# *Isoptericola cucumis* sp. nov., isolated from the root tissue of cucumber (*Cucumis sativus*)

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A Gram-stain-positive, aerobic organism, showing an irregular cell morphology, was isolated from the root tissue of cucumber (*Cucumis sativus*) and investigated in detail for its taxonomic position. On the basis of the 16S rRNA gene sequence analysis, strain AP-38$^T$ was shown to be most closely related to *Isoptericola variabilis* (99.1 %) and *Isoptericola nanjingensis* (98.9 %). The 16S rRNA gene sequence similarity to all other species of the genus *Isoptericola* was $\leq$98.5 %. DNA–DNA relatedness to *Isoptericola variablis* DSM 10177$^T$ and *Isoptericola nanjingensis* DSM 24300$^T$ was 31(reciprocal 41 %) and 34 (reciprocal 34 %), respectively. The diagnostic diamino acid of the peptidoglycan was L-lysine. The quinone system contained predominantly menaquinones MK-9($H_4$) and MK-9($H_2$). In the polar lipid profile, major compounds were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and two phosphatidylinositol mannosides. The polyamine pattern contained the major components spermidine and spermine and significant amounts of tyramine. In the fatty acid profile, anteiso-$C_{15:0}$ and iso-$C_{15:0}$ were present in major amounts. These data support the allocation of the strain to the genus *Isoptericola*. The results of physiological and biochemical characterization additionally provide phenotypic differentiation of strain AP-38$^T$ from *I. variabilis* and *I. nanjingensis*. AP-38$^T$ represents a novel species of the genus *Isoptericola*, for which we propose the name *Isoptericola cucumis* sp. nov., with AP-38$^T$ (= LMG 29223$^T$=CCM 8653$^T$) as the type strain.

The genus *Isoptericola* was initially proposed by Stackebrandt *et al.* (2004) to accommodate an atypical species formerly assigned to the genus *Cellulosimicrobium* as *Cellulosimicrobium variabile* (Bakalidou *et al.*, 2002). At the time of writing, the genus *Isoptericola* includes seven species with validly published names: *Isoptericola variabilis* (Stackebrandt *et al.*, 2004), *Isoptericola hypogeus* (Groth *et al.*, 2005), *Isoptericola halotolerans* (Zhang *et al.*, 2005), *Isoptericola dokdonensis* (Yoon *et al.*, 2006), *Isoptericola jiangsuensis* (Wu *et al.*, 2010), *Isoptericola chiayiensis* (Tseng *et al.*, 2011) and *Isoptericola nanjingensis* (Huang *et al.*, 2012). Two further species have been proposed but their names have not yet been validly published, 'Isoptericola salitolerans' (Guan *et al.*, 2013) and '*Isoptericola rhizophila*' (Kaur *et al.*, 2014). In this study, the bacterial strain AP-38$^T$,isolated in the early 1990s as an endophyte from the healthy internal root tissue of cucumber (*Cucumis sativus*), was investigated.

The strain produced single, coccoid to rod-shaped, sometime irregular cells forming yellow colonies with a smooth surface after 48 h at 28 °C on tryptone soy agar (TSA; Oxoid). Cell morphological features were recorded with cells grown on TSA at 28 °C by phase contrast microscopy. During exponential growth, irregularly shaped cells of strain AP-38$^T$ were observed, 0.9–1.5 μm wide and 1.5–2.5 μm sometimes 3–4 μm long, which showed no motility. Cells stained weakly Gram-positive (analysed as described by Gerhardt *et al.*, 1994) and were negative for cytochome oxidase, determined by using an oxidase test (Merck).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AP-38$^T$ is KU201961.

Three supplementry figures are available with the online Supplementary Material.

 

Endospores could not be observed. Temperature-dependent growth was investigated after growth on TSA at 4, 15, 25, 28, 32, 37 and 42 °C. Salinity- and pH-dependent growth was analysed in tryptic soy broth (TSB; Difco) either supplemented with 1 to 10 % (w/v) NaCl or adjusted to pH values between pH 4 and 10 (increasing in 0.5 pH units by the addition of HCl or NaOH and stabilized by the addition of 5 mM phosphate buffer adjusted to the same pH); both were cultured at 28 °C.

