Taibaiella coffeisoli sp. nov., isolated from the soil of a coffee plantation

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A Gram-stain-negative, obligately aerobic, non-motile, non-sporulating, rod-shaped bacterium, designated TZCO2^T, was isolated from the soil of an irrigated coffee plantation in Arusha, Tanzania, East Africa. Phylogenetic analysis, based on 16S rRNA gene sequences, indicated that the isolate is affiliated with the genus *Taibaiella* in the family *Chitinophagaceae*. Its closest relative is *Taibaiella koreensis* THG-DT86^T (96.7 %). The pH and temperature ranges for growth were pH 6.0–8.5 (optimum 7.0–7.5) and 10–35 °C (optimum 30 °C, respectively. The predominant fatty acids were iso-$C_{15:0}$ (32.4 %), iso-$C_{15:1}$ G (22.6 %), iso-$C_{17:0}$ (15.1 %) and iso-$C_{17:0}$ 3-OH (10.0 %) The only isoprenoid quinone detected in strain TZCO2^T was menaquinone-7 (MK-7); the major polar lipids were phosphoaminolipid, phosphatidylethanolamine, unidentified aminolipids and lipids. The DNA G+C content was 51.9 mol%. Physiological and chemotaxonomic data further confirmed that strain TZCO2^T is distinct from other members of the genus *Taibaiella*. Thus, strain TZCO2^T is considered to represent a novel species of the genus, for which the name *Taibaiella coffeisoli* sp. nov. is proposed. The type strain is TZCO2^T (=NCAIM B 02601^T=CCM 8601^T).

The genus *Taibaiella* is a member of the family *Chitinophagaceae* (Euzéby, 1997) and was proposed by Zhang *et al.* (2013). At the time of writing the genus *Taibaiella* included four species with validly published names: *Taibaiella smilacinae* isolated from a stem of *Smilacina japonica* (Zhang *et al.*, 2013), *Taibaiella koreensis* (Son *et al.*, 2014) isolated from the soil of a ginseng field, *Taibaiella chishuiensis* (Tan *et al.*, 2014) isolated from freshwater and *Taibaiella yonginensis* isolated from urban soil (Singh *et al.*, 2015). Species of the genus *Taibaiella* are Gram-stain-negative, rod-shaped, strictly aerobic, non-motile and oxidase-positive; they produce flexirubin-type pigments. Their only respiratory quinone is MK-7. The major polar lipid is phosphatidylethanolamine. The major fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ and iso-$C_{17:0}$ 3-OH. The DNA G+C content of the type strain (*T. smilacinae* PTJT-5^T) of the genus is 40.3 mol%. 16S rRNA gene sequence analysis showed that the novel strain, designated TZCO2^T, belongs to the genus *Taibaiella*. This paper describes the taxonomic characterization of this novel *Taibaiella*-like bacterial strain.

In January 2014 a coffee plantation was sampled in Arusha, Tanzania (3° 22′ 30.72″ S 36° 39′ 6.84′ E). Strain TZCO2^T was isolated from the upper soil of a newly planted and irrigated coffee tree (*Coffea arabica*). A solution was made from 10 g of the sample in 90 ml physiological saline (NaCl at 0.9 %, w/v) with glass beads and stirred thoroughly for 20 min. Serial dilutions were made and plated on TGY-5 agar plates that were composed of 5 g l^{-1} triptone, 2.5 g l^{-1} yeast extract, 5 g l^{-1} glucose (all from BioLab) and 15 g l^{-1} agar (Merck) in distilled water (dH_2O) at pH 7.0, incubated at 30 °C for 72 h. Colonies were selected randomly and subsequently purified twice on TGY-10 agar medium (the same as TGY-5, but containing 10 g l^{-1} glucose) at 30 °C.

Genomic DNA was extracted by using an UltraClean Microbial DNA Isolation kit (MoBio Laboratories). Subsequently the 16S rRNA gene was amplified using 27F and 1492R primers (Lane, 1991). Amplification was performed by using an Eppendorf Mastercycler (Eppendorf).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TZCO2^T is KM505048.

