Trabulsiella odontotermis sp. nov., isolated from the gut of the termite *Odontotermes formosanus* Shiraki

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Bacterial strains Eant 3-9^T and Eant 3-3, isolated from the gut of the termite *Odontotermes formosanus*, were characterized using a polyphasic taxonomic approach. Cells of these organisms were Gram-negative, non-pigmented, rod-shaped, non-endospore-forming and facultatively anaerobic. Phenotypic, physiological and genetic characteristics of the two strains were highly similar. Phylogenetic analyses using 16S rRNA gene sequences showed that strain Eant 3-9^T formed a monophyletic branch towards the periphery of the evolutionary radiation occupied by the genus *Trabulsiella*; its closest neighbour was *Trabulsiella guamensis* DSM 16940^T (98.1 % similarity). Genomic DNA–DNA relatedness between strains Eant 3-9^T and Eant 3-3 was 96 ± 4 %, indicating that they belong to a single species. Eant 3-9^T displayed DNA–DNA relatedness values of 48 ± 5 and 14 ± 3 % with *T. guamensis* DSM 16940^T and *Salmonella typhimurium* ATCC 13311^T, respectively, indicating that Eant 3-9^T warrants novel species status in the genus *Trabulsiella*. Eant 3-9^T and Eant 3-3 could be further distinguished from *T. guamensis* DSM 16940^T using whole-cell protein profiles and a range of physiological and biochemical characteristics. It is evident from genotypic and phenotypic data that Eant 3-9^T represents a novel species in the genus *Trabulsiella*, for which the name *Trabulsiella odontotermis* sp. nov. is proposed. The type strain is Eant 3-9^T (=BCRC 17577^T=LMG 23580^T); Eant 3-3 is a reference strain.

The genus *Trabulsiella* proposed by McWhorter *et al.* (1991) belongs to the family *Enterobacteriaceae*, a member of the *Gammaproteobacteria*. At the time of writing, the genus *Trabulsiella* encompasses a single species, *Trabulsiella guamensis*. Five similar strains, including the type strain of *T. guamensis*, were isolated from dust collected inside a vacuum cleaner, whereas other strains similar to *T. guamensis* have been isolated from soil samples (one strain) and human faeces (two strains) (McWhorter *et al.*, 1991). Strains of *T. guamensis* were previously classified as Centers for Disease Control and Prevention (CDC) Enteric Group 90 (based on their biochemical similarity to strains of *Salmonella*). However, strains of *T. guamensis* did not agglutinate any of the *Salmonella* typing antisera O or H. Based on DNA relatedness results, McWhorter *et al.* (1991)

clearly separated *T. guamensis* from 62 tested strains in the family *Enterobacteriaceae*. In the addendum section, these workers described two different biochemical patterns in the *T. guamensis* strains, but they did not name them as biogroups. The eight strains from the USA and Guam were negative for indole production, gelatin hydrolysis and aesculin hydrolysis, whereas the four strains from Germany and Malaysia were positive for these reactions, and whole genome DNA–DNA hybridization between these four strains and the type strain was 98 %. Hence, based on these data, two different biogroups of *T. guamensis* were identified (Lindquist & Farmer, 2005). Recently, J. J. Farmer (personal communication) found a second group of three strains in the Enterics Reference Laboratory at CDC that were H₂S-negative and may represent a second species in this genus (Lindquist & Farmer, 2005). The aim of the present study was to determine the taxonomic position of two novel H₂S-negative strains, Eant 3-9^T and Eant 3-3, isolated from the termite gut.

Eant 3-9^T and Eant 3-3 were isolated from the gut of the termite *Odontotermes formosanus* Shiraki, collected from a decayed bamboo tree located in Pingtung County in

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains Eant 3-9^T and Eant 3-3 are DQ453129 and DQ453130, respectively.

Electrophoretic protein patterns of the novel strains and related bacteria and tables showing phenotypic characteristics and antibiograms of members of the genus *Trabulsiella* are available as supplementary material in IJSEM Online.

southern Taiwan. For this purpose, the termites were surface-sterilized with 75 % ethanol for 10 s and 0.1 % (w/v) mercuric chloride for 10 min, rinsed several times in sterile distilled water, crushed and streaked on desoxycholate agar (BD Difco) plates and incubated at 25 °C. The isolates thus obtained were further maintained and subcultivated on nutrient agar (BD Difco).

