

Citricoccus parietis sp. nov., isolated from a mould-colonized wall and emended description of *Citricoccus alkalitolerans* Li et al. 2005

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A Gram-positive, coccoid-shaped organism (strain 02-Je-010^T), forming yellow-pigmented colonies was isolated from the wall of an indoor environment. On the basis of 16S rRNA gene sequence similarity studies, it was shown that strain 02-Je-010^T belongs to the genus *Citricoccus* with sequence similarities of 98.9% to *Citricoccus alkalitolerans* DSM 15665^T and 98.6% to *Citricoccus muralis* DSM 14442^T. Cell-wall sugars were mannose and glucose. The diagnostic diamino acid of the peptidoglycan was lysine. The major menaquinones detected were MK-9(H₂) and MK-8(H₂). The polar lipid profile consisted of the major lipids diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol and moderate amounts of two unknown phospholipids and two unknown glycolipids. The fatty acid profile comprised major amounts of anteiso-C_{15:0}, anteiso-C_{17:0} and iso-C_{15:0}. All these data supported the affiliation of strain 02-Je-010^T to the genus *Citricoccus*. The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain 02-Je-010^T from the two recognized *Citricoccus* species. For these reasons, strain 02-Je-010^T represents a novel species, for which the name *Citricoccus parietis* sp. nov. is proposed, with the type strain 02-Je-010^T (=CCUG 57388^T=CCM 7609^T).

The genus *Citricoccus*, originally proposed by Altenburger et al. (2002) comprises the two species *Citricoccus muralis*, isolated from a medieval wall painting (Altenburger et al., 2002), and *Citricoccus alkalitolerans*, isolated from a desert soil sample collected in Egypt (Li et al., 2005). Both species were isolated from dry samples.

Strain 02-Je-010^T was enriched and recovered from a wall colonized with mould. After extraction of a 1 g material sample by shaking for 15 min in 10 ml 0.9% NaCl solution containing 0.01% (v/v) Tween 80, aliquots of this suspension were spread on plates containing organic medium M79 agar [containing (l⁻¹): 10 g glucose, 10 g peptone (Bacto), 2 g casein hydrolysate, 2 g yeast extract, 6 g NaCl and 15 g agar; pH 7.0]. The plates were incubated for 2 weeks at 28 °C. The isolated strain was maintained on organic medium M79 and preserved at –80 °C by mixing in a 1:1 ratio of well-grown cultures in organic medium M79 broth with glycerol preservation medium (Salser, 1978),

containing (w/v): 1.26% K₂HPO₄, 0.36% KH₂PO₄, 0.01% MgSO₄·H₂O, 0.09% sodium citrate, 0.18% (NH₄)₂SO₄ and 8.8% glycerol. Stock cultures of the isolates in liquid M79 supplemented with 5% DMSO were additionally maintained in the vapour phase of liquid nitrogen. Morphological properties, Gram-staining and cell morphology were observed microscopically as described by Kämpfer & Kroppenstedt (2004). Isolation of the DNA was performed with a commercialized DNA extraction kit (GenElute Plant Genomic DNA kit; Sigma) after disruption of cells by using a 1 min bead-beating step with 1 g 0.1 mm Ø Zirconia beads at maximum speed.

Multiple sequence alignment and analysis of the data were performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 4 (Tamura et al., 2007). Genetic distances were calculated using the Kimura-2 model. Clustering by using the neighbour-joining (Fig. 1) and maximum-parsimony (results not shown) methods were performed after bootstrap analysis based on 1000 replications. The 16S rRNA gene sequence of strain 02-Je-010^T was a continuous stretch of 1415 bp.

Sequence similarity calculations after the neighbour-joining analysis indicated that the closest relatives of strain

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 02-Je-010^T is FM992367.

A table showing the major fatty acid compositions of strain 02-Je-010^T and the type strains of species of the genus *Citricoccus* is available as supplementary material with the online version of this paper.

