Izhakiella capsodis gen. nov., sp. nov., in the family Enterobacteriaceae, isolated from the mirid bug Capsodes infuscatus

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Gram-stain-negative, oxidase-negative, facultatively anaerobic, motile, rod-shaped, non-pigmented bacterial strains (N6PO6T, N8PO1 and N8PI1) were isolated from the mirid bug Capsodes infuscatus captured on Asphodelus aestivus plants. The 16S rRNA gene sequences of the strains shared 94.7–95.7 % similarity with species of the genus Pantoea and 95.6 % or less with species from other genera in the family Enterobacteriaceae. A polyphasic approach that included determination of phenotypic properties and phylogenetic analysis based on 16S rRNA, rpoB, gyrB and atpD gene sequences supported the classification of strains N6PO6T, N8PO1 and N8PI1 as representing a novel species of a new genus in the family Enterobacteriaceae. Strain N6PO6T, and the two reference strains of the novel species, grew at 1–37°C, and in the presence of NaCl (up to 7.5 %, w/v) and sucrose (up to 60 %). Their major cellular fatty acids were C₁₆:0, C₁₇:0 cyclo, C₁₈:1ω7c, summed feature 2 (C₁₄:0 3-OH and/or iso-C₁₆:1ω7c) and summed feature 3 (C₁₆:1ω7c and/or iso-C₁₅:0 2-OH). The DNA G+C content of strain N6PO6T was 49.9 mol%. On the basis of phenotypic properties and phylogenetic distinctiveness, the mirid bug isolates are classified as representing a novel species in a new genus Izhakiella, in the family Enterobacteriaceae, for which the name Izhakiella capsodis is proposed. The type strain of Izhakiella capsodis is N6PO6T (=LMG 28430T=DSM 29293T).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strains N6PO6T, N8PO1 and N8PI1 are KP436763, KP436763 and KP436672, respectively.

Two supplementary figures are available with the online Supplementary Material.
To amplify internal fragments of the 16S rRNA gene, we used the universal bacterial primers as described by Weisburg et al. (1991). Purified PCR products were sequenced at MCLAB (San Francisco, CA, USA), using primers 8F, 534r, 968F and 1512r as described in detail by Raats & Halpern (2007). This resulted in sequences of about 1500 bp in length. The identification of phylogenetic neighbours was initially carried out using the BLASTN program (Altschul et al., 1997) against the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012). Sequences with the highest scores were then selected for the calculation of pairwise sequence similarity using a global alignment algorithm, which was implemented at the EzTaxon-e server (Kim et al., 2012). Sequence alignment was performed using the CLUSTAL W program, and a phylogenetic tree was generated using the neighbour-joining and maximum-parsimony methods in the MEGA version 4.1 software (Tamura et al., 2007) (Fig. 1) (only the neighbour-joining tree is shown).

Strains N6PO6T, N8PO1 and N8PI1 shared 99.9 % 16S rRNA gene sequence similarity with each other and 95.7 % or less with other members of the family Enterobacteriaceae. Strain N6PO6T shared 95.73 % with Pantoea gaviniae A18/07T, 95.67 % with Pantoea deleyi LGM 24200T and less than 95.60 % with the type strains of species of other genera in the family Enterobacteriaceae. In the 16S rRNA gene phylogenetic tree (Fig. 1), the strains clustered together and formed a distinct lineage, suggesting they belong to a single species within a new genus.

In the family Enterobacteriaceae, species which belong to different genera share 97.2 % 16S rRNA gene sequence similarity or less. For example, the type species Phaseolibacter flectens and Rosenbergiella nectarea, and Mangrovibacter plantisponsor and Cronobacter mgytensis share 97.0 and 97.2 % 16S rRNA gene sequence similarity, respectively (Rameshkumar et al., 2010; Halpern et al., 2013a, b). As strains N6PO6T, N8PO1 and N8PI1 share 95.73 % or less 16S rRNA gene sequence similarity with species from other genera in the family Enterobacteriaceae, they are considered to represent a novel species of a new genus.

To further support the proposed phylogenetic position of strains N6PO6T, N8PO1 and N8PI1 as a novel species of a new genus, their phylogenetic position was further analysed based on partial rpoB, gyrB and atpD gene sequences (Fig. 2, and Figs S1 and S2, available in the online Supplementary Material). Phylogenetic trees were generated using the neighbour-joining and maximum-parsimony methods in MEGA version 4.1 (Tamura et al., 2007) but only the neighbour-joining trees are shown.

The amplification and sequencing of these genes (accession numbers KM841418–20, KM267156–61) were performed according to Brady et al. (2008) and Ko et al. (2007).