Bacterial cultures were observed in the lag, exponential and stationary phases of growth under a phase-contrast microscope to ascertain the shape and motility (hanging drop method) of the cells. Gram staining was tested using a Gram Stain Set S kit (BD). The optimum pH range for growth was examined by measuring the OD₅₉₅ of the culture grown in tryptic soy broth (BD Difco) and nutrient broth adjusted to various pH values (pH 4–10 at intervals of 0.5 pH units) using the appropriate biological buffers (Chung *et al.*, 1995). Tolerance of strains to various NaCl levels was tested in nutrient broth adjusted to different NaCl concentrations (0, 0.5 % and 1.0–5.0 %, w/v, at intervals of 1.0 %). The optimum temperature for growth of the strains was examined in tryptic soy broth and nutrient broth adjusted to pH 7. Growth was examined by measuring the turbidity of cultures grown at various temperatures (4–45 °C). Growth under anaerobic conditions was determined after incubating the strains in the Oxoid AnaeroGen system. Growth was monitored by measuring the optical density of the cultures with respect to time.

Phenotypically and morphologically, strains Eant 3-9^T and Eant 3-3 showed similar characteristics. Good growth was observed aerobically and anaerobically in complex media including nutrient medium, Luria–Bertani medium and tryptic soy medium, indicating that strains Eant 3-9^T and Eant 3-3 were facultatively anaerobic. Cells of Eant 3-9^T and Eant 3-3 were Gram-negative, motile, non-spore-forming and rod-shaped (0.5–0.7 µm in diameter and 1.0–1.5 µm in length). Eant 3-9^T and Eant 3-3 formed visible circular, convex, semi-transparent colonies with entire edges. The colony size of Eant 3-9^T and Eant 3-3 was 0.5–2.0 mm in diameter on nutrient agar after 48 h incubation at 28 °C. Good growth was observed at 15–42 °C, 0–5 % NaCl and pH 5–10. Optimum growth was observed at 28–35 °C, 0–1 % NaCl and pH 7.0.

Extraction of genomic DNA, PCR amplification and 16S rRNA gene sequencing was carried out as described previously (Chen *et al.*, 2001). Sequence analysis was achieved using a DNA sequencer (ABI PRISM 310; Applied Biosystems) and sequence assembly was carried out by using the Fragment Assembly System program from Wisconsin Package 9.1 supplied by the National Health Research Institute of Taiwan. The resultant sequences were compared with available 16S rRNA gene sequences in the EMBL, GenBank and RDP II databases. Multiple sequence alignment of strains Eant 3-9^T and Eant 3-3 with their closest relatives was performed using BIOEDIT software (Hall, 1999). Phylogenetic trees were inferred using the maximum-parsimony and neighbour-joining (Saitou & Nei, 1987)

tree-making algorithms. An evolutionary distance matrix was generated for the neighbour-joining algorithm using the Jukes & Cantor (1969) distance model and bootstrap analysis (1000 resamplings).

Nearly complete 16S rRNA gene sequences of 1450 and 1449 nt, respectively, were obtained for strains Eant 3-9^T and Eant 3-3. The 16S rRNA gene sequence similarity between Eant 3-9^T and Eant 3-3 was 99.6 %. A comparison of the sequences with those of representatives of genera classified in the family *Enterobacteriaceae* of the *Gamma-proteobacteria* showed that the organisms fell within the evolutionary radiation occupied by the genus *Trabulsiella* (Fig. 1). The overall topologies of phylogenetic trees obtained by the neighbour-joining and maximum-parsimony (data not shown) methods were similar. According to sequence similarity calculations, strain Eant 3-9^T was phylogenetically most closely related to *T. guamensis* DSM 16940^T (98.1 % similarity), *Citrobacter koseri* CDC 3613-63^T (97.6 % similarity), *Salmonella enterica* subsp. *enterica* ATCC 9150 (97.6 % similarity), *Salmonella typhimurium* ATCC 13311^T (97.6 % similarity), *Raoultella planticola* ATCC 33558^T (97.0 % similarity), *Enterobacter cloacae* subsp. *cloacae* ATCC 13047^T (96.7 % similarity), *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 13883^T (96.6 % similarity) and *Serratia liquefaciens* JCM 1245^T (96.6 % similarity). The high bootstrap value (100 %) provides strong support for the inclusion of strain Eant 3-9^T in the genus *Trabulsiella*.

