Comamonas odontotermitis sp. nov., isolated from the gut of the termite *Odontotermes formosanus*

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A bacterial strain, designated Dant 3-8^T, isolated from the gut of the termite *Odontotermes formosanus*, was investigated by a polyphasic taxonomic approach. The cells were rod-shaped, Gram-negative, non-pigmented, non-spore-forming and non-fermentative. Phylogenetic analyses using the 16S rRNA gene sequence showed that the strain formed a monophyletic branch towards the periphery of the evolutionary radiation occupied by the genus *Comamonas*, its closest neighbours being *Comamonas testosteroni* DSM 50244^T (96.4 % sequence similarity), *Comamonas koreensis* KCTC 12005^T (96.0 %) and *Comamonas terrigena* DSM 7099^T (96.2 %). Strain Dant 3-8^T was clearly distinguished from all of these strains by using phylogenetic analysis, DNA–DNA hybridization, whole-cell protein profiles, fatty acid composition data and a range of physiological and biochemical characteristics. It is evident from the genotypic and phenotypic data that Dant 3-8^T represents a novel species in the genus *Comamonas*, for which the name *Comamonas odontotermitis* sp. nov. is proposed. The type strain is Dant 3-8^T (= BCRC 17576^T = LMG 23579^T).

The genus *Comamonas*, proposed by De Vos *et al.* (1985), belongs to family *Comamonadaceae* of the class *Betaproteobacteria*. At the time of writing, the genus encompasses eight recognized species: *Comamonas terrigena*, *C. aquatica*, *C. badia*, *C. denitrificans*, *C. kerstersii*, *C. koreensis*, *C. nitrativorans* and *C. testosteroni*. The aim of the present study was to determine the taxonomic position of strain Dant 3-8^T, isolated from the gut of the termite *Odontotermes formosanus* (Shiraki).

Strain Dant 3-8^T was isolated from the gut of termites collected from a decayed bamboo tree located in Pingtung County in southern Taiwan. For this purpose, termites were surface-sterilized with 75 % ethanol for 10 s and with 0.1 % (w/v) mercuric chloride for 10 min, rinsed several times in sterile distilled water, crushed and streaked on desoxycholate agar plates (BD Difco) and incubated at 25 °C. The strain was further maintained and subcultivated on nutrient agar (BD Difco) at 25 °C. Type strains of *C. badia*,

C. denitrificans, *C. koreensis*, *C. nitrativorans* and *C. testosteroni* were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and type strains of *C. aquatica*, *C. kerstersii* and *C. terrigena* were obtained from the Laboratorium voor Microbiologie – Bacteriënverzameling (LMG) for comparison.

Cultural and morphological characteristics were observed on nutrient agar. Cell morphology was observed under a light microscope. The motility of cells was tested by the hanging drop method. The Gram stain kit-S (BD Difco) was used for Gram staining. The optimum pH range for growth was examined by measuring the OD₅₉₅ of cultures 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 appropriate biological buffers (Chung *et al.*, 1995). Tolerance of the strain to various levels of NaCl was tested in nutrient broth, which was adjusted to different NaCl concentrations (w/v; 0, 0.5 % and 1.0–5.0 % at intervals of 1.0 %). The optimum temperature for growth was examined in tryptic soy broth and nutrient broth adjusted to pH 7. Growth was examined by measuring the OD₅₉₅ of cultures grown at various temperatures (4–45 °C). Growth under anaerobic conditions was determined after incubating the strains in the Oxoid AnaeroGen system.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Dant 3-8^T is DQ453128.

Whole-cell protein profiles of strain Dant 3-8^T and related strains are available as supplementary material in IJSEM Online.

Cells of strain Dant 3-8^T were Gram-negative, motile, non-spore-forming rods, 0.5–1.0 µm in diameter and 2.0–2.5 µm long. No accumulation of poly-β-hydroxybutyrate granules as inclusion bodies was observed. Strain Dant 3-8^T formed visible circular colonies with an umbonate elevation, semi-transparent with irregular edges. The colony diameter was approximately 1.5–3.0 mm on nutrient agar after 48 h of incubation at 25 °C. Strain Dant 3-8^T grew well at 15–37 °C, 0–2 % NaCl and pH 6–9. Optimum growth was observed at 28–35 °C, 0–1 % NaCl and pH 7.0. Strain Dant 3-8^T was unable to grow at 25 °C after 120 h of incubation under anaerobic conditions.