Seven supplementary figures are available with the online Supplementary Material.

PCR products were purified with a PCR Advanced PCR Clean Up kit (Viogene). The almost complete 16S rRNA gene sequence of the strain was determined by using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's instructions. Sequencing products were separated on a model 3130 Genetic Analyzer (Applied Biosystems). The 16S rRNA gene sequence of strain TZCO2^T was compared to those of the type strains of all members of the genus *Taibaiella* and of representative members of related genera obtained from GenBank (Kim *et al.*, 2012). Multiple alignments of 16S rRNA gene sequences were made with CLUSTAL X (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed using the maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) methods with Kimura's two-parameter calculation model and the maximum-parsimony algorithm (Kimura, 1980) using MEGA version 5.0 (Tamura *et al.*, 2011). Tree topologies and distances were evaluated by bootstrap analysis based on 1000 replicates.

Almost complete 16S rRNA gene (1420 bp) sequence analysis revealed that strain TZCO2^T is a member of the genus *Taibaiella*, showing closest similarity (96.7 %) with *T. koreensis* THG-DT86^T. Since all of the strains of species of the genus *Taibaiella* that were compared showed less than 97 % 16S rRNA gene sequence similarity to strain TZCO2^T, it can be concluded that they differ at the species level (Stackebrandt & Goebel, 1994; Tindall *et al.*, 2010). On the basis of the 16S rRNA gene sequence analysis, the phylogenetic position of strain TZCO2^T among the other members of the genus *Taibaiella* is unique and distinct (Fig. 1.). The overall topology of the maximum-likelihood tree was similar to that of the neighbour-joining and maximum-parsimony trees (Figs. S1 & S2, available in the online Supplementary Material).

Samples were taken for electron-microscopic morphology from 48 h-old cultures grown in TGY-5 broth at 30 °C. Negative staining was carried out on a carbon-coated Formvar film with 1 % (w/v) uranyl acetate, pH 4.4 (Szoboszlay *et al.*, 2008). After staining, the sample was examined with a Morgagni 268D electron microscope at 100 kV. Electron-microscopic morphology of strain TZCO2^T is shown in Figs S3–6. The rod-shaped cells were approximately 1.5 µm long and 0.9 µm in diameter. The cell surfaces were totally smooth and without flagella. Electron micrographs of strain TZCO2^T closely resembled available pictures of *T. koreensis* THG-DT86^T (Son *et al.*, 2014) and *T. chishuiensis* AY17^T (Tan *et al.*, 2014). From these results it can be speculated that a lack of flagella is a common characteristic of members of the genus *Taibaiella*. Cell morphology was also observed at 1000 × magnification with a light microscope (Zeiss; Docuval) using cells grown for 72 h at 30 °C on TGY-10 agar plates. Colonies of the strain grown on TGY-10 agar were smooth, circular, convex, 1.2 × 0.6 mm in diameter, opaque and yellowish-ochre. On TGY-10, TSA and R2A agar they formed regular and rounded colonies, but on TSA agar plates colony diameters were smaller. The most reliable growth of strain TZCO2^T was observed on TGY-10 agar.

