylogenetically 106 split, although this is not unusual as *Pantoea* has been shown to be polyphyletic 107 (Brady et al., 2008). The majority of the Pantoea "core" species are contained in a 108 cluster supported by high bootstrap support, whilst P. dispersa and a Pantoea species 109 from Brenner DNA group IV cluster amongst Erwinia species. The "Japanese" 110 species are situated in a distinctly separate clade with a different branch point, also 111 with high bootstrap support. These findings support those of Grimont & Grimont 112 (2005), based on 16S rRNA gene and *rpoB* sequence comparisons that the "Japanese" 113 species cluster at a lower level to the *Pantoea* "core" clade. Interestingly, LMG 7888<sup>T</sup> 114 the type strain of *Tatumella ptyseos* clusters closely with the type strain of *Pantoea* 115 terrea, within the "Japanese" species clade prompting further examination of the 116 relationship of the "Japanese" species with T. ptyseos. The additional P. citrea strains 117 LMG 23359 and LMG 23360, which cause pink disease of pineapple, do not cluster 118 with the type strain of this species (LMG 22049<sup>T</sup>), but are found on a separate branch 119 suggesting these strains do not belong to P. citrea. The 16S rRNA gene sequence 120 similarity of LMG 23360 was greater than 98 % to the type strains of *P. punctata*, 121 *P. citrea*, *Pantoea* sp. (Brenner DNA group II) and *T. ptyseos*.

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#### 124 gyrB, rpoB, atpD, and infB gene sequencing

125 MLSA was performed on all strains, as previously described (Brady et al., 2008), to 126 provide further support for the removal of the "Japanese" species from Pantoea. 127 Sequence analysis and tree construction was performed as described above. Several 128 *Erwinia* species were included in the MLSA study and housekeeping gene sequences 129 for Erwinia tasmaniensis, Escherichia coli, Shigella dysenteriae, Klebsiella 130 pneumoniae, Citrobacter rodentium, Citrobacter koseri and Cronobacter sakazakii 131 were obtained from genome sequencing databases (http://www.ncbi.nlm.nih.gov, 132 http://www.sanger.ac.uk, http://asap.ahabs.wisc.edu/asap). In Fig. 2, a MSLA 133 phylogenetic tree based on the concatenated sequences of gyrB, rpoB, atpD and infB 134 genes, Pantoea strains form a monophyletic cluster within the Enterobacteriaceae. 135 This cluster contains two sub lineages which are supported by high bootstrap values 136 of 100 %. The first sub lineage contains the Pantoea "core" species including 137 Brenner DNA groups II, IV and V. The second sub lineage consists of the "Japanese" 138 species and the type strain of T. ptyseos. The type strain of T. ptyseos clusters closely 139 with P. terrea strains, specifically with LMG 23565 and its duplicate CCUG 30163. 140 This result indicates that LMG 23565 (= CCUG 30163) was erroneously classified 141 and in fact belongs to T. ptyseos. LMG 23359 and LMG 23360, two supposed 142 P. citrea strains causing pink disease of pineapple referred to as MLSA group J 143 (Brady et al., 2008), group with LMG 22049<sup>T</sup>, the type strain of *P. citrea* but are 144 situated on a separate branch with a long branch length. This supports the probability 145 that these two strains do not belong to *P. citrea*, but to a novel species. All species 146 clusters within both sub lineages of *Pantoea* are supported with 100 % bootstrap 147 values. All four housekeeping- and 16S rRNA-gene sequences were examined for 148 heterogenous nucleotides which could be used as signature nucleotide positions to

149 differentiate between Tatumella and Pantoea. Eight signature nucleotides were 150 identified in the 16S rRNA gene sequences which differ between Tatumella and 151 Pantoea. The gene sequences of atpD revealed the most heterogeneity with 23 152 signature nucleotides which are conserved in *Tatumella* species and differ from all 153 Pantoea species. The signature nucleotides for the 16S rRNA- and atpD-genes are 154 listed in Supplementary Table S1, available on IJSEM online. The E. coli numbering 155 positions are used to designate the nucleotide positions (Brosius et al., 1978, 156 http://www.ncbi.nlm.nih.gov).

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# 158 **DNA-DNA hybridizations**

159 High quality DNA for DNA-DNA hybridization of strains was prepared by the 160 method of Wilson (1987), with minor modifications (Cleenwerck et al., 2002). DNA-161 DNA hybridizations were performed using the microplate method (Ezaki et al., 1989) 162 with some modifications (Cleenwerck et al., 2002). The hybridization temperature 163 was 45 °C  $\pm$  1 °C, reciprocal reactions (A x B and B x A) were performed for every 164 DNA pair from all strains and their variation was within the limits of this method 165 (Goris et al., 1998). The values presented are based on a minimum of four replicates. 166 Representative strains from each "Japanese" Pantoea species were selected for 167 hybridization based on the 16S rRNA gene sequence- and MLSA- phylogenetic trees. 168 The type strains of the "Japanese" species were hybridized amongst each other, and 169 with the type strain of *Tatumella ptyseos*, their closest phylogenetic neighbour, as well 170 as with the type strains of P. agglomerans, P. ananatis, P. vagans, P. stewartii ssp. 171 stewartii and P. dispersa. A summary of the hybridization results is presented in Table 2. The DNA relatedness between the type strains of *P. citrea* LMG 22049<sup>T</sup>, 172 *P. punctata* LMG 22050<sup>T</sup> and *P. terrea* LMG 22051<sup>T</sup> ranges from 13 to 21 %, which 173