Extraction of chromosomal DNA, PCR amplification and sequencing of the 16S rRNA gene was carried out as described previously (Chen *et al.*, 2001). Sequence analysis was achieved using a DNA sequencer (ABI PRISM 310 instrument; Applied Biosystems) and sequences were assembled by using the Fragment Assembly System program from the Wisconsin package 9.1. The resultant sequence was compared with available 16S rRNA gene sequences from the RDP and GenBank databases. Multiple-sequence alignment including strain Dant 3-8^T and its closest relatives was performed using BioEdit software (Hall, 1999). Phylogenetic trees were inferred using the maximum-parsimony (Kluge & Farris, 1969) 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).

A nearly complete 16S rRNA gene sequence (1453 bp) was obtained for strain Dant 3-8^T. A comparison of the sequence with those of representatives of genera classified in the family *Comamonadaceae* of the class *Betaproteobacteria* showed that the organism fell within the evolutionary radiation occupied by the genus *Comamonas* (Fig. 1). According to sequence similarity calculations, the organism was most closely related to *C. testosteroni* DSM 50244^T (96.4 % similarity), *C. koreensis* KCTC 12005^T (96.0 %), *C.*

terrigena DSM 7099^T (96.2 %), *C. kerstersii* LMG 3475^T (95.9 %), *C. nitrivorans* 23310^T (95.4 %), *C. aquatica* LMG 2370^T (95.3 %), *C. denitrificans* 123^T (95.0 %) and *C. badia* IAM 14839^T (93.1 %). The 16S rRNA gene sequence similarity of strain Dant 3-8^T with other species within the class *Betaproteobacteria* was less than 95 %.

Whole genome DNA–DNA hybridization experiments were performed with photobiotin-labelled probes as described by Ezaki *et al.* (1989). The degree of hybridization was calculated by means of triplicate experiments. The separate species status of strain Dant 3-8^T was demonstrated by the hybridization values obtained when it was hybridized with *C. terrigena* LMG 5929^T, *C. koreensis* DSM 18232^T and *C. testosteroni* DSM 50244^T, which respectively showed 56.0 ± 3.3 , 40.2 ± 5.4 and 14.5 ± 3.5 % DNA–DNA relatedness. The DNA–DNA relatedness between strain Dant 3-8^T and its closest phylogenetic neighbours was well below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987). Based on the above DNA–DNA relatedness data, isolate Dant 3-8^T warrants separate species status in the genus *Comamonas*.

For G + C content calculations, DNA samples were prepared in duplicate and degraded enzymically into nucleosides as described by Mesbah *et al.* (1989). The obtained nucleoside mixture was then separated by HPLC. The DNA G + C content of strain Dant 3-8^T was 61.6 mol% (individual measurements of 61.1 and 62.1 mol%).

Further differentiation of strain Dant 3-8^T from its closest phylogenetic neighbours was examined by protein electrophoretic patterns and fatty acid profiling. Biomass for fatty acid studies was grown in nutrient medium for 2 days at 28 °C as described by Chang *et al.* (2002) and Tago & Yokota (2004). Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (MIDI Inc.). Preparation of whole-cell proteins and SDS-PAGE were performed as described by Pot

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses) constructed after multiple alignments of data showing the position of strain Dant 3-8^T in the genus *Comamonas*. Distances were calculated and clustering with the neighbour-joining method was 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.

et al. (1994). Electrophoretic protein patterns were recorded on a Umax Astra 1220 S scanner (Umax Systems) and analysed with the Universal Software 1D Advanced from Advanced American Biotechnology & Imaging (Fullerton, CA, USA) for normalization of the protein profiles and numerical analysis using UPGMA. A simple band-matching method was used for clustering. Bands with migration distance differences of less than 3 % were considered as the same. Correlation coefficients are expressed as percentage similarity.