Carbon-source utilization and enzyme activities were tested by using API 20E, API 20NE, API ID 32GN, API 50CH and API ZYM test kits (bioMérieux), according to the manufacturer's instructions. All API tests were carried out in parallel (Table 1) with strains TZCO2^T and *T. koreensis* THG-DT86^T. Examination of the OF test, citrate utilization, catalase activity, aesculin hydrolysis, nitrate reduction, Tween 80 hydrolysis, gelatin hydrolysis, growth in 5 % (w/v) NaCl, malonate utilization, phenylalanine deamination and hydrogen sulphide production were carried out by the methods of Barrow & Feltham (1993), verifying the API tests. The Gram-reaction was performed using the non-staining method, as described by Buck (1982). Growth at different temperatures (7, 10, 15, 20, 28, 30, 35, 37 and 40 °C) and pH values (pH 2.0–10.0 in increments of 0.5 pH units) was assessed after 5 days incubation at 30 °C on TGY agar (Merck; for temperature) or broth (for pH). After autoclaving TGE broth, pH was controlled (S220 SevenCompact) and adjusted by adding sterile solutions of HCl or NaOH (1 M each). Salt tolerance was tested after 5 days incubation on TGY-10 agar supplemented with 1–10 % (w/v) NaCl (at 30 °C). Growth on nutrient agar, trypticase soy agar (TSA), R2A agar, marine agar, yeast malt extract agar, MRS agar, LB agar and MacConkey agar (Merck) was also evaluated at 30 °C. All of the tests described above were performed by the National Collection of Agricultural and Industrial Micro-organisms, Budapest, Hungary. Growth under anaerobic conditions was determined in TGY-10 broth with and without the addition of 0.15 % (w/v) KNO_3 at 30 °C. To ensure anaerobic conditions, 100 ml serum bottles (Glasgerätebau Ochs) with 75 ml of TGY-10 broth were crimp-sealed and sparged with N_2 under sterile conditions. The dissolved oxygen concentration in the bottles was measured non-invasively by using a Fibox 3 trace v3 fibre optic oxygen metre with PSt3 sensor spots (PreSens).

Strain TZCO2^T grew slowly on all of the above-mentioned nutrient agars excluding yeast malt extract agar (YMA) and did not grow on MacConkey agar. TGY-10 and modified TSA with $CaCl_2$ and Tween 80 (Son *et al.*, 2014) were the preferred media for multiplication. No anaerobic growth was observed. Lack of nitrate reduction and trypsin activity are unique features of TZCO2^T. In most cases the phenotypic characteristics, measured by API tests, resulted in no reaction, thus these features are able to differentiate this strain from its closest phylogenetic relatives. Other physiological characteristics of strain TZCO2^T are summarized in the species description. Chemotaxonomic analyses (quinone and fatty acid methyl ester analysis) and G+C determination were carried out by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunsweig, Germany. *T. koreensis* THG-DT86^T and TZCO2^T had been grown under the same conditions before the fatty acid composition,

International Journal of Systematic and Evolutionary Microbiology ijsem000873.3d 19/1/2016 19:6:32