is considerably lower than the 28-43 % reported by Kageyama *et al.* (1992) and could
be due to different hybridization methods used. LMG 23359 and LMG 23360, the
strains which can cause pink disease of pineapple (Cha *et al.*, 1997) shared 96 %
DNA relatedness when hybridized against each other, whilst LMG 23360 displayed
only 42 % DNA similarity with the type strain of *P. citrea* LMG 22049<sup>T</sup>. These
results together with the 16S rRNA gene sequence- and MLSA-data prove that LMG
23360 and LMG 23359 do not belong to *P. citrea*.

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Pantoea citrea LMG 22049<sup>T</sup> and P. punctata LMG 22050<sup>T</sup> displayed 14 and 21 % 182 183 DNA relatedness when hybridized to *T. ptyseos* LMG 7888<sup>T</sup>, respectively. In contrast, 184 the T. ptyseos type strain shared 66 % DNA relatedness with P. terrea LMG 22051<sup>T</sup>, 185 and 87 % with LMG 23565, originally considered to be P. terrea. LMG 23565 186 demonstrated only 55 % DNA similarity, when hybridized to *P. terrea* LMG 22051<sup>T</sup>. 187 These results are in favour of the reclassification of LMG 23565 (= CCUG 30163) as 188 T. ptyseos and confirm the close phylogenetic relationship between P. terrea and 189 T. ptyseos, observed in both the 16S rRNA gene sequence- and MLSA- phylogenetic 190 trees (Figs. 1 & 2). The DNA relatedness between the type strains of the "Japanese" 191 Pantoea species and the type strains of P. agglomerans, P. ananatis, P. vagans, 192 P. stewartii and P. dispersa was below 10 %. These low hybridization values are 193 also reflected in the phylogenetic distance between the "Japanese" species and the 194 Pantoea "core" species in both Figures 1 and 2.

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**196 G** + **C content** 

197 The mole % G + C content of the type strains, determined by HPLC (Mesbah et al.,

198 1989), are as follows: *Pantoea citrea* (LMG  $22049^{T}$ ) = 49.8 mol %; MLSA group J

199 (LMG  $23360^{T}$ ) = 50.2 mol %; *Pantoea punctata* (LMG  $22050^{T}$ , LMG 22098) = 50.7

200 mol %, Pantoea terrea (LMG 22051<sup>T</sup>, LMG 23564) = 52.6-52.8 mol % and

201 *Tatumella ptyseos* (LMG 7888T, LMG 23565) = 51.7-52.1 mol %.

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## 203 **Phenotypic assays**

204 Differences between the Pantoea "core" species and the "Japanese" species have been 205 previously noted, not only phylogenetically but also phenotypically (Grimont & 206 Grimont, 2005, Delétoile et al., 2008). API 20 E, API 50 CHB/E, Biotype 100 207 (bioMérieux) and GN2 MicroPlate (Biolog) tests were performed on all Tatumella 208 and *Pantoea* strains used in this study, to verify that the phenotypic data agrees with 209 those available in literature. Cell suspensions were prepared from strains grown on 210 tryptic soy agar for 12 hours. All phenotypic test strips and plates were incubated for 211 24-48 hours at 28 °C, except the Biotype 100 strips which were incubated for up to 212 six days. Scoring was performed according to the manufacturer's instructions. 213 Phenylalanine deaminase activity was determined by adding 10 % aqueous FeCl<sub>3</sub> to 214 24 hour cultures grown on (D)-phenylalanine agar (Merck). The development of a 215 green colour indicated a positive reaction. A high correlation was observed between 216 the phenotypic results generated in this study, the phenotypic data presented in 217 Bergey's Manual of Systematic Bacteriology (Grimont & Grimont, 2005) and the 218 original species descriptions of P. citrea, P. punctata, P. terrea (Kageyama et al., 219 1992) and Tatumella ptyseos (Hollis et al., 1981). T. ptyseos and the "Japanese" 220 species can be distinguished from the genus Pantoea by a positive reaction for 221 arginine dihydrolase (except P. terrea and T. ptyseos) and 2-ketogluconate 222 dehydrogenase, their inability to produce acid from arbutin, myo-inositol and L-223 rhamnose and their inability to utilize D-galacturonic acid, D-glucoronic acid, myoinositol,  $\beta$ -methyl-D-glucoside, L-rhamnose, D-sacccharic acid and *meso*-tartrate (except *T. ptyseos*). A summary of the most useful phenotypic and biochemical characteristics for differentiating *T. ptyseos* and the "Japanese" species from *Pantoea* species is presented in Table 3. Table 3 was assembled using data generated in this study (except for DKGA), of which the majority agrees with that previously published.

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231 A summary of biochemical and phenotypic characteristics which can differentiate 232 between T. ptyseos, the "Japanese" species and the pink disease-causing strains LMG 233 23360 and LMG 23359 is listed in Table 4. The strains causing pink disease of 234 pineapple can be differentiated from *P. citrea* by a positive phenylalanine deaminase 235 reaction, acid production from amidon, their ability to utilise adonitol and trigonelline, 236 and their inability to utilize gentiobiose and lactulose. P. terrea can be differentiated 237 from T. ptyseos, its closest phylogenetic neighbour, by negative phenylalanine 238 deaminase reaction, the ability to utilize formic acid and pyruvic acid methyl ester, 239 and the inability to utilize quinic acid and L-tartrate.