The 16S rRNA gene of strain AP-38$^T$ was PCR-amplified and subsequently sequenced for phylogenetic analysis with the universal primers 8F and 1492R (Lane, 1991). The sequence was corrected manually by removing unclear 5′ and 3′ sequence ends and by detailed control of the electropherograms. The final sequence had a size of 1432 nucleotides spanning gene termini 8–1474 [numbered according to the *rrnB* of *Escherichia coli* (Brosius *et al.*, 1978)]. The EzTaxon type strain 16S rRNA gene sequence database (Kim *et al.*, 2014) was used to determine pairwise sequence similarities to the most closely related type strains. Highest sequence similarity was obtained to *I. variabilis* (99.1 %) and *I. nanjingensis* (98.9 %). Sequence similarity to all other species of the genus *Isoptericola* was ≤98.5 %.

The phylogenetic placement of strain AP-38$^T$ was investigated using ARB release 5.2 (Ludwig *et al.*, 2004) and the 'All-Species Living Tree' Project (LTP) (Yarza *et al.*, 2008) database release 123 of July 2015. The sequence was aligned using SINA (v1.2.9; Pruesse *et al.*, 2012) according to the SILVA seed alignment (http://www.arb-silva.de; Pruesse *et al.*, 2007) and implemented into the LTP database. The sequence alignment including all type strains of the family *Promicromonosporaceae* was checked manually before sequence analysis. Phylogenetic trees were calculated with the neighbour-joining method (ARB neighbour-joining) using the Jukes–Cantor correction (Jukes & Cantor, 1969), the maximum-likelihood method using RAxML v7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis, and the maximum-parsimony method using DNAPARs version 3.6 (Felsenstein, 2005). All trees were based on 16S rRNA gene sequences between gene termini 96 and 1440 (according to *E. coli* numbering; Brosius *et al.*, 1978) and 100 repetitions (bootstrap analysis; Felsenstein, 1985).

Strain AP-38$^T$ clustered independently of the treeing method applied with the type strains of *I. hypogeus*, *I. nanjingensis* and *I. variabilis* (Figs 1, and S1 and S2 available in the online Supplementary Material). But, a monophyletic cluster of all species of the genus *Isoptericola* was only obtained by the neighbour-joining method. This was consistent with the analysis shown in the proposals of all other species of the genus *Isoptericola,* which always based their conclusions on the analysis performed with the neighbour-joining method (Groth *et al.*, 2005; Zhang *et al.*, 2005; Yoon *et al.*, 2006; Wu *et al.,* 2010; Tseng *et al.*, 2011; Huang *et al.*, 2012; Guan *et al.*, 2013; Kaur, *et al.*, 2014). Other treeing methods (maximum-likelihood, maximum-parsimony), however, did not support the monophyly of the genus (Figs S1 and S2).