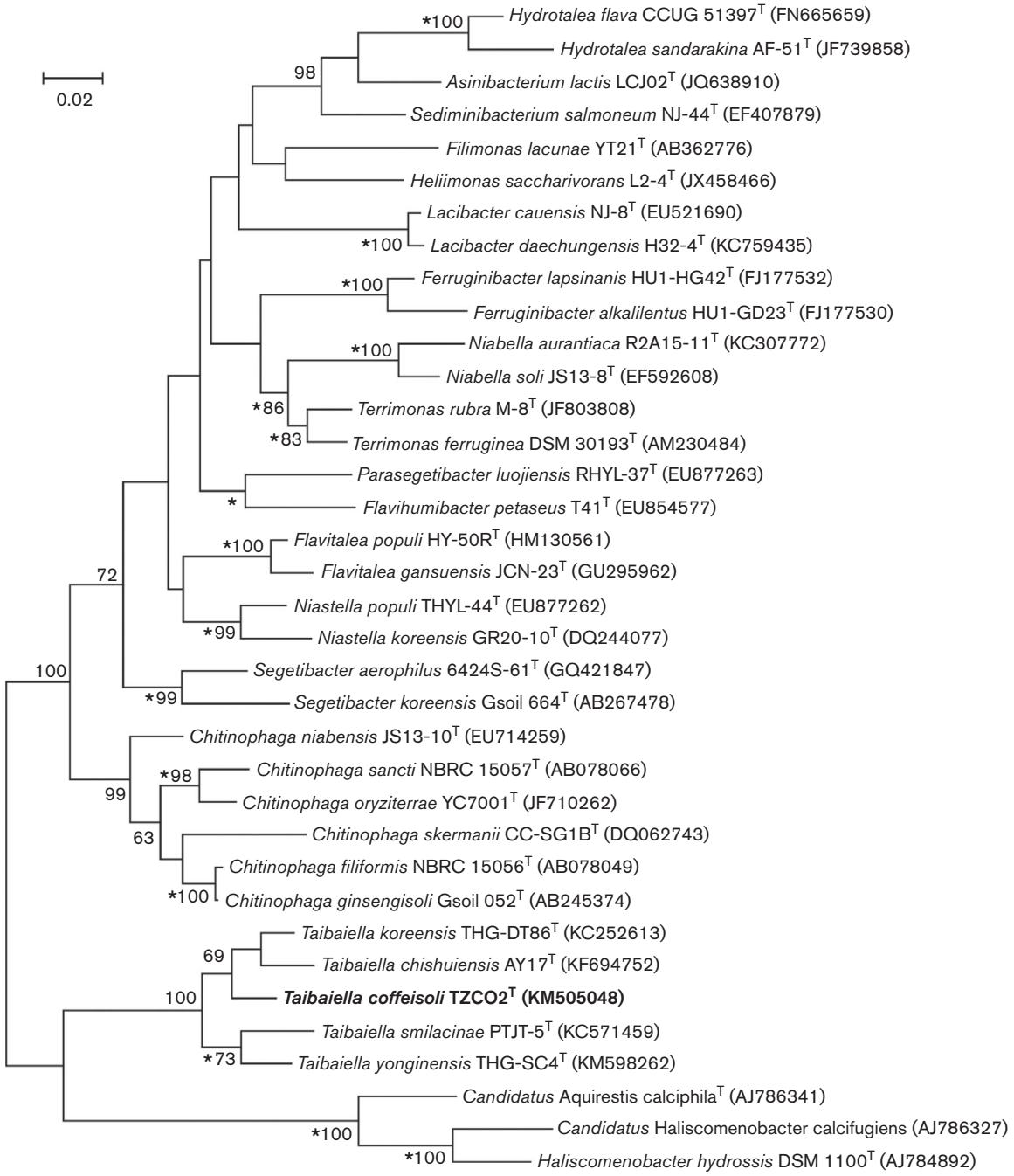

Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequence analysis showing the relative position of *Taibaiella coffeisoli* TZCO2^T among various representative members of the family *Chitinophagaceae*. Branches with an asterisk occurred with every tree-making algorithm used in the study. Bootstrap values are shown as percentages of 1000 replicates; only values ≥50 % are shown. Bar, 0.02 substitutions per nucleotide position.

respiratory lipoquinone and polar lipid analyses (Fig. S7). Sufficient cells of comparable physiological age could be harvested from half of the plates. Fatty acid compounds were estimated with the TSBA40 method by the Identification Service of the DSMZ (Miller, 1982; Kuykendall *et al.*, 1988; Kämpfer & Kroppenstedt, 1996). Respiratory lipoquinones and polar lipids were extracted from 100 mg of freeze-dried cell material using the two-stage method described by Tindall (1990a, b; Tindall *et al.*, 2007). DNA for determination of the genomic G+C content was extracted based on the method of Cashion *et al.* (1977). The G+C ratio determination was carried out

International Journal of Systematic and Evolutionary Microbiology ijsem000873.3d 19/1/2016 19:6:32

Table 1. Differential characteristics between strain TZCO2^T and type strains of related taxa

Taxa: 1, strain TZCO2^T; 2, *T. koreensis* THG-DT86^T. Data are from this study. +, Positive; −, negative. Both strains were positive for flexirubyn-type pigments, hydrolysis of Tween 80, DNA, CM cellulose, gelatin and casein, the oxidase test, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and acid phosphatase. Both strains were negative for the following characteristics: Gram-staining, motility and sporulation, hydrolysis of starch and aesculin. They were also negative for cystine arylamidase, lipase (C14), α-chymotrypsin, α- galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. They were negative for the assimilation of gluconate, L-arabinose, adipate, citrate and for acid production from: glycerol, D-arabinose, D-ribose, D-galactose, D-fructose, L-rhamnose, methyl-α-D-glucopyranosidase, methyl-α-D-mannopyranosidase, amygdalin, arbutin, salicin, D-cellobiose, sucrose, D-melezitose, D-turanose, D-lactose, D-melibiose, D-trehalose, inulin, D-raffinose, gentiobiose and L-fucose.