240

A selection of 50 phenotypic characteristics was used to construct a UPGMA dendrogram (Fig. 3) in PAUP 4.0b10 (Swofford, 2000). The dendrogram shows a clear distinction of *T. ptyseos* and the "Japanese" species from the *Pantoea* "core" species, lending further support for the re-classification of these three species in the genus *Tatumella*, and the description of a novel species for the strains causing pink disease of pineapple.

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#### 249 **Conclusions**

The many previous re-arrangements seen in the "*Erwinia herbicola-Enterobacter agglomerans* complex" were, for the most part, based on DNA-DNA hybridization data, biochemical characterization and protein-profiling. When *P. citrea*, *P. punctata* and *P. terrea* were described as belonging to the genus *Pantoea*, molecular techniques such as 16S rRNA gene sequencing and MLSA were not readily accessible. With rapid gene sequencing now a reality, it is possible to observe true phylogentic relationships and to resolve evident taxonomic issues.

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258 The 16S rRNA gene- and MLSA-trees show a clear division of the Pantoea "core" 259 species from the "Japanese" species and T. ptyseos. Furthermore, MLSA group J 260 (strains causing pink disease of pineapple) was shown to cluster independently from 261 the type strain of *P. citrea* in both phylogenetic trees. These results were supported 262 equally by DNA-DNA hybridization values, which share a high correlation with 263 MLSA data (Brady et al., 2008), and phenotypic data. Although the genus Tatumella 264 is reported to have few distinguishing phenotypic properties (Farmer, 2005), a number 265 of biochemical characteristics were identified which are shared by the "Japanese" 266 species and which can be used to differentiate these species from *Pantoea* (see Table 267 3).

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An important characteristic distinguishing *P. citrea*, *P. punctata*, *P. terrea*, *T. ptyseos* and MLSA group J from *Pantoea* species is their ability to produce 2-ketogluconate dehydrogenase which oxidizes 2-ketogluconate to DKGA. The production of DKGA is responsible for the discolouration of fruit tissue typical of pink disease of pineapple (Pujol & Kado, 2000), and therefore plays an important role in disease expression.

274 Noticeably, not one *Pantoea* "core" species has this ability (Bouvet et al., 1989). 275 Several objections for not including the "Japanese" species in the genus Tatumella 276 were stated in the original description, despite a personal communication from P. 277 Grimont suggesting that the "Japanese" species were phenotypically similar to 278 Tatumella (cited by Kageyama et al., 1992). These included acid production from D-279 xylose and L-arabinose, arginine dihydrolase and phenylalanine deaminase activity, 280 methyl red and Voges-Proskauer reaction, esculin hydrolase activity, citrate utilization 281 and lower G + C content. However, closer examination of all available data revealed 282 that all taxa are positive for acid production from D-xylose and L-arabinose, methyl 283 red and Voges-Proskauer reactions and citrate utilization. T. ptyseos and P. terrea are 284 both negative for arginine dihydrolase activity, whilst P. citrea and P. punctata are 285 both positive. The G + C content of *T. ptyseos* is 53-54 mol % which is slightly higher 286 compared to the "Japanese" species which ranges from 49.7 to 51.9 mol %. However, 287 the G + C contents of Pantoea "core" species range from 53 to 61 mol %, which is 288 considerably higher. Therefore the current data, both phylogenetic and phenotypic, 289 supports the transfer of the "Japanese" species from the genus Pantoea to the genus 290 Tatumella.

291

We propose to transfer *P. citrea*, *P. punctata* and *P. terrea* to the genus *Tatumella* as *Tatumella citrea* comb. nov., *Tatumella punctata* comb. nov. and *Tatumella terrea* comb. nov. We further propose *Tatumella morbirosei* sp. nov. for strains LMG 23359 and LMG 23360, the causal agent of pink disease of pineapple.

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# 299 Emended description of the genus *Tatumella* Hollis, Hickman & Fanning, **1982**

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301 (Ta.tum.el'la. M.L. dim. Neut. *–ella* ending; M.L. fem. n. *Tatumella* named to honour
302 Harvey Tatum, an American bacteriologist who made many contributions to our
303 understanding of the classification and identification of fermentative and
304 nonfermentative bacteria of medical importance.) The description below is based on
305 the data of Hollis *et al.* (1981), Kageyama *et al.* (1992) and this paper.