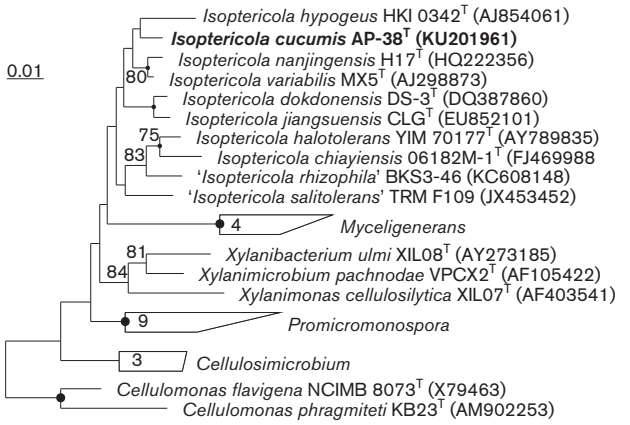


**Fig. 1.** Neighbour-joining tree showing the phylogenetic placement of strain AP-38$^T$ with type strains of species of the genus *Isoptericola* and type strains of species of all genera belonging to the same family, the *Promicromonosporaceae*. The tree was generated in ARB using with the neigbour-joining method. The analysis was based on nucleotide sequences spanning 16S rRNA gene sequence positions 96–1440 (according to *E. coli* numbering). Bootstrap values ≥70 % are given at nodes. Filled circles at nodes represent nodes also present in the maximum-parsimony and maximum-likelihood trees (Figs S1 and S2). Larger circles represent those nodes that were also supported with high bootstrap values by the other analyses. Numbers within a cluster represent the number of species which are included in the cluster. Outgroups were the type strain sequences of *Cellulomonas flavigena* and *Cellulomonas phragmiteti*. Bar, 0.01 nucleotide substitutions per nucleotide position.

Biomass subjected to analyses of polyamines, quinones and polar lipids was grown in PYE broth (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) at 28 °C. For polyamine analysis, biomass was harvested at the late exponential growth phase as recommended by Busse & Auling (1988), whereas biomass use for extraction of diamino acids, quinones and polar lipids was harvested at the stationary growth phase. Polyamines were extracted as reported by Busse & Auling (1988) and Altenburger *et al.* (1997) and analysed using HPLC conditions described by Busse *et al.* (1997). Quinones and polar lipids were extracted and analysed as described by Tindall (1990a, b) and Altenburger *et al.* (1996). The HPLC apparatus used was described by Stolz *et al.* (2007). Strain AP-38$^T$ showed a quinone system which contained 46.6 % menaquinone MK-9($H_4$), 34.7 % MK-9($H_2$), 13.5 % MK-9($H_6$), 3.8 % MK-8($H_4$) and 1.5 % MK-8($H_2$). The polar lipid profile (Fig. S3) consisted of the major lipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and two phosphatidylinositol mannosides. Additionally, three polar lipids were detected that did not contain a sugar residue, an amino group or a phosphate group. The polyamine pattern contained [in μmol (g dry

weight)$^{-1}$]: 12.3 spermidine, 8.2 spermine, 1.3 tyramine, 0.20 cadaverine and 0.1 putrescine.

Diamino acid extraction from cells grown on TSB (Difco) for 24 h at 28 °C was carried out according to the method of Schumann (2011). The amino acids were analysed by HPLC on a C18-column with pre-column derivatization (*o*-phthaldialdehyde) and fluorescence detection. Gradient elution was used with 20 mM potassium phosphate, pH 7.2 (A), and acetonitrile/methanol (50 : 50, v/v) as mobile phase. The cell wall contained alanine, glutamic acid, glycine and the diagnostic diamino acid L-lysine in a molar ratio of 1 : 1 : 0.2 : 0.2, peptidoglycan type A4$\alpha$ according to Schleifer & Kandler, (1972). This peptidoglycan structure was found only in *I. hypogeus* (Groth *et al.*, 2005) and differs from those of most of the described species of the genus in having D-Glu instead of D-Asp. The acyl type of the muramic acid was detected as described by Schumann (2011), and the peptidoglycan contained *N*-acetylated muramic acid.

Fatty acids analysis of cells, grown on TSA at 28 °C, was performed as described by Kämpfer & Kroppenstedt (1996). The fatty acid profile comprised mainly iso- and anteiso-branched fatty acids and was similar to those of the most closely related species (Table 1).

The results of the physiological characterization, performed using methods described previously (Kämpfer, 1990; Kämpfer *et al.*, 1991), are given in Table 2 and in the species description. Strain AP-38$^T$ was able to utilize many sugars and sugar-related compounds, similar to both of the most closely related species; however, a distinct physiological biochemical profile allowed differentiation from the type strains of *I. variabilis* and *I. nanjingensis*. DNA–DNA hybridisation experiments were performed with strain AP-38$^T$ and the type strains of the most closely related species, *I. variabilis* DSM 10177$^T$ and *I. nanjingensis* DSM 24300$^T$, according to the method of Ziemke *et al.* (1998) (except that for nick translation 2 µg of DNA as labelled during 3 h of incubation at 15 °C). Strain AP-38$^T$ showed low DNA–DNA relatedness to *I. variabilis* DSM 10177$^T$ and *I. nanjingensis* DSM 24300$^T$ of 31 % (reciprocal 41 %) and 34 % (reciprocal 34 %), respectively.