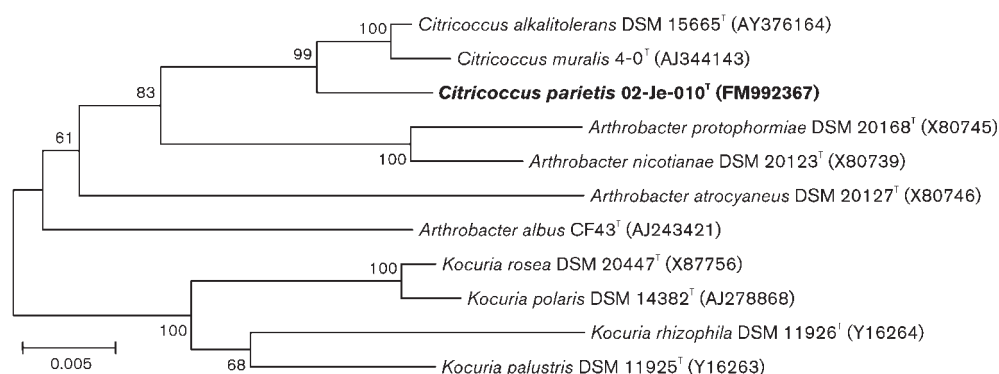


Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory database (accession numbers are given in parentheses). Multiple alignment, distances (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were performed by using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 4 (Tamura *et al.*, 2007). Percentage bootstrap values based on 1000 replications are given at branch points. Bar, 0.005 substitutions per nucleotide position.

02-Je-010^T were *C. alkalitolerans* DSM 15665^T (98.9%), and *C. muralis* DSM 14442^T (98.6%).

Bacterial biomass of the isolate for chemotaxonomic investigations was prepared by cultivating strain 02-Je-010^T for 24–48 h in shake flasks in liquid organic medium M79 at 180 r.p.m. at 28 °C, except for the fatty acid analyses where cells were grown in tryptic soy broth.

Standard paper chromatography, HPLC and TLC procedures were used to determine the quinone system (Collins *et al.*, 1977; Groth *et al.*, 1996), whole-organism sugars (Becker *et al.*, 1965) and polar lipids (Minnikin *et al.*, 1979). Analysis of the amino acids in peptidoglycan hydrolysates was carried out according to Schleifer & Kandler (1972) and as described by Groth *et al.* (1996). Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The diagnostic diamino acid of the peptidoglycan was lysine together with glutamic acid and glycine (variation A4 α); whole-organism hydrolysates contained sugars composed of mannose and glucose.

Strain 02-Je-010^T exhibited a quinone system with the predominant menaquinones MK-9(H₂) and MK-8(H₂) (57 and 25%, respectively). Minor amounts of MK-9 (5%) and MK-8 (3%) were also detected.

The polar lipid profile was rather complex, consisting of seven components. The major lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol; two unknown glycolipids and two unknown phospholipids were also present. Such a complex pattern of phospholipids, with the three major components and some unknown glyco- and phospholipids, was also described for *C. muralis*, whereas only two unknown glycolipids were found in *C. alkalitolerans*.

The fatty acid profile of strain 02-Je-010^T was very similar to those of *C. alkalitolerans* DSM 15665^T and *C. muralis* DSM 14442^T (Supplementary Table S1, available in IJSEM

Online). The chemotaxonomic markers of strain 02-Je-010^T analysed were typical for the genus *Citricoccus* and supported the affiliation to this genus.

Strain 02-Je-010^T was grown on PYES medium (Altenburger *et al.*, 2002) for the observation of growth at the following temperatures: 4, 10, 20, 28, 37, 40 and 45 °C. Tolerance to NaCl and pH was determined as described by Altenburger *et al.* (2002). Growth was observed between 4 and 36 °C (but not above that temperature), at initial pH values 6.5–12.0, with optimum growth at pH 8.0–9.0, and in 1–10% NaCl.

Results of further comparative physiological characterization, using identical test conditions, are given in Table 1 and the species description, with methods as described previously (Kämpfer *et al.*, 1991). DNA–DNA hybridization experiments were performed with strain 02-Je-010^T and *C. alkalitolerans* DSM 15665^T and *C. muralis* DSM 14442^T using the method described by Ziemke *et al.* (1998), with a minor variation in the nick translation step, where 2 µg DNA was labelled during a 3 h incubation at 15 °C.