306 Gram-negative, non-capsulated, non-sporeforming small rods that are 0.6-1.2 x 0.9-307 3.0 µm in size. Cells are motile by means of polar, subpolar or lateral flagella or non-308 moltile at 36 °C. Facultatively anaerobic, fermentative, catalase positive (weak and 309 slow), oxidase negative. Non-pigmented, or pale beige to pale orange. Glucose 310 dehydrogenase, gluconate dehydrogenase and 2-ketogluconate dehydrogenase are 311 produced. Reduce nitrate to nitrite. Indole, urease and gelatin tests are negative. 312 Positive for Voges-Proskauer (Coblentz), methyl red and citrate (Simmons); 313 phenylalanine, arginine dihydrolase and ONPG tests are variable. Negative for H<sub>2</sub>S 314 (TSI), lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, KCN 315 test, lipase and DNase. Acid is produced from L-arabinose, D-galactose, D-glucose, 316 glycerol, D-mannitol, D-mannose, melibiose, D-ribose, D-trehalose and D-xylose 317 (variable reaction for *T. morbirosei*), but not from amygdalin, dulcitol, erythritol, 318 glutarate, glycogen, histamine, myo-inositol, α-methyl-D-glucoside, propionate, L-319 rhamnose, sorbitol and L-sorbose. N-acetyl-D-glucosamine, 4-aminobutyrate, L-320 aspargine, L-aspartic acid, bromosuccinic acid, D-fructose, fumarate,  $\beta$ -321 galactoppyranoside, D-gluconic acid,  $\alpha$ -D-glucose-1-phosphate,  $\alpha$ -D-glucose-6-322 phosphate, L-glutamic acid, glycerol, inosine, 5-ketogluconate, D-mannose, L-323 proline, D-psicose, D-ribose, succinic acid, D-trehalose, thymidine and uridine are

324	utilized as sole sources of carbon and energy. Susceptible to many antibiotics.
325	Isolated from human clinical samples, fruit and soil. The G + C content of the DNA
326	ranges from 49.8 to 53 mol %. The type species is Tatumella ptyseos Hollis,
327	Hickman & Fanning, 1982.
328	
329	Description of Tatumella citrea (Kageyama, Nakae, Yagi & Sonoyama 1992)
330	comb. nov.
331	Tatumella citrea (ci'tre.a. M.L. adj. citrea, of citrus)
332	
333	Cells are Gram-negative, short rods (0.8-1.2 x 1.0-3.0 $\mu$ m) occurring singly or in
334	pairs, non-motile and non-sporeforming. Colonies are pale beige to pale orange,
335	round, convex and smooth with entire margins. Nicotinic acid or nicotinamide are
336	required for growth. Facultatively-anaerobic, oxidase negative, catalase positive,
337	glucose dehydrogenase, gluconate dehydrogenase and 2-ketogluconate dehydrogenase
338	are produced. Indole, urease and phenylalanine deaminase are negative, whilst
339	arginine dihydrolase is positive. Reduce nitrate to nitrite. Acid is produced from: L-
340	arabinose, D-fucose, D-galactose, D-glucose, glycerol, maltose, D-mannose, D-
341	mannitol, D-tagatose and D-trehalose, but not from amidon, D-raffinose or sucrose.
342	The following carbon sources are utilized at 28 °C: N-acetyl-D-glucosamine, cis-
343	aconotic acid, L-arabinose, L-aspargine, L-aspartic acid, citrate, dextrin, erythritol,
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formic acid, D-fructose, D-galactose, gentiobiose (weak), D-gluconic acid, D-glucose,
5-ketogluconate, lactose, lactulose, D-malate, D-maltose, maltotriose, D-mannitol,
pyruvic acid methyl ester, succinic acid and D-tagatose. The following carbon sources
are not utilized at 28 °C: D-adonitol, L-alanine, D-arabinose, betaine, dulcitol, L-

348 fucose,  $\alpha$ -methyl-D-glucoside,  $\beta$ -methyl-D-glucoside, D-galacturonic acid, D-

349 glucoronic acid, glutarate, histdine, *myo*-inositol, D-melibiose, L-proline, propionate, 350 quinic acid, D-raffinose, L-rhamnose, D-sorbitol, L-serine, sucrose, L-tartrate, *meso*-351 tartrate, and trigonelline. The G + C content of the type strain is 49.8 mol %. The 352 type strain is LMG 22049<sup>T</sup> (= BD  $875^{T}$  = ATCC  $31623^{T}$  = SHS 2003<sup>T</sup>) and was 353 isolated from mandarin orange in Japan.

354

355 Description of *Tatumella punctata* (Kageyama, Nakae, Yagi & Sonoyama 1992)
356 comb. nov.

*Tatumella punctata* (punc.ta'ta. L. N. *punctum*, a point; M.L. adj. *punctata*, full of
points)

359

360 Cells are Gram-negative, short rods (1.1-1.2 x 1.3-2.3 µm) occurring singly or in 361 pairs, non-motile and non-sporeforming. Colonies are pale beige to pale orange, 362 round, convex and smooth with entire margins. Nicotinic acid or nicotinamide are 363 required for growth. Facultatively-anaerobic, oxidase negative, catalase positive, 364 glucose dehydrogenase, gluconate dehydrogenase and 2-ketogluconate dehydrogenase 365 are produced. Indole, urease and phenylalanine deaminase are negative, whilst 366 arginine dihydrolase is positive. Reduce nitrate to nitrite. Acid is produced from: L-367 arabinose, D-fructose, D-fucose, D-galactose, D-glucose, glycerol, D-mannose, D-368 raffinose, sucrose and D-trehalose, but not from amidon or D-mannitol. The following 369 carbon sources are utilized at 28 °C: N-acetyl-D-glucosamine, D-arabinose, L-370 aspargine, L-aspartic acid, cellobiose (variable), citrate, D-fructose, D-galactose, 371 gentiobiose, D-gluconic acid, D-glucose, 5-ketogluconate, D-mannitol, D-mannose, 372 succinic acid, sucrose and D-trehalose. The following carbon sources are not utilized at 28 °C within three to six days: cis-aconotic acid, D-adonitol, L-alanine, L-373