DNA–DNA hybridisation experiments with other strains showing a 16S rRNA gene sequence similarity were not performed because the 16S rRNA gene sequence similarities were <98 %. This level of agreement is below the value for which the probability of 0.25 is less than 1 % (Meier-Kolthoff *et al.* 2013). Kim *et al.* (2014) recently recommended a threshold of 98.65 %.

From the results of the phylogenetic and chemotaxonomic analyses, it is obvious that strain AP-38$^T$ represents a novel species, which is for now allocated to the genus *Isoptericola.* For this species we propose the name *Isoptericola cucumis* sp. nov.

## Description of *Isoptericola cucumis* sp. nov.

*Isoptericola cucumis* (cu.cu′mis. L. gen. n. *cucumis* of the cucumber).

Cells are Gram-stain-positive, strictly aerobic, irregular rods (0.9–1.5×1.5–2.5 µm) and non-motile. Colonies grown on TSA are circular, convex and yellow. Optimal temperature for growth is 28 °C; growth occurs at 10–45 °C but not at 5 °C or 50 °C on TSA. Optimal pH for growth is 7.0; growth occurs at pH 5.5–8. Growth occurs in the presence of 1–9 % NaCl but not at higher concentrations in TSB. Test for catalase is positive; oxidase activity is negative. Casein, gelatine, hypoxanthine are hydrolysed, but not urea and xanthine. Acid is produced from D-glucose and raffinose. No acid

**Table 1.** Fatty acid profiles (>1 %) of strain AP-38$^T$ and the most closely related type strains of the genus *Isoptericola*

Data for strains 1–3 from this study. Data for strains 4–7 from Huang *et al.* (2012). Data for strains 2 and 3 were exactly in congruence with those reported by Huang *et al.* (2012) obtained from biomass grown at 30 °C but cultivated on the same medium prior to extraction. Strains: 1, AP-38$^T$; 2, *I. nanjingensis* DSM 24300$^T$ (H17$^T$); 3, *I. variabilis* DSM 10177$^T$ (MX5$^T$); 4, *I. hypogeus* HKI 0342$^T$; 5, *I. jiangsuensis* CLG$^T$; 6, *I. dokdonensis* DS-3$^T$; 7, *I. halotolerans* YIM 70177$^T$; 8, *I. chiayiensis* 06182M-1$^T$.

| Fatty acid (%) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| $C_{14:0}$ | 3.0 | 2.2 (5.97) | (6.6) | – | 1.17 | 3.3 | 2.33 | 1.75 |
| $C_{15:0}$ | 1.4 | – (–) | – (–) | – | 2.64 | 2.6 | – | – |
| $C_{16:0}$ | 3.7 | (8.32) | 3.3 (7.2) | – | 3.13 | 9.0 | 20.05 | 9.72 |
| $C_{17:0}$ | – | – (–) | (–) | – | 1.24 | – | – | 1.48 |
| iso- $C_{13:0}$ | – | – (–) | (–) | – | 3.98 | 1.1 | – | – |
| iso- $C_{14:0}$ | 3.3 | 3.9 (2.13) | 4.8 (1.5) | 8.4 | 3.40 | 4.2 | – | 3.01 |
| iso- $C_{15:0}$ | 12.4 | 23.3 (22.35) | 24.2 (17.0) | 18.1 | 14.29 | 11.5 | 7.02 | 12.02 |
| iso- $C_{16:0}$ | 4.4 | 8.9 (7.57) | 9.9 (6.0) | 22.6 | 1.39 | 3.0 | 1.44 | 5.02 |
| iso- $C_{17:0}$ | – | – (–) | – (–) | – | 1.09 | – | – | 1.37 |
| anteiso- $C_{15:0}$ | 66.2 | 54.7 (48.63) | 53.7 (53.6) | 44.0 | 60.06 | 58.6 | 54.46 | 55.04 |
| anteiso- $C_{17:0}$ | 7.5 | 3.9 (3.45) | 3.9 (7.0) | 3.1 | 5.44 | 41.0 | 10.69 | 8.77 |

