Thorsellia anophelis gen. nov., sp. nov., a new member of the *Gammaproteobacteria*

Peter Kämpfer,¹ Jenny M. Lindh,² Olle Terenius,² Siamak Haghdoust,² Enevold Falsen,³ Hans-Jürgen Busse⁴ and Ingrid Faye²

Correspondence

Peter Kämpfer
peter.kaempfer@agrar.
uni-giessen.de

¹Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, IFZ-Heinrich-Buff-Ring 26–32, D-35392 Giessen, Germany

²Department of Genetics, Microbiology and Toxicology, S-106 91 Stockholm University, Stockholm, Sweden

³CCUG, Culture Collection University of Göteborg, Göteborg, Sweden

⁴Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, Wien, Austria

A Gram-negative, rod-shaped organism (CCUG 49520^T) was isolated from the midgut of the mosquito *Anopheles arabiensis*. 16S rRNA gene sequence analysis demonstrated that this isolate is unique, showing <93% similarity to species of the families *Enterobacteriaceae* and *Vibrionaceae*. The quinone system consisted exclusively of ubiquinone Q-8; the polar lipid profile consisted of the major compounds phosphatidylethanolamine and phosphatidylglycerol, a moderate to minor amount of two unknown aminophospholipids, an unknown phospholipid and two unknown polar lipids; the polyamine pattern was characterized by the predominant compound 1,3-diaminopropane and showed some significant differences when compared with members of the *Enterobacteriaceae* and *Vibrionaceae*. On the basis of 16S rRNA gene sequence analysis in combination with chemotaxonomic data, strain CCUG 49520^T is considered to represent a new genus and species, for which the name *Thorsellia anophelis* gen. nov., sp. nov. is proposed. The type strain is CCUG 49520^T (= CIP 108754^T).

During the characterization of organisms from the midgut of the mosquito *Anopheles arabiensis* originating from Kenya, strain H2.1^T was recovered (Lindh *et al.*, 2005). Subcultivation was performed on nutrient agar (Oxoid) at 37 °C for 24 h for up to 7 days and strain CCUG 49520^T was recovered. On this agar, CCUG 49520^T was able to grow at 10–37 °C, but not at 4 or 45 °C. Growth was slow in general. The organism was able to grow on nutrient agar, tryptone soy agar and R2A agar, and also weakly on MacConkey agar (all from Oxoid). Tests for growth were performed in Luria–Bertani broth at 10–50 °C (5 °C intervals) with shaking at 160 r.p.m. Growth occurred at 15–45 °C with an optimum at 30 °C. The generation time at 30 °C was 100 min. Gram-staining was performed as described by Gerhardt *et al.* (1994). Preparation of bacterial DNA for G+C content estimations was performed using the blood and cell culture

DNA midi kit from Qiagen according to the manufacturer's instructions. G+C content was determined by HPLC (Svoboda & Harms-Ringdahl, 2002). Cell morphology was observed under a Zeiss light microscope at ×1000, using cells that had been grown for 3 days at 37 °C on nutrient agar; results are given under the species description below.

The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). Analysis of the sequence data was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001), after multiple alignments of the data using CLUSTAL X (Thompson *et al.*, 1997). A distance matrix method (distance options according to the Kimura two-parameter model), including clustering by neighbour-joining (Fig. 1a), and a discrete character-based maximum-parsimony method (Fig. 1b) were used. In each case, bootstrap values were calculated based on 1000 replications. The 16S rRNA gene sequence of strain CCUG 49520^T was a continuous stretch of 1498 bp. Sequence similarity calculations indicated that strain CCUG 49520^T showed the greatest degree of similarity to *Serratia rubidaea* DSM 4480^T (GenBank accession no. AJ233436; 92.1%). Lower sequence similarities (<92%) were found with all other species of the family *Enterobacteriaceae*.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CCUG 49520^T is AY837748.