374 arabinose, D-arabitol, L-arabitol, betaine, dextrin, dulcitol, erythritol, formic acid, L-375 fucose, D-galacturonic acid, D-glucoronic acid,  $\alpha$ -methyl-D-glucoside,  $\beta$ -methyl-D-376 glucoside, glutarate, histidine, myo-inositol, lactose, lactulose, D-maltose, maltotriose, 377 D-melibiose, L-proline, propionate, pyruvic acid methyl ester, quinic acid, D-378 raffinose, L-rhamnose, D-sorbitol, L-serine, L-tartrate, meso-tartrate and trigonelline. The G + C content of the type strain is 50.7 mol %. The type strain is LMG  $22050^{T}$ 379 (= BD  $876^{T} =$  ATCC  $31626^{T} =$  SHS  $2006^{T}$ ) and was isolated from mandarin orange in 380 381 Japan.

382

# 383 Description of *Tatumella terrea* (Kageyama, Nakae, Yagi & Sonoyama 1992) 384 comb. nov.

385 Tatumella terrea (ter're.a. L. n. terra, soil; L. adj. terrea, of soil)

386

387 Cells are Gram-negative, short rods (0.8-0.9 x 1.2-2.0 µm) occurring singly or in 388 pairs, motile by means of one or two lateral flagella and non-sporeforming. Colonies 389 are pale beige to pale orange, round, convex and smooth with entire margins. 390 Nicotinic acid or nicotinamide are required for growth. Facultatively-anaerobic, 391 oxidase negative, catalase positive, glucose dehydrogenase, gluconate dehydrogenase 392 and 2-ketogluconate dehydrogenase are produced. Indole, urease, phenylalanine 393 deaminase and arginine dihydrolase are negative. Reduce nitrate to nitrite. Acid is 394 produced from: L-arabinose, D-fructose, D-galactose, D-glucose, glycerol, D-395 mannose, sucrose and D-trehalose, but not from amidon, D-mannitol or D-raffinose. 396 The following carbon sources are utilized at 28 °C: N-acetyl-D-glucosamine, L-397 aspargine, L-aspartic acid, citrate, D-fructose, formic acid, D-galactose, D-gluconic 398 acid, D-glucose, 5-ketogluconate, D-mannose, pyruvic acid methyl ester, succinic 399 acid and D-trehalose. The following carbon sources are not utilized at 28 °C: cis-400 aconotic acid, D-adonitol, L-alanine, D-arabinose, L-arabinose, D-arabitol, L-arabitol, 401 betaine, cellobiose, dextrin, dulcitol, erythritol, L-fucose, D-galacturonic acid, 402 gentiobiose, D-glucoronic acid,  $\alpha$ -methyl-D-glucoside,  $\beta$ -methyl-D-glucoside, 403 glutarate, histidine, myo-inositol, lactose, lactulose, D-maltose, maltotriose, D-404 melibiose, L-proline, propionate, quinic acid, D-raffinose, L-rhamnose, D-sorbitol, L-405 serine, sucrose, L-tartrate and trigonelline. The G + C content of the type strain is 52.8 mol %. The type strain is LMG  $22051^{T}$  (= BD  $877^{T}$  = ATCC  $31628^{T}$  = SHS  $2008^{T}$ ) 406 407 and was isolated from soil in Japan.

408

# 409 Description of *Tatumella morbirosei* sp. nov.

410 *Tatumella morbirosei* (mor.bi.ró.se.i. L.N. *morbus*, disease; L.Adj. *roseus*, rosy,
411 pink. N.L. gen. N. *morbirosei*, of the pink disease, referring to the causal agent of
412 pink disease of pineapple)

413

414 Cells are Gram-negative, short rods (0.8-1.2 x 1.0-3.0  $\mu$ m) occurring singly or in 415 pairs, non-motile and non-sporeforming. Colonies are pale beige, round, convex and 416 smooth with entire margins. Facultatively-anaerobic, oxidase negative, catalase 417 positive, glucose dehydrogenase, gluconate dehydrogenase and 2-ketogluconate 418 dehydrogenase are produced. Indole and urease are negative, whilst phenylalanine 419 deaminase is positive and arginine dihydrolase is weakly positive. Reduce nitrate to 420 nitrite. Acid is produced from: amidon, L-arabinose, D-fucose, D-fructose, D-421 galactose, D-glucose, glycerol, 5-ketogluconate, melibiose, D-mannitol, D-mannose, 422 D-tagatose and D-trehalose but not from D-raffinose or sucrose. The following carbon 423 sources are utilized at 28 °C: N-acetyl-D-glucosamine, D-adonitol, D-arabinose, L-