Levels of rpoB, gyrB and atpD gene sequence similarity between strains N6PO6T, N8PO1 and N8PI1 were 99.8–100, 100 and 98.7–99.1 %, respectively. None of the strain pairs were identical in all three genes. The high levels of similarity of the genes between all three strains indicate that they belong to the same species. Similar results were found for Rosenbergiella nectarea strains for which all strains shared >99.4 % sequence similarity in their 16S rRNA, rpoB, gyrB and atpD genes (Halpern et al., 2013b).

Nevertheless, species from different genera in the family Enterobacteriaceae share sequence similarity of 86 % or less in their rpoB, gyrB and atpD genes. For example: rpoB, 86 % or less among Erwinia species; gyrB, 81 % or less among species from the genera Erwinia and Pantoea; and atpD, 83–86 % among Pantoea species and 81 % or less among Erwinia species. It has already been shown for other members of the family Enterobacteriaceae that species from different genera share 86.5 % or less rpoB, gyrB and atpD gene sequence similarity (Rameshkumar et al., 2010; Halpern et al., 2013a, b), thus demonstrating that strains N6PO6T, N8PO1 and N8PI1 belong to a novel species of a new genus.

Strains N6PO6T, N8PO1 and N8PI1 formed a distinct lineage and clustered together with each other when phylogenetic trees based on these gene sequences were generated (Fig. 2 and Figs S1 and S2), demonstrating that they belong to the same species in a new genus. The three strains revealed a clear division of the suggested genus from other genera in the family Enterobacteriaceae.

Electron microscopy was used to determine cell morphology and size. For this, bacteria were grown on LB agar (HiMedia) supplemented with 10 % sucrose and then suspended in saline. The samples were fixed to a carbon-coated grid and stained with 2 % uranyl acetate and photographed under a JEM-1200EX electron microscope (JEOL). Cells measured 0.32–0.74 μm in width and 1.2–2.4 μm in length.

For determination of the DNA G+C content, genomic DNA of strain N6PO6T was extracted according to a modified version of the procedure of Wilson (1987). The G+C content of the DNA sample was determined in three independent analyses using the HPLC technique (Mesbah et al., 1989), by the BCCM/LMG Bacteria Collection Identification Service (Laboratory of Microbiology, Ghent University, Ghent, Belgium). The DNA G+C content of strain N6PO6T was 49.9 mol%.

For determination of the whole-cell fatty acid composition, cells were grown for 24 h at 28 °C under aerobic conditions on TSA medium (BBL 11768). Inoculation and harvesting of cells and fatty acid extraction and analysis were performed according to the recommendations of the commercial identification system MIDI (Microbial Identification System). The whole fatty acid composition was determined by GC at the BCCM/LMG Bacteria Collection Identification Service. The peak naming table MIDI TSBA50 (revision 5.0) was used. The major cellular fatty acids (>10 %)
of strain N6PO6\textsuperscript{T} were C\textsubscript{16}:0 (37.5 %), C\textsubscript{17}:0 cyclo (14.9 %), C\textsubscript{18}:1\textsuperscript{\textit{v}7c} (11.9 %), and summed feature 3 (C\textsubscript{16}:1\textsuperscript{\textit{v}7c and/or iso-C\textsubscript{15}:0 2-OH, 10.9 %).

A genus-specific signature region was found based on an alignment of the 16S rRNA gene sequences of strains N6PO6\textsuperscript{T}, N8PO1 and N8PI1 against 16S rRNA gene sequences from the NCBI database. The sequence 5'-AGGAGGAAAAAGGTAGCAGTTAGCCGTGGTCG was specific for the suggested genus as representative species from other genera showed base mismatches when they were aligned with this sequence.

**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences, showing the nearest neighbours of Izhakiella capsodis strains. Arsenophonus nasoniae ATCC 49151\textsuperscript{T} was included as an outgroup. Bootstrap values (\(\geq50\%\)) resulting from 1000 replicates are indicated at branch nodes. Asterisks indicate branches of the tree that were also formed by using the maximum-parsimony method. Bar, 0.005 substitutions per nucleotide position.
For phenotypic characterization of strains N6PO6T, N8PO1 and N8PI1, LB agar was used as the basal growth medium. Growth at 1, 4, 8, 20, 28, 30, 35, 37, 41 and 44°C was measured using LB agar supplemented with 10% sucrose. Salt tolerance was determined at 28°C in LB agar containing 0–15% (w/v) NaCl. The ability to grow in the presence of sucrose was measured on LB supplemented with 0–60% sucrose. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on LB agar supplemented with 10% sucrose and 0.5% glucose. Biochemical tests were performed by using API 20E, 20NE and 50CH identification systems (bioMérieux), according to the manufacturer’s instructions except that the incubation temperature was 28°C for 2 days. Catalase activity was evidenced by bubble production in a 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined using 1% N,N,N’,N’-tetramethyl-p-phenylenediamine dihydrochloride (Sigma-Aldrich; T3134). Growth was tested on MacConkey agar (HiMedia). The phenotypic traits of the three isolates are given in the species description and compared with their closest relatives in Tables 1 and 2.