Predominant fatty acids of strain Dant 3-8^T were 16:0 (33.6 %), 18:1 ω 7c (16.2 %) and summed feature 3 (16:1 ω 7c and/or 15:0 iso 2-OH; 33.9 %). The fatty acid pattern of strain Dant 3-8^T is shown in Table 1 in comparison with those of representative *Comamonas* species. Although strain Dant 3-8^T showed a profile typical of *Comamonas* (Chang *et al.*, 2002; Wauters *et al.*, 2003; Tago & Yokota, 2004), it could be clearly distinguished from the type strains of its closest phylogenetic neighbours *C. koreensis*, *C. terrigena* and *C. testosteroni* by the absence of 14:0, 15:0 and 17:0 (Table 1). The whole-cell protein profile of strain Dant 3-8^T was comparable to those of the type strains of *C. koreensis*, *C. testosteroni*, *C. terrigena* and *C. kerstersii* within 87 % similarity (Supplementary Fig. S1 available in IJSEM Online), but few band positions unique to Dant 3-8^T could be identified. These results further warrant a separate position for strain Dant 3-8^T within the genus *Comamonas*.

Strain Dant 3-8^T was examined for a broad range of phenotypic properties. Additional biochemical tests were

performed to assess the carbon source utilization pattern by the Biolog GN2 and API ZYM, API 20E and API 20NE (bioMérieux) microtest systems according to the methods outlined by the manufacturers. Sensitivity of strain Dant 3-8^T to various antibiotics was examined by disc diffusion assays (Bauer *et al.*, 1966). The antibiotic discs contained ampicillin (10 μ g), chloramphenicol (30 μ g), gentamicin (10 μ g), kanamycin (30 μ g), nalidixic acid (30 μ g), novobiocin (30 μ g), rifampicin (5 μ g), penicillin G (10 U), streptomycin (10 μ g) or tetracycline (30 μ g). The effect of antibiotics on cell growth was assessed after 3 days of incubation.

Detailed results of biochemical characterization and antibiotic sensitivity are provided in Table 2 and in the species description. It is evident from Table 2 that there are several phenotypic characters that readily separate strain Dant 3-8^T from phylogenetically related species. Taking together the results of 16S rRNA gene sequencing, DNA–DNA hybridization and chemotaxonomic analyses, it is evident that strain Dant 3-8^T should be classified within a novel species in the genus *Comamonas*, for which the name *Comamonas odontotermitis* sp. nov. is proposed.

Description of *Comamonas odontotermitis* sp. nov.

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

Table 1. Comparison of the fatty acid composition of strain Dant 3-8^T with those of other *Comamonas* species

Strains: 1, Dant 3-8^T (*C. odontotermitis* sp. nov.); 2, *C. koreensis* DSM 18232^T; 3, *C. testosteroni* DSM 50244^T; 4, *C. terrigena* LMG 1253^T; 5, *C. denitrificans* DSM 17887^T; 6, *C. nitrivorans* DSM 13191^T; 7, *C. aquatica* LMG 2370^T; 8, *C. kerstersii* LMG 3475^T; 9, *C. badia* DSM 17552^T. Data in columns 2–4 and 9 were obtained from Chang *et al.* (2002) and Tago & Yokota (2004). Values are percentages of total fatty acids; –, fatty acids representing less than 0.5 %.

Fatty acid	1	2	3	4	5	6	7	8	9
10:0 3-OH	3.8	3.5	4.8	5.3	4.2	5.0	5.0	4.5	2.4
12:0	2.7	2.3	2.4	2.8	3.0	2.9	3.0	2.6	2.9
14:0	–	1.0	1.0	3.3	3.2	3.4	3.9	2.9	1.5
15:0	–	9.4	1.0	3.7	–	–	–	–	–
15:0 2-OH	–	0.6	–	–	–	–	–	–	–
16:0	33.6	29.9	30.4	27.5	17.8	21.2	25.2	23.4	33.3
16:0 2-OH	2.5	2.2	2.0	–	–	–	–	–	2.3
16:1 2-OH	–	–	0.6	–	–	–	–	–	–
17:0	–	2.6	0.8	1.5	–	–	–	0.6	–
17:0 cyclo	5.9	12.3	3.8	2.4	–	–	–	0.7	–
17:1	–	0.7	–	–	–	–	–	–	–
18:1/18:1 ω 7c	16.2	9.6	17.9	14.9	22.4	23.8	19.0	36.1	13.1
19:0 cyclo	–	–	0.9	–	–	–	–	–	–
20:0 iso	–	–	1.1	–	–	–	–	–	–
Summed feature 3*	33.9	26.1	33.1	38.4	48.6	42.9	42.4	28.2	41.9

*Summed feature 3 comprises 16:1 ω 7c and/or 15:0 iso 2-OH.