Characteristic	1	2
Colony colour	Yellowish ochre	Yellow
Motility	−	−
Growth at:	−	−
7 °C	−	−
10 °C	+	+
35 °C	+	+
37 °C	−	+
42 °C	−	−
NaCl tolerance (%, w/v)	0–1.5	0–1.5
Oxidase/Catalase activities	+/+	+/+
pH range for growth	6.5–8.5	6.0–8.5
Flexirubin-type pigments	+	+
Hydrolysis of:		
Starch	−	−
Aesculin	−	−
Tween 80	+	+
DNA	+	+
CM cellulose	+	+
Nitrate reduction	−	+
Enzymatic activities (API ZYM)		
Valine arylamidase	−	+
Trypsin	+	−
Assimilation of (API 20NE):		
D-Mannitol	−	+
Malate	−	+
DNA G+C content (mol%)	51.9	50.1

according to Tamaoka & Komagata (1984) and Mesbah *et al.* (1989). To investigate the presence of flexirubin-type carotenoids in strain TZCO2^T one gram of frozen bacterial cell suspension was crushed in a crucible mortar in the presence of quartz sand to rupture cells. Pigments were extracted by the gradual addition of 10 ml cold chloroform during crushing. The supernatant was decanted and residues were further extracted by crushing and the addition of 10 ml chloroform. The two extracts were pooled and chloroform evaporated under vacuum at a maximum temperature of 40 °C. The pigment was redissolved in 5 ml of HPLC-grade methanol and purified by passing through a 0.22 µm PTFE syringe filter before injection onto an HPLC column. HPLC separation was performed on a Purospher STAR, C18, 3 µm, 240 × 4.6 mm column using a gradient elution of: (A) methanol/water 93 : 7 (v/v), and (B) methanol/acetonitrile/2-propanol 10 : 35 : 55 (by vol.) (Daood & Biacs, 2005). Detection was carried out at 450 nm. Peak identification was based on the retention time and the spectral characteristics as compared to those reported in the literature using similar methods of determination (Venil *et al.*, 2014). The Chromaster Hitachi HPLC instrument consisting of a model 5430 diode-array detector, a model 5210 autosampler and a model 5110 gradient pump was used for the determination of carotenoid-type pigments. The instrument and data processing were operated by EZChrom Elite software.

The DNA G+C content of strain TZCO2^T was 51.9 mol%. The only respiratory quinone was MK-7 (100 %). At the same time, the fatty acid profile of strain TZCO2^T, iso-$C_{15:0}$ (32.4 %), iso-$C_{15:1}$ G (22.6 %), iso-$C_{17:0}$ (15.1 %) and iso-$C_{17:0}$ 3-OH (10.0 %), showed characteristic differences from those of its closest relatives (Table 2). Although similar fatty acid profiles were observed for strains of

Table 2. Cellular fatty acid composition (as a percentage of the total present) of strain TZCO2^T and closely related taxa

Taxa: 1, strain TZCO2^T; 2, *T. koreensis* THG-DT86^T; ~~3, *T. chishuiensis* AY17^T; 4, *T. smilacinae* PTJT-5^T~~. Fatty acids amounting to less than 1 % of the total fatty acids in all strains are not listed. All data are from the present study unless otherwise indicated. TR, Trace amounts (<1 %); −, not detected.