**Table 2.** Phenotypic characteristics of strain AP-38$^T$ and the most closely related type strains of the genus *Isoptericola*

Data for strains 1–3 from this study. Data for strains 4–7 from Huang *et al.* (2012). Data for strains 2 and 3 were exactly in congruence with those reported by Huang *et al.* (2012). Strains: 1, AP-38$^T$; 2, *I. nanjingensis* DSM 24300$^T$ (=H17$^T$); 3, *I. variabilis* DSM 10177$^T$ (=MX5$^T$); 4, *I. hypogeus* HKI 0342$^T$; 5, *I. jiangsuensis* CLG$^T$; 6, *I. dokdonensis* DS-3$^T$; 7, *I. halotolerans* YIM 70177$^T$; 8, *I. chiayiensis* 06182M-1$^T$.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Oxidase | − | + | + | − | − | + | + | − |
| Growth at/with: | | | | | | | | |
| 42 °C | + | − | + | − | − | − | − | − |
| 10 % NaCl (w/v) | − | + | + | − | + | − | + | + |
| Hydrolysis of: | | | | | | | | |
| Casein | + | − | + | + | − | − | − | − |
| Gelatin | + | − | + | + | − | + | − | − |
| Hypoxanthine | + | + | + | + | − | − | − | − |
| Urea | − | − | + | − | − | − | − | − |
| Xanthine | − | + | + | + | − | − | − | − |
| Nitrate reduction | − | − | + | + | + | + | − | − |
| Utilization of: | | | | | | | | |
| D-Arabinose | + | − | + | − | − | − | W | W |
| D-Ribose | + | + | + | − | + | − | + | W |
| L-Rhamnose | + | + | + | − | W | − | − | + |
| D-Mannitol | + | + | + | − | − | − | + | − |
| Lactose | + | + | + | − | + | − | − | − |
| Melibiose | + | + | − | − | + | − | − | − |
| Trehalose | + | + | − | − | + | + | − | W |
| Raffinose | + | + | + | − | + | − | − | − |
| D-Arabitol | + | + | + | W | − | − | − | W |
| *N*-Acetylglucosamine | + | + | + | − | + | − | − | − |

production from sugars can be observed from D-xylose, lactose, sucrose, D-mannitol, dulcitol, salicin, D-adonitol, *myo*-inositol, D-sorbitol, L-arabinose, L-rhamnose, maltose, trehalose, cellobiose, erythritol, melibiose or D-arabitol. Several carbohydrates are utilized: D-arabinose, *N*-acetyl-D-glucosamine, D-fructose, D-cellobiose, D-galactose, D-gluconate, D-glucose, maltose, D-mannose, L-rhamnose, sucrose, melibiose, arbutin, *N*-acetyl-D-galactosamine, ribose, D-adonitol, D-arabitol, *myo*-inositol, D-mannitol, lactose, raffinose, trehalose and D-xylose. Salicin and D-sorbitol are not utilized. Major fatty acids are iso-$C_{15:0}$ and anteiso-$C_{15:0}$. The diagnostic diamino acid of the peptidoglycan is L-lysine; the acyl type of the muramic acid is acetyl. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and two phosphatidylinositol mannosides. Furthermore, minor amounts of three polar lipids are present that do not contain a sugar, phosphate or amino residue. The quinone system is predominantly composed of menaquinones MK-9($H_4$) and MK-9($H_2$), with moderate amounts of MK-9($H_6$) and minor amounts of MK-8($H_4$) and MK-8 ($H_2$). In the polyamine pattern, spermidine and spermine predominate. In addition, moderate amounts of tyramine and traces of cadaverine and putrescine are present.

The type strain, AP-38$^T$ (=LMG 29223$^T$=CCM 8653$^T$), was isolated in the early 1990s as an endophyte from the healthy internal root tissue of cucumber (*Cucumis sativus*) grown in a greenhouse at Auburn University, Auburn, AL, USA.

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