**Fig. 2.** Neighbour-joining tree based on atpD gene sequences, showing the nearest neighbours of *Izhakiella capsodis* strains. Bootstrap values (≥50%) resulting from 1000 replicates are indicated at branch nodes. Asterisks indicate branches of the tree that were also formed by using the maximum-parsimony method. Bar, 0.02 substitutions per nucleotide position.
Table 1. Biochemical differentiation between Izhakiella capsodis gen. nov., sp. nov. and other species from the closest genera in the family Enterobacteriaceae

Taxa: 1, Izhakiella capsodis gen. nov., sp. nov. (n = 3); 2, Tatumella pyseos LMG 7888T; 3, T. cirea LMG 22049T; 4, T. morbiolesi LMG 23360T; 5, T. puncticula LMG 2205T; 6, T. terrae LMG 22051T; 7, Pantoea agglomerans DSM 3493T; 8, P. dispersa LMG 2603T; 9, P. gaviniae A18/07T; 10, P. calida 1400/07T; 11, P. ananatis LMG 2665T; 12, P. stewartii LMG 2715T; 13, P. allii LMG 24248T; 14, P. deleyi LMG 24200T; 15, P. vagans LMG 24199T; 16, P. wallisii LMG 26277T; 17, Erwinia amylovora ATCC 15580T; 18, E. toletana A37T; 19, E. psidii LMG 7034T; 20, Roseobacter nectarans LMG 26121T; 21, Phaseolibacter flectens ATCC 12775T. Data are from the current study (taxon 1), Hollis et al. (1981) (2), Gavini et al. (1989) (7,8), Kaygage et al. (1992) (3,7), Mergaert et al. (1993) (7,8,11,12), Hauben et al. (1998) (17, 19), Rojas et al. (2004) (18), Brenner & Farmer (2005) (2), Hauben & Swings (2005) (7,17,19), Brady et al. (2009) (14,15), Brady et al. (2010) (2–6), Popp et al. (2010) (7,9,10), Brady et al. (2011) (13), López et al. (2011) (17), Brady et al. (2012) (16), Halpern et al. (2013a, b) (20,21), +, Positive; w, weakly positive; −, negative; v, variable (reaction for the type strain of the type species in parentheses); ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| Voges–Proskauer reaction | + | + | + | + | + | + | + | + | + | V(−) | + | + | ND | + | − | − | − | + | + |
| β-Galactosidase | − | − | ND | ND | ND | + | + | + | + | + | + | + | + | + | ND | + | ND | V(+) | − |
| Arginine dihydrolase | − | V(−) | + | W | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Ornithine decarboxylase | − | − | − | − | − | − | − | − | − | ND | ND | ND | + | − | − | − | − | − | − | − |
| Lysine decarboxylase | + | − | − | − | − | − | − | − | − | ND | ND | − | − | − | − | − | − | − | − | − |
| Production of H2S | − | − | − | − | − | − | − | − | − | ND | ND | ND | − | − | − | − | − | − | − | − |
| Citrate utilization | − | + | + | + | + | + | + | + | + | V(−) | + | ND | ND | + | + | + | ND | + | ND | V(+) | V(+) |
| Urease | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Indole | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Gelatinase | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Reduction of nitrate to nitrogen | − | − | − | − | − | − | − | − | − | ND | ND | − | − | ND | − | ND | − | − | − | − | − | − |
| Acid production from: | | | | | | | | | | | | | | | | | | | | | | |
| Lactose | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| α-Mannitol | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Sorbitol | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Sucrose | − | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Inositol | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Arabinose | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Trehalose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | V | + | + | V(−) | − | − | − |
| Growth at 41 °C | − | − | ND | ND | ND | ND | V(−) | + | W | − | − | ND | ND | + | − | − | − | − | − | − | − |
| Motility | + | + | − | − | − | − | − | − | − | − | ND | − | − | − | − | − | − | − | − | − | − | − |

*API20E test results.
†At 25 °C but not at 36 °C (according to Hollis et al., 1981).