Whole genome DNA–DNA hybridization experiments were performed with photobiotin-labelled probes as described previously by Ezaki *et al.* (1989) between strains Eant 3-9^T, Eant 3-3, *T. guamensis* DSM 16940^T and *Salmonella typhimurium* ATCC 13311^T. The degree of hybridization was calculated by means of triplicate experiments. The DNA–DNA relatedness value between Eant 3-9^T and Eant 3-3 was 96 ± 4 %. The high degree of DNA–DNA relatedness and the high levels of 16S rRNA gene similarity between the two isolates indicate that the two strains represent a single species. The separate species status of strain Eant 3-9^T was demonstrated by the DNA–DNA hybridization values obtained with *T. guamensis* DSM 16940^T and *Salmonella typhimurium* ATCC 13311^T (48 ± 5 and 14 ± 3 %, respectively). The thermal stability of DNA was determined further by the methods outlined by Fernandez *et al.* (1989). A lower melting temperature (ΔT_m value, less than 1.0 °C) was observed between Eant 3-9^T and Eant 3-3, whereas a higher melting temperature (ΔT_m value, 6.5 ± 1.0 °C) was observed between *T. guamensis* DSM 16940^T and Eant 3-9^T. The DNA–DNA relatedness between strain Eant 3-9^T and its closest phylogenetic neighbour, *T. guamensis* DSM 16940^T, is well below the 70 % (ΔT_m value, 5 °C) cut-off point recommended for assignment of strains to the same genomic species (Wayne *et al.*, 1987). Based on the above DNA–DNA relatedness and DNA thermal stability data, isolate Eant 3-9^T warrants separate species status in the genus *Trabulsiella*.

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 data showing the position of strains Eant 3-9^T and Eant 3-3 in the genus *Trabulsiella*. Distances and clustering with the neighbour-joining method were performed by using the software package BIOEDIT. Numbers at nodes are percentage bootstrap values based on 1000 resampled datasets; only values above 50% are given. Bar, 1% sequence dissimilarity. The sequence of *Pseudoxanthomonas kaohsiungensis* J36^T was used as an outgroup.

For G + C content calculations, a DNA sample was prepared and degraded enzymically into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture obtained was then separated by HPLC. The G + C contents of strains Eant 3-9^T and *T. guamensis* DSM 16940^T were 55.7 ± 0.2 and 54.9 ± 0.3 mol%, respectively.

Further differentiation of strains Eant 3-9^T and Eant 3-3 from their closest phylogenetic neighbours was examined by protein electrophoretic patterns and fatty acid profiling. Preparation of whole-cell proteins and SDS-PAGE were carried out as described by Pot *et al.* (1994). Whole-cell protein profiles of strains Eant 3-9^T and Eant 3-3 were highly

similar, but differed from those of *T. guamensis* DSM 16940^T and *Salmonella typhimurium* ATCC 13311^T (Supplementary Fig. S1, available in IJSEM Online). Biomass for the preparation of fatty acid methyl esters was grown in shake flasks of nutrient broth for 24 h at 28 °C, checked for purity, harvested by centrifugation, washed twice with distilled water and freeze-dried. Fatty acid methyl esters were then prepared, separated and identified according to the instructions of the Microbial Identification System (MIDI; Microbial ID). The fatty acid compositions of strains Eant 3-9^T and *T. guamensis* DSM 16940^T are shown in Table 1. The fatty acid profile of Eant 3-9^T was similar to that of *T. guamensis* DSM 16940^T, but the fatty acid proportions differed (Table 1).

The sensitivity of the cultures to different antibiotics was checked by the disc diffusion method (Bauer *et al.*, 1966) (Supplementary Table S1). *T. guamensis* DSM 16940^T, Eant 3-9^T and Eant 3-3 were susceptible to ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), rifampicin (5 µg), streptomycin (10 µg), sulfamethoxazole (23.75 µg) plus trimethoprim (1.25 µg) and tetracycline (30 µg), but were resistant to penicillin G (10 U).

Strains Eant 3-9^T and Eant 3-3 and *T. guamensis* DSM 16940^T were examined for a broad range of phenotypic properties, notably for the features known to be of value in *Enterobacteriaceae* systematics (Farmer *et al.*, 1985) using conventional tests. Additional biochemical tests were performed by Biolog-GN2, API ZYM (bioMérieux) and API 20E (bioMérieux) microtest systems according to the

Table 1. Major fatty acid compositions (%) of strain Eant 3-9^T and *T. guamensis* DSM 16940^T

Summed feature 1 comprises 13:0 3-OH and/or 15:1 i i/H; summed feature 2 comprises 14:0 3-OH and/or 16:1 iso I; summed feature 3 comprises 16:1 ω7c and/or 15:0 iso 2-OH. Fatty acid values less than 0.5 % are not given. Both strains were grown on nutrient broth (BD Difco) at 28 °C for 24 h prior to fatty acid analysis.

Fatty acid	Eant 3-9 ^T	<i>T. guamensis</i> DSM 16940 ^T
12:0	3.8	4.0
13:0	0.8	0.9
14:0	8.4	6.9
16:0	30.0	26.8
17:0	1.4	1.9
17:0 cyclo	16.8	11.5
18:1 ω7c	15.9	21.1
19:0 cyclo ω8c	3.5	1.2
Summed feature 1	0.6	0.7
Summed feature 2	8.1	8.1
Summed feature 3	9.9	14.5
Unknown 14.502	0.7	0.6

methods outlined by the manufacturers. The results of physiological and biochemical characteristics of the strains are listed in Supplementary Table S2 and in the species description. It is apparent from the results (Table 2) that strains Eant 3-9^T and Eant 3-3 can be distinguished clearly from their closest phylogenetic relative *T. guamensis* DSM 16940^T by nitrate reduction, an H₂S-negative reaction, acetoin production (Voges–Proskauer reaction) and weak tyrosine clearing activity (Table 2). Despite physiological and biochemical attributes, results of DNA–DNA hybridization, fatty acid composition and whole-cell protein profiles clearly distinguished strains Eant 3-9^T and Eant 3-3 from *T. guamensis* DSM 16940^T.