We assumed a G+C content of the DNA of 68 mol% for strain 02-Je-010^T, as reported for *C. muralis* (Altenburger *et al.*, 2002), in the DNA–DNA hybridization experiments. Because this G+C content is about 4 mol% higher than the value reported for *C. alkalitolerans* by Li *et al.* (2005), the G+C content of the type strain of *C. alkalitolerans* was reanalysed at the DSMZ. The G+C content of *C. alkalitolerans* DSM 15665^T was 67.8 mol%.

Strain 02-Je-010^T showed relatively low DNA–DNA relatedness to *C. alkalitolerans* DSM 15665^T (58.8%, reciprocal 62.7%) and *C. muralis* DSM 14442^T (40.4%, reciprocal 34.8%). The observed physiological differences between these type strains (Table 1) clearly warrant the creation of a separate species.

Table 1. Differential physiological characteristics of the type strains of *Citricoccus* species

Taxa: 1, strain 02-Je-010^T (*Citricoccus parietis* sp. nov.); 2, *C. alkalitolerans* DSM 15665^T; 3, *C. muralis* DSM 14442^T. All data are from this study. All strains were positive for utilization of *cis*-aconitate, L-aspartate, citrate, fumarate, D-glucose*, 3-hydroxybenzoate, DL-3-hydroxybutyrate, L-leucine, L-malate, D-maltitol, maltose*, D-mannose*, 2-oxoglutarate, L-phenylacetate, L-proline, pyruvate, sucrose* and trehalose. All strains were negative for utilization of *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine*, D-adonitol, β-alanine*, L-arabinose†, arbutin, cellobiose, D-fructose*, D-galactose†, *myo*-inositol, itaconate, D-mannitol, melibiose, mesaconate, L-ornithine, L-rhamnose, ribose†, D-sorbitol, sucrose, L-tryptophan* and D-xylose. +, Positive; –, negative.

Characteristic	1	2	3
Assimilation of:			
Adipate, azelate, glutarate, L-histidine*, putrescine, suberate	+	–	–
Acetate, 4-hydroxybenzoate, L-phenylalanine, propionate, L-serine	+	–	+
<i>trans</i> -Aconitate, L-alanine	–	+	–
4-Aminobutyrate	–	+	+
DL-Lactate	+	–	+

*Data congruent with those reported by Li *et al.* (2005) for *C. alkalitolerans*.

†Data not congruent with those reported by Li *et al.* (2005) for *C. alkalitolerans*.

Description of *Citricoccus parietis* sp. nov.

Citricoccus parietis (pa.ri'e.tis. L. gen. n. *parietis* of the wall of a house).

Coccoid cells, about 1.3 μm in diameter. Gram-positive, and oxidase-positive and catalase-positive, showing an oxidative metabolism. Good growth occurs after 3 days incubation on tryptone soy agar, R2A agar and nutrient agar at 25–30 °C. Colonies are yellow-pigmented, glistening, circular and opaque. Growth was observed at 4–36 °C (but not above that temperature), at pH 6.5–12.0, with optimum growth at pH 8.0–9.0, and in 1–10 % NaCl. The quinone system is composed of MK-9(H₂) and MK-8(H₂).

The peptidoglycan is of the Lys–Glu–Gly type (variation A4α). The polar lipid profile consists of the major lipids diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol, and two unknown glycolipids and two unknown phospholipids.

Major fatty acids are iso- and anteiso-branched fatty acids, such as anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}. Small amounts of iso-C_{16:0}, iso-C_{17:0} and C_{16:0} are also found. Carbon source utilization (including differential characteristics determined under identical conditions) is indicated in Table 1.

The type strain, 02-Je-010^T (=CCUG 57388^T=CCM 7609^T), was isolated in Jena, Germany, from the wall of a house colonized with mould.

Emended description of *Citricoccus alkalitolerans* Li *et al.* 2005

The description is that of Li *et al.* (2005) with the following emendations. The G+C content of the DNA is 67.8 mol% (HPLC method). Negative for the utilization of D-galactose, D-arabinose and D-ribose on the basis of the method of Kämpfer *et al.* (1991). Results of some physiological tests are different based on the method of Kämpfer *et al.* (1991).

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