424 arabinose, L-aspargine, L-aspartic acid, citrate, dextrin, erythritol, formic acid (weak), 425 D-fructose, D-galactose, D-gluconic acid, D-glucose, 5-ketogluconate, D-malate, D-426 maltose, maltotriose, D-mannitol, D-mannose, pyruvic acid methyl ester (weak), 427 succinic acid, D-tagatose, D-trehalose and trigonelline. The following carbon sources 428 are not utilized at 28 °C: *cis*-aconotic acid, L-alanine, L-arabitol, betaine, cellobiose, 429 dulcitol, L-fucose, gentiobiose,  $\alpha$ -methyl-D-glucoside,  $\beta$ -methyl-D-glucoside, D-430 galacturonic acid, D-glucoronic acid, glutarate, histidine, myo-inositol, lactose, 431 lactulose, D-melibiose, L-proline, propionate, quinic acid, D-raffinose, L-rhamnose, 432 D-sorbitol, L-serine, L-tartrate and *meso*-tartrate. The G + C content of the type strain is 50.2 mol %. The type strain is LMG  $23360^{T}$  (= BD  $878^{T}$  = NCPPB  $4036^{T}$  = 433 434  $CMC6^{T}$ ) and was isolated from pineapple in the Philippines.

435

## 436 Acknowledgements

437

This study was partially supported by the South African-Flemish Bilateral Agreement, the National Research Foundation (NRF), the Tree Protection Co-operative Programme (TPCP) and the THRIP support programme of the Department of Trade and Industry, South Africa. The BCCM/LMG Bacteria collection is supported by the Federal Public Planning Service-Science Policy, Belgium. The authors wish to thank Katrien Engelbeen for technical assistance and Dr J.P. Euzeby for suggesting the name "morbirosei".

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- 559 Publishing and Wiley-Interscience

- 560 **Table 1**: Strains of *Tatumella* and *Pantoea* used in this study, LMG = BCCM/LMG Bacteria Collection, Ghent University, Belgium, ATCC =
- 561 American Type Culture Collection, Rockville, Maryland, U.S.A., CCUG = Culture Collection, University of Göteborg, Sweden, BD = Plant
- 562 Pathogenic and Plant Protecting Bacteria (PPPPB) Culture Collection, ARC-PPRI, Pretoria, South Africa,
- 563  $^{T}$  = type strain,  $^{a}$  = Genbank accession numbers, Br HG = Brenner hybridization group

Species	Strain	Source	Location	gyrB <sup>a</sup>	rpoB <sup>a</sup>	$atpD^a$	inf <b>B</b> <sup>a</sup>
Tatumella citrea	$LMG 22049^{T} = BD 875^{T}$	Mandarin orange	Japan	EF988802	EF988974	EF988715	EF988888
Tatumella morbirosei	$LMG 23360^{T} = BD 878^{T}$	Pineapple	Philippines	EU344760	EU344768	EU344756	EU344764
	LMG 23359 = NCPPB 4035	Pineapple	Philippines	EU344759	EU344767	EU344755	EU344763
Tatumella punctata	$LMG 22050^{T} = BD 876^{T}$	Mandarin orange	Japan	EF988803	EF988975	EF988716	EF988889
	LMG 22097	Mandarin orange	Japan	EF988805	EF988977	EF988718	EF988891
	LMG 22098	Persimmon	Japan	EF988806	EF988978	EF988719	EF988892
	LMG 23562	Mandarin orange	Japan	EF988807	EF988979	EF988720	EF988893
	LMG 23563	Mandarin orange	Japan	EF988808	EF988980	EF988721	EF988894
	CCUG 30157	Mandarin orange	Japan	EF988795	EF988967	EF988708	EF988881
	CCUG 30160	Mandarin orange	Japan	EF988796	EF988968	EF988709	EF988882
Tatumella terrea	$LMG 22051^{T} = BD 877^{T}$	Soil	Japan	EF988804	EF988976	EF988717	EF988890
	LMG 23564	Soil	Japan	EF988809	EF988981	EF988722	EF988895
	CCUG 30162	Soil	Japan	EF988797	EF988969	EF988710	EF988883
Tatumella ptyseos	$LMG 7888^{T} = ATCC 33301^{T}$	Human	USA	EU145260	EU145292	EU145244	EU145276
	LMG 23565	Soil	Japan	EU344758	EU344766	EU344754	EU344762
	CCUG 30163	Soil	Japan	EU344757	EU344765	EU244753	EU344761
Pantoea agglomerans	LMG 1286 <sup>T</sup>	Human	Zimbabwe	EF988798	EF988970	EF988711	EF988884
	LMG 2660	Wisteria floribunda	Japan	EF988823	EF988995	EF988736	EF988909
Pantoea ananatis	$LMG 2665^{T}$	Pineapple	Brazil	EF988824	EF988996	EF988737	EF988910
	LMG 20103	Eucalyptus	South Africa	EF988799	EF988971	EF988712	EF988885
Pantoea stewartii ssp. stewartii	$LMG 2715^{T}$	Maize	USA	EF988831	EF989003	EF988744	EF988917
_	LMG 2718	Maize	USA	EF988832	EF989004	EF988745	EF988918
Pantoea stewartii ssp. indologenes	$LMG 2632^{T}$	Fox millet	India	EF988822	EF988994	EF988735	EF988908
	LMG 2673	Pineapple	Hawaii	EF988827	EF988999	EF988740	EF988914
Pantoea dispersa	LMG 2603 <sup>T</sup>	Soil	Japan	EF988818	EF988990	EF988731	EF988904