Being Gram-stain-negative, facultatively anaerobic, chemoheterotrophic, oxidase-negative, catalase-positive, glucose-fermentative, rod-shaped bacteria, the novel strains share typical characteristics with other members of the family Enterobacteriaceae. Strains N6P06T, N8PO1 and N8P11 share some phenotypic traits with their closest related genera Pantoea, Tatumella and Erwinia. Most members of these genera are motile, Voges–Proskauer-positive, negative for decarboxylation of ornithine, do not reduce nitrate to nitrogen and do not produce H2S. However, the new genus can be distinguished from these closely related genera by its positive urease activity, inability to utilize citrate and positive lysine decarboxylase activity.

Strains N6P06T, N8PO1 and N8P11 shared >98.7 % sequence similarity in their 16S rRNA, rpoB, gyrB and atpD gene sequences. The strains clustered together in all the phylogenetic trees based on these four gene sequences, supporting the fact that they belong to the same species in the family Enterobacteriaceae (Figs 1 and 2 and Figs S1 and S2). However, they comprised a separate group from all other genera of this family and thus belong to a new genus (Figs 1, 2 and Figs S1 and S2).

The results of this study support the recognition of a novel species of a new genus, for which the name Izhakiella capsodis gen. nov., sp. nov. is proposed.

Description of Izhakiella gen. nov.

Izhakiella (I.zha.ik’la. N.L. fem. dim. n. Izhakiella named to honour Professor Ido Izhaki, an Israeli ecologist, in recognition of his work and achievements in ecology). Cells are Gram-stain-negative, motile rods. Colonies are non-pigmented and transparent. Growth is facultatively anaerobic,
Chemoheterotrophic. Catalase activity is positive and oxidase activity is negative. D-Glucose is fermented, and lysine decarboxylase is present. Urea is hydrolysed, acetoin is produced, D-glucose, D-mannose, D-mannitol, D-fructose, D-mannose, L-arabitol, potassium gluconate are assimilated; L-lyxose, L-xylose, D-adonitol, methyl D-xylopyranoside, L-sorbitose, dulcitol, inositol, D-sorbitol, methyl D-mannopyranoside, methyl D-glucopyranoside, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate.

The type strain is N6PO6T (=LMG 28430T =DSM 29293T), isolated from a mirid bug (Capsodes infuscatus) that was captured on the plant Asphodelus aestivus in northern Israel. Strains N8PO1 (=LMG 28431 =DSM 29292) and N8PI1 are reference strains of the species.

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References

Description of Izhakiella capsodis sp. nov.
Izhakiella capsodis [cap.so’dis. N.L. gen. n. capsodis of Capsodes, isolated from a mirid bug (Capsodes infuscatus)].

After 48 h of incubation on LB agar or on LB supplemented with 10 % sucrose at 28 °C, colonies are small, circular, smooth, transparent and colourless. Cells are peritrichously flagellated and measure 0.32–0.74 μm in width and 1.2–2.4 μm in length. Optimum temperature for growth is 28–30 °C after 10 days of incubation. Growth occurs at temperatures between 1 and 37 °C but not at 41 °C. Growth is observed on LB agar supplemented with 0–7.5 % (w/v) NaCl (poor growth with 0 and 7.5 %, optimum growth with 3 % NaCl) and with 0–60 % sucrose (poor growth with 0 %, optimum growth with 10–25 % sucrose). Growth is not observed on MacConkey agar. Shows the following characteristics after 48 h of incubation at 28 °C: amygdalin fermentation is variable (positive for the type strain); sorbitol, sucrose, melibiose and arabino- nol are not fermented; aesculin is hydrolysed; D-glucose, D-mannose, D-mannitol, N-acetyl-glucosamine and potassium gluconate are assimilated; L-arabinose, maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not assimilated. Acid production is positive for D-ribose (weakly), D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose (weakly for the type strain), D-mannitol, N-acetylglucosamine, aesculin (weakly), trehalose and D-tagatose. Acid production does not occur for glycerol, erythritol, D-arabinose, L-arabinose, D-xyllose, L-xyllose, D-Adonitol, methyl β-D-xlylopyranoside, L-sorbitose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-fucose, L-fucose, D-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate.

The type strain is N6PO6T (=LMG 28430T =DSM 29293T), isolated from a mirid bug (Capsodes infuscatus) that was captured on the plant Asphodelus aestivus in northern Israel. Strains N8PO1 (=LMG 28431 =DSM 29292) and N8PI1 are reference strains of the species.

Table 2. The presence of some major fatty acids in Izhakiella capsodis gen. nov., sp. nov. and in other species from closely related genera in the family Enterobacteriaceae

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*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 2 contains C14 : 0 3-OH and/or iso-C16 : 1ω; summed feature 3 contains C16 : 1ω7c and/or iso-C15 : 0 2-OH; summed feature 7 contains one or more of C18 : 1ω12t, C18 : 1ω9t and C18 : 1ω7c.