A two-dimensional thin-layer chromatogram of the polar lipids of strain CCUG 49520^T is available as supplementary material in IJSEM Online.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers given in parentheses) constructed after multiple alignments of the data by CLUSTAL x (Thompson *et al.*, 1997). (a) Neighbour-joining tree. Distances (distance options according to the Kimura two-parameter model) were calculated and clustering with the neighbour-joining method was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branch points. Bar, 2% sequence divergence. (b) Phylogenetic tree constructed under the maximum-parsimony criterion performed by using the software package MEGA with the following settings. For maximum-parsimony tree search options: the heuristic search (Close-Neighbour-Interchange). For scoring changes: the MEGA standard method (in which all nucleotide changes are weighted equally). Tree length is given by the sum of minimum numbers of substitutions. Bootstrap values based on 1000 replications are listed as percentages at branch points. Bar, 50 substitutions.

Chemotaxonomic analyses were made of the respiratory quinones (Tindall, 1990; Altenburger *et al.*, 1996), polar lipids (Tindall, 1990), fatty acids (Kämpfer & Kroppenstedt, 1996) and polyamines (Busse & Auling, 1988; Busse *et al.*, 1997).

The fatty acid profile of strain CCUG 49520^T (given in the species description) was similar to those of other species of the *Enterobacteriaceae*.

The detection of a polar lipid profile with the major compounds phosphatidylethanolamine and phosphatidylglycerol (see Supplementary Fig. S1 available in IJSEM Online) is in good agreement with characteristics of representatives

of the *Enterobacteriaceae* and *Vibrionaceae* (for review see Wilkinson, 1988). However, species of the *Enterobacteriaceae* were reported also to contain significant amounts of diphosphatidylglycerol, while this lipid was shown to be present only in trace amounts in species of the *Vibrionaceae*. No diphosphatidylglycerol was detected in polar lipid extracts of strain CCUG 49520^T, in contrast to results for *Serratia* (Wilkinson, 1988). The polar lipid profile of strain CCUG 49520^T is more similar to those of members of the *Vibrionaceae*.

Detection of the quinone system Q-8 is in agreement with the phylogenetic affiliation of CCUG 49520^T because Q-8 has been reported to be present in all representatives of the

Enterobacteriaceae and *Vibrionaceae* (Yokota *et al.*, 1992). The detection of a polyamine pattern with the single major compound 1,3-diaminopropane clearly distinguishes strain CCUG 49520^T from species of the *Vibrionaceae*, which were reported characteristically to contain *sym*-norspermidine (Yamamoto *et al.*, 1983, 1991; Hamana, 1997). Among more than 70 analysed species of *Enterobacteriaceae*, a similar polyamine pattern has only been reported for five strains of *Erwinia amylovora* (Zherebilo *et al.*, 2001) and *Serratia marcescens* IAM 12142^T [only when grown on a highly specified cell culture medium (Hamana, 1996)]. However, the polyamine pattern of this latter strain also contained moderate amounts of putrescine and cadaverine (> 10 % of the total polyamine content). On a more common medium (nutrient broth), the same *Serratia* strain showed a polyamine pattern with the predominant compound cadaverine and moderate to major amounts of 1,3-diaminopropane and putrescine. All *Serratia* species examined by Hamana (1996) exhibited as a common feature in the polyamine pattern the presence of moderate to major amounts of cadaverine (> 10 % of the total polyamine content) and most of them also contain at least moderate amounts of putrescine. In the polyamine pattern of CCUG 49520^T, by contrast, cadaverine was completely lacking and putrescine could only be detected in trace amounts. Hence, the polyamine pattern alone allows differentiation of strain CCUG 49520^T from the indicated relatives of the family *Enterobacteriaceae*, including the genus *Serratia*.

Results of the physiological characterization of strain CCUG 49520^T are given in the species description, determined using the methods of Kämpfer (1990) and Kämpfer *et al.* (1991). Strain CCUG 49520^T was able to produce acid from various carbohydrates, but carbon substrate utilization tests showed weak results, even after incubation for several days. On the basis of the results presented we propose that strain CCUG 49520^T constitutes a member of a new genus and species, for which the name *Thorsellia anophelis* gen. nov., sp. nov. is proposed.