	LMG 2604	Wild rose	Netherlands	EF988819	EF988991	EF988732	EF988905
Pantoea anthophila	LMG 2558 <sup>T</sup>	Impatiens balsamina	India	EF988812	EF988984	EF988725	EF988898
-	LMG 2560	Tagetes erecta	Unknown	EF988813	EF988985	EF988726	EF988899
Pantoea vagans	LMG 24199 <sup>T</sup>	Eucalyptus	Uganda	EF988768	EF988940	EF988715	EF988854
	LMG 24201	Maize	South Africa	EF988792	EF988964	EF988705	EF988878
Pantoea eucalypti	LMG 24197 <sup>T</sup>	Eucalyptus	Uruguay	EF988762	EF988934	EF988675	EF988848
	LMG 24198	Eucalyptus	Uruguay	EF988763	EF988935	EF988676	EF988849
Pantoea deleyi	LMG 24200 <sup>T</sup>	Eucalyptus	Uganda	EF988770	EF988950	EF988683	EF988856
Pantoea sp. (Br HG II)	LMG 5345 <sup>T</sup>	Human, stool	New Jersey, USA	EU145272	EU145304	EU145256	EU145288
	LMG 24526	Human, blood	New York, USA	EU145261	EU145293	EU145245	EU145277
Pantoea sp. (Br HG IV)	LMG 2781 <sup>T</sup>	Human, trachea	Connecticut, USA	EU145271	EU145303	EU145255	EU145287
	LMG 24529	Human, cyst	Georgia, USA	EU145264	EU145296	EU145248	EU145280
Pantoea sp. (Br HG V)	LMG 5343 <sup>T</sup>	Human, urethra	Montana, USA	EU145270	EU145302	EU145254	EU145286
	LMG 24532	Human, sputum	Wisconsin, USA	EU145267	EU145299	EU145251	EU145283
Pantoea sp. (Br HG V)	LMG 24534 <sup>T</sup>	Human, blood	Paris, France	EU145269	EU145301	EU145253	EU145285
Erwinia billingiae	LMG 2613 <sup>T</sup>	Pear	UK	EU145275	EU145307	EU145259	EU145291
Erwinia psidii	LMG 7034	Guava	Brazil	FJ187834	FJ187844	FJ187829	FJ187839
Erwinia rhapontici	LMG 2688 <sup>T</sup>	Rhubarb	UK	EF988838	EF989010	EF988751	EF988924
Erwinia toletana	$LMG 24162^{T}$	Olive tree	Spain	EU145274	EU145306	EU145258	EU145290

- 569 Table 2: DNA-DNA hybridization values between T. citrea comb. nov., T. morbirosei sp. nov., T. punctata comb. nov., T. terrea comb. nov.,
  - Tatumella citrea 1. LMG 22049<sup>T</sup> Tatumella morbirosei 2. LMG 23360<sup>T</sup> 3. LMG 23359 4. LMG 22050<sup>T</sup> Tatumella punctata 5. LMG 22098 6. LMG  $22051^{T}$ Tatumella terrea 7. LMG 23564 8. LMG 7888<sup>T</sup> Tatumella ptyseos 9. LMG 23565 10. LMG 1286<sup>T</sup> Pantoea agglomerans 11. LMG 2665<sup>T</sup> Pantoea ananatis 12. LMG 24199<sup>T</sup> Pantoea vagans 13. LMG 2715<sup>T</sup> Pantoea stewartii ssp. stewartii 14. LMG 2603<sup>T</sup> Pantoea dispersa
- *T. ptyseos* and selected species belonging to *Pantoea*.

578	2 = Tatumella citrea comb. nov. (1 strain), 3 = Tatumella morbirosei sp. nov. (2 strains), 4 = Tatumella punctata comb. nov. (7 strains),
579	5 = Tatumella terrea comb. nov. (3 strains), 6 = Pantoea agglomerans (3 strains), 7 = Pantoea ananatis (4 strains), 8 = Pantoea anthophila (2
580	strains), 9 = Pantoea deleyi (1 strain), 10 = Pantoea dispersa (2 strains), 11 = Pantoea eucalypti (2 strains), 12 = Pantoea stewartii ssp. stewartii
581	(1 strain), 13 = Pantoea stewartii ssp. indologenes (2 strains), 14 = Pantoea vagans (7 strains). All data presented was generated during this
582	study, except for 2-ketogluconate dehydrogenase for which the results were taken from Kageyama et al., 1992 and Bouvet et al., 1989.
583	+, positive; -, negative; (+), weakly positive; +/-, variable reaction, ND, not determined
584	* T. ptyseos is non-motile at 36 °C but many strains are motile at 25 °C. Flagella of Tatumella species are polar, sub-polar or lateral rather than

Table 3: Selected phenotypic characteristics useful for distinguishing *Tatumella* species from *Pantoea* species. 1 = *Tatumella* ptyseos (1 strain),

585 peritrichous like *Pantoea* species.