It is clear from the genotypic and phenotypic data that strains Eant 3-9^T and Eant 3-3 represent a novel species in the genus *Trabulsiella*. The name *Trabulsiella odontotermis* sp. nov. is proposed for this taxon, with Eant 3-9^T as the type strain.

Description of *Trabulsiella odontotermis* sp. nov.

Trabulsiella odontotermis (o.don.to.ter'mi.tis. N.L. n. *Odontotermes* the scientific name of a genus of termite; N.L. gen. n. *odontotermis* of a termite of the genus *Odontotermes*).

Facultatively anaerobic, Gram-negative, motile, non-spore-forming and rod-shaped. After 24 h growth on nutrient agar at 25 °C, the mean cell size is about 0.5–0.7 × 1.0–1.5 µm. Growth is observed at 28, 30, 37 and 42 °C. Positive (API 20E) for catalase, ONPG test, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, acetoin production and acid production from glucose, mannitol, sorbitol, rhamnose, amygdalin and arabinose. Negative for oxidase, nitrate reduction, H₂S production, urease, tryptophan deaminase, indole production, gelatinase, inositol, sucrose and melibiose. Positive (API ZYM) for alkaline phosphatase, C4 esterase, leucine arylamidase, trypsin, α-chymotrypsin,

Table 2. Phenotypic characteristics that differentiate strains Eant 3-9^T and Eant 3-3 from *T. guamensis* DSM 16940^T

Strains: 1, Eant 3-9^T (*T. odontotermis* sp. nov.); 2, Eant 3-3 (*T. odontotermis* sp. nov.); 3, *T. guamensis* DSM 16940^T. All results for *T. guamensis* DSM 16940^T were determined in the present study. +, Positive; –, negative; W, weak reaction.

Characteristic	1	2	3
H ₂ S production*	–	–	+
Voges–Proskauer	+	+	–
Nitrate reduction	–	–	+
Tyrosine clearing	W	W	+
G + C content (mol%)	55.7	55.5	54.9

*Identical results were obtained with API 20NE, triple-sugar iron agar and peptone iron agar.

acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -glucosidase, β -glucosidase and α -fucosidase, but negative for C8 lipase, C14 lipase, valine arylamidase, α -galactosidase and β -glucuronidase. The following compounds (Biolog GN2) are oxidized: dextrin, glycogen, Tween 40, Tween 80, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, D-cellobiose, D-fructose, D-galactose, gentiobiose, α -D-glucose, maltose, D-mannitol, D-mannose, D-psicose, L-rhamnose, D-sorbitol, D-trehalose, methyl pyruvate, monomethyl succinate, acetic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, *p*-hydroxyphenylacetic acid, DL-lactate, D-saccharic acid, succinic acid, bromosuccinic acid, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, L-ornithine, L-phenylalanine, L-proline, L-serine, urocanic acid, inosine, uridine, thymidine, putrescine, glycerol, DL- α -glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate. The following compounds are not oxidized: α -cyclodextrin, adonitol, D-arabitol, *i*-erythritol, *myo*-inositol, α -D-lactose, lactulose, D-melibiose, methyl β -D-glucoside, D-raffinose, sucrose, turanose, xylitol, *cis*-aconitic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, malonic acid, propionic acid, sebacic acid, succinamic acid, glucuronamide, L-alaninamide, hydroxy-L-proline, L-leucine, L-pyroglutamic acid, D-serine, L-threonine, DL-carnitine, γ -aminobutyric acid, phenylethylamine, 2-aminoethanol and 2,3-butanediol. Utilization of L-fucose, formic acid and quinic acid is strain-specific and differs between the two strains isolated. The major fatty acids are 16:0, 17:0 cyclo, 18:1 ω 7c, summed feature 2 (14:0 3-OH and/or 16:1 iso I) and summed feature 3 (16:1 ω 7c and/or 15 iso 2-OH).

The type strain is Eant 3-9^T (=BCRC 17577^T=LMG 23580^T). Strain Eant 3-9^T and reference strain Eant 3-3 were isolated from the gut of the termite *Odontotermes formosanus* Shiraki from southern Taiwan. The DNA G+C contents of strains Eant 3-3 and Eant 3-9^T are 55.5 and 55.7 mol%, respectively.

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