Fatty acid	1	2
Satured		
$C_{16:0}$	TR	TR
Unsatured		
$C_{16:1}\omega11c$	TR	1.5
$C_{17:1}\omega6c$	TR	–
Branched chain		
iso-$C_{15:0}$	32.4	39.4
iso-$C_{15:0}$ 3-OH	1.8	3.3
iso-$C_{15:1}$ G	22.6	19.2
iso-$C_{16:0}$	1.4	TR
iso-$C_{16:1}$ G	TR	–
iso-$C_{17:0}$	15.1	12.1
iso-$C_{17:0}$ 3-OH	10.0	12.6
iso-$C_{17:1}\omega10c$	TR	TR
anteiso-$C_{15:0}$	1.9	1.1
anteiso-$C_{17:0}$	4.0	TR
Unknown		
ECL 13.565	2.8	3.7
ECL 16.582	1.1	1.8

~~*Data from Tan *et al.* (2014) and Zhang *et al.* (2013).~~

International Journal of Systematic and Evolutionary Microbiology ijsem000873.3d 19/1/2016 19:6:33

species belonging to the genus *Taibaiella*, in which iso-$C_{15:0}$ iso-$C_{15:1}$ G, iso-$C_{17:0}$ and iso-$C_{17:0}$ 3-OH are the major fatty acids, significant differences in the proportions were observed. This finding also confirms that strain TZCO2^T differs at the species level from other members of the genus *Taibaiella*. Flexirubin-type pigments were detectable in strain TZCO2^T, indicating that these may be a common characteristic of members of the genus *Taibaiella*.

Therefore, on the basis of the low 16S rRNA gene sequence similarities between strain TZCO2^T and its closest relatives within the genus *Taibaiella*, and the results of the chemotaxonomic, biochemical and physiological analysis, strain TZCO2^T is considered to represent a novel species within the genus *Taibaiella* for which the name *Taibaiella coffeisoli* sp. nov. is proposed.

Description of *Taibaiella coffeisoli* sp. nov.

Taibaiella coffeisoli [cof.fe.i.so'li. N.L. fem. n. *Coffea* the coffee plant; N. neut. n. *solum* soil; N.L. gen. n. *coffeisoli* of soil of the coffee plant].

4 Cells are Gram-stain-negative, obligately aerobic, non-spore-forming, non-motile, 0.6–0.7 × 1.2–1.4 μm in size and devoid of flagella. Within 5 days at 30 °C colonies grown on TGY-10 agar plates are smooth, circular, convex, non-luminescent with regular margins, opaque and yellowish-ochre in colour. The most reliable growth is observed on TGY-10 and modified TSA agar. Slow and unambiguous growth is observed on R2A, marine and nutrient agars. Colonies are also formed on MRS and LB agars, but at an even slower rate. No growth is observed on YMA or MacConkey agar. Growth is observed at temperatures of between 10 and 35 °C and between pH 6.0 and 8.5. The optimal growth temperature and pH are 30 °C and pH 7.0–7.5, respectively. Growth occurs in the absence of NaCl and in the presence of 0.5–1.5% (w/v) NaCl, thus not in the presence of 2.0% (w/v) NaCl. Oxidase and catalase-positive. Flexirubin-type pigments are present. Casein, Tween 80, DNA and CM-cellulose are hydrolysed, but starch and aesculin are not. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Negative reactions are observed for lipase (C14), valine-arylamidase, cystine arylamidase, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Gelatin assimilation was positive, but none of the carbohydrates tested are assimilated (API 50CH and API ID 32GN). The major fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ and iso-$C_{17:0}$ 3-OH. Menaquinone-7 is the only (100%) respiratory lipoquinone present. The polar lipids of strain TZCO2^T consist of phosphoaminolipid, phosphatidylethanolamine, nine unidentified lipids (L1–9), three unidentified aminolipids (AL1–3) and one unidentified phospholipid.

The type strain, TZCO2^T (=NCAIM 02601^T=CCM 8601^T) was isolated from a soil sample of an irrigated coffee plantation in Arusha, Tanzania, East Africa. The DNA G+C content of the type strain is 51.9 mol%. 5

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International Journal of Systematic and Evolutionary Microbiology ijsem000873.3d 19/1/2016 19:6:33

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