586	<sup><i>a</i></sup> API 50 CHB/E <sup><i>b</i></sup> Biotype 100 GN2 MicroPlate
500	

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
motility (36 °C)*	-	-	-	-	+	+	+	-	-	+	-	-	+	-
arginine dihydrolase	-	+	(+)	+	-	-	-	-	-	-	-	-	-	-
2-ketogluconate	+	+	+	+	+	-	-	ND	ND	-	ND	-	-	ND
dehydrogenase														
Acid from $^{a}$ :														
arbutin	-	-	-	-	-	+	+	+	-	+	+	+	+	+
myo-inositol	-	-	-	-	-	(+)	+	+	-	+	+	-	+	+

L-rhamnose	-	-	-	-	-	+	+	+	+	+	+	-	+	+
Utilization of <sup><i>b</i></sup> :														
cis-aconitic acid	-	+	-	-	-	+	+	+	+	+	+	+	+	+
D-alanine	-	-	-	-	-	+	+	+	+	+	+	+	+	+
L-alanine	-	-	-	-	-	+	+	+	+	+	+	+	+	+
cellobiose	-	-	-	+/-	-	+	+	+	+	+	+	+	+	+
D-galacturonic acid	-	-	-	-	-	+	+	+	+	+	+	+	+	+
D-glucoronic acid	-	-	-	-	-	+	+	+	+	+	+	+	+	+
L-histidine	-	-	-	-	-	+	+	+	+	+	+	+	+	+
myo-inositol	-	-	-	-	-	+	+	+	+	+	+	+	+	+
D,L-lactic acid	-	-	-	-	-	+	+	+	+	+	+	+	+	+
maltose	-	+	+	-	-	+	+	+	+	+	+	-	+	+
β-methyl-D-glucoside	-	-	-	-	-	+	+	+	+	+	+	+	+	+
L-proline	-	-	-	-	-	+	+	+	+	+	+	+	+	+
L-rhamnose	-	-	-	-	-	+	+	+	+	+	+	+	+	+
D-saccharic acid	-	-	-	-	-	+	+	+	+	+	+	+	+	+
L-serine	-	-	-	-	-	+	+	+	+	+	+	+	+	+
meso-tartrate	(+)	-	-	-	-	+	+	+	+	+	+	+	+	+

- **Table 4:** Phenotypic characteristics distinguishing *T. citrea* comb. nov., *T. morbirosei*
- 589 sp. nov., *T. punctata* comb. nov. and *T. terrea* comb. nov. from each other and from
- 590 T. ptyseos
- 1 = T. ptyseos LMG 7888<sup>T</sup>, LMG 23565, 2 = T. citrea LMG 22049<sup>T</sup>,
- 3 = T. morbirosei LMG 23360<sup>T</sup>, LMG 23359, 4 = T. punctata LMG 22050<sup>T</sup>,
- 593 LMG 22097, LMG 22098, LMG 23563, 5 = T. terrea LMG 22051<sup>T</sup>, LMG 23564.
- 594 +, positive; (+), weakly positive; -, negative
- 595 All data presented was generated during this study.

Characteristic	1	2	3	4	5
arginine dihydrolase	-	+	(+)	+	-
phenylalanine deaminase	+	-	+	-	-
Acid from:					
amidon (starch)	-	-	+	-	-
D-mannitol	-	+	+	-	-
D-raffinose	-	-	-	+	-
sucrose	+	-	-	+	+
Utilization of:					
adonitol	-	-	+	-	-
L-arabinose	-	+	+	-	-
dextrin	-	+	+	-	-
erythritol	-	+	+	-	-
formic acid	-	+	(+)	-	+
gentiobiose	-	(+)	-	+	-
lactulose	-	+	-	-	-
pyruvic acid methyl ester	-	(+)	(+)	-	+
quinic acid	+	-	-	-	-
L-tartrate	+	-	-	-	-
trigonelline	-	-	+	-	-

- 601 **Supplementary Table S1:** Signature nucleotides of the 16S rRNA- and *atpD*-gene
- 602 sequences for the differentiation of *Tatumella* species from *Pantoea* species. *E. coli*

E.coli numbering position	Tatumella	Pantoea
16S rRNA gene signature nucleotides:		
136	Т	С
593	С	Т
619	С	Т
624	С	Т
637	С	Т
652	А	Т
1115	С	Т
1366	Т	С
<i>atpD</i> gene signature nucleotides:		
198	С	Т
201	Т	G
204	G	М
207	G	Н
330	Т	А
338	А	С
351	Т	С
360	А	Т
361	А	Т
366	С	Т
374	Т	А
376	G	Т
385	А	С
387	Т	G
549	А	Y
603	G	С
613	С	А
615	G	С
693	Т	С
699	Т	G
739	G	А
804	Т	R
828	А	S

603 numbering positions are used.

**Figure 1:** Maximum likelihood tree based on 16S rRNA gene sequences of *Tatumella* species. Bootstrap values after 1000 replicates are expressed as percentages. *Escherichia coli* was included as an outgroup.

**Figure 2:** Maximum likelihood tree based on the concatenated nucleotide sequences of *gyrB*, *rpoB*, *atpD* and *infB* genes of *Tatumella* and *Pantoea* strains. Bootstrap values after 1000 replicates are expressed as percentages. *Cronobacter sakazakii* was included as an outgroup. Gene sequences for *Erwinia tasmaniensis*, *Escherichia coli*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Citrobacter rodentium*, *Citrobacter koseri* and *Cronobacter sakazakii* were obtained from genome sequencing databases (http://www.ncbi.nlm.nih.gov, http://www.sanger.ac.uk, http://asap.ahabs.wisc.edu/asap).

**Figure 3:** UPGMA dendrogram constructed from 50 phenotypic characteristics useful for the differentiation of the "core" *Pantoea* species from species of the genus *Tatumella*.





0.1