Description of *Thorsellia* gen. nov.

Thorsellia (Thor.sel'li.a. N.L. fem. n. *Thorsellia* named in honour of Walborg Thorsell, a pioneer on mosquito repellent research in Sweden).

Cells are Gram-negative, facultatively anaerobic, motile and rod-shaped. Growth on all tested media is slow. Growth occurs after 48 h incubation on tryptone soy agar and nutrient agar at 37 °C; good growth also occurs at 30 °C. Growth occurs in Luria–Bertani broth at 15–45 °C with an optimum at 30 °C, at which the generation time is 100 min. 1,3-Diaminopropane is the characteristic compound in the polyamine pattern, ubiquinone Q-8 is the single quinone and in the polar lipid profile phosphatidylethanolamine and phosphatidylglycerol are predominant. The major fatty acids are C_{16:0}, C_{18:1}ω7c and C_{14:0}. The DNA G + C content is 46 mol%. The type species is *Thorsellia anophelis*.

Description of *Thorsellia anophelis* sp. nov.

Thorsellia anophelis (a.no'phe.lis. N.L. gen. n. *anophelis* from a mosquito of the genus *Anopheles*).

Exhibits the following properties in addition to those given in the genus description. Slow growth occurs after 24 h incubation on tryptone soy agar and nutrient agar at 37 °C. The fatty acid profile comprises C_{16:0} (32.9 %), C_{18:1}ω7c (35.9 %), C_{14:0} (12.2 %), C_{18:0} (1.0 %), C_{12:0} 3-OH (0.8 %), summed feature 2 (C_{14:0} 3-OH/iso C_{16:1}, 5.4 %) and summed feature 3 (C_{16:1}ω7c/iso-C_{15:0} 2-OH, 8.0 %). The polar lipid profile contains moderate to minor amounts of two unknown aminophospholipids, an unknown phospholipid and two unknown polar lipids; when the organism is grown under strictly aerobic conditions, the quinone system consists exclusively of ubiquinone Q-8; the polyamine pattern consists of the predominant compound 1,3-diaminopropane [94.1 μmol (g dry weight)⁻¹], spermidine [1.5 μmol (g dry weight)⁻¹] and traces [< 1.0 μmol (g dry weight)⁻¹] of putrescine and *sym*-homospermidine. Aesculin is hydrolysed. Positive for acid production from glucose (O/F positive after 48 h incubation), lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, rhamnose, maltose, trehalose, cellobiose, D-arabitol and D-mannose. No acid is produced from sorbitol, L-arabinose, raffinose, D-xylose, methyl D-glucoside, erythritol or melibiose. The following carbon sources are utilized (only after prolonged incubation, 7 days): *p*-arbutin, D-gluconate, D-glucose, sucrose, D-trehalose, D-xylose, adonitol, *myo*-inositol, maltitol, D-mannitol, acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, fumarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, mesaconate, pyruvate, L-alanine and L-leucine. The following substrates are not utilized as carbon sources: *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, salicin, D-sorbitol, putrescine, 4-aminobutyrate, azelate, citrate, glutarate, itaconate, oxoglutarate, suberate, β-alanine, L-aspartate, L-histidine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. Negative for hydrolysis of *p*-nitrophenyl (pNP) β-D-glucopyranoside, *o*-nitrophenyl (oNP) β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-xylopyranoside, bis-pNP phosphate, pNP phenylphosphonate, pNP phosphorylcholine, 2-deoxythymidine-5'-pNP phosphate, L-alanine *p*-nitroanilide (pNA), L-glutamate-γ-3-carboxy pNA and L-proline pNA.

The type strain is CCUG 49520^T (= CIP 108754^T), isolated from the midgut contents of the mosquito *Anopheles arabiensis*.

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