Table 2. Genotypic, phenotypic and nutritional characteristics that distinguish strain Dant 3-8^T from other *Comamonas* species

Strains: 1, Dant 3-8^T (*C. odontotermis* sp. nov.); 2, *C. koreensis* DSM 18232^T; 3, *C. testosteroni* DSM 50244^T; 4, *C. terrigena* LMG 1253^T; 5, *C. denitrificans* DSM 17887^T; 6, *C. nitrivorans* DSM 13191^T; 7, *C. aquatica* LMG 2370^T; 8, *C. kerstersii* LMG 3475^T; 9, *C. badia* DSM 17552^T. Data for motility and G+C content for reference strains were obtained from Chang *et al.* (2002), Etchebehere *et al.* (2001), Gumaelius *et al.* (2001), Tago & Yokota (2004) and Wauters *et al.* (2003); the remaining data were obtained in this study. +, Positive; –, negative; R, resistant; S, sensitive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Source	Termite gut	Wetland	Soil	Hay-infusion filtrate	Activated sludge	Sludge	Freshwater	Human	Activated sludge
Motility	+	–	+	+	+	+	+	+	+
Nitrite reduction to nitrogen	–	–	–	–	+	–	–	–	–
Alkaline phosphatase	+	+	+	+	–	–	–	–	–
Acid phosphatase	+	+	+	+	+	+	+	+	–
C8 lipase	+	+	+	+	–	+	+	+	+
C14 lipase	–	+	–	–	–	–	–	–	–
Valine arylamidase	+	+	+	+	+	+	–	+	+
Cystine arylamidase	–	–	+	–	–	–	+	–	–
Assimilation of (API 20NE):									
D-Gluconate	+	+	+	+	–	–	+	–	+
Caprate	–	–	+	–	–	–	–	–	–
Adipate	–	+	+	+	–	+	+	+	+
Malate	+	+	+	–	–	+	+	+	+
Citrate	+	+	+	–	–	–	–	–	+
Phenylacetate	+	–	–	–	–	+	–	–	+
Oxidation of (Biolog GN2):									
Acetate	+	+	+	–	–	+	+	+	–
cis-Aconitate	–	–	–	+	+	+	–	–	–
Propionate	+	+	+	+	–	+	+	–	+
γ-Hydroxybutyrate	+	+	–	+	+	–	+	+	+
Itaconate	–	–	–	–	–	–	+	+	–
D- and L-Serine	–	+	–	–	–	–	–	–	+
L-Threonine	+	+	+	–	+	+	–	+	–
L-Phenylalanine	+	+	–	–	–	+	–	+	–
Hydroxy-L-proline	+	+	–	–	–	–	–	–	–
L-Ornithine	+	–	–	–	–	–	–	–	+
Glycyl L-aspartate	+	–	+	–	+	–	–	–	+
Glycyl L-glutamate	+	+	+	–	–	–	–	–	+
N-Acetyl-D-galactosamine	–	–	–	–	–	+	–	–	+
Tween 40	+	+	+	–	–	+	–	+	+
Tween 80	+	+	+	–	–	+	+	+	–
Susceptibility to:									
Ampicillin	R	R	R	S	S	S	S	R	R
Gentamicin	S	S	R	S	S	S	S	S	S
Penicillin G	R	R	R	S	S	S	S	S	S
Rifampicin	R	S	S	S	S	S	S	S	R
Streptomycin	S	S	R	S	S	S	S	S	R
DNA G+C content (mol%)	61.6	66	62.5–64.5	64	60.8	ND	64	61	66.3

Odontotermes, referring to the isolation of the type strain from the termite *O. formosanus*).

Aerobic, Gram-negative, non-spore-forming, motile and rod-shaped. After 24 h growth on nutrient agar at 25 °C, the mean cell size is 0.5–1.0 µm width and 2.0–2.5 µm length.

Optimum growth occurs at 28–35 °C, 0–1 % NaCl and pH 7.0. In API 20NE tests, shows positive reactions for oxidase (weak), catalase (weak), nitrate reduction and assimilation of gluconate, malate, citrate and phenylacetate and negative reactions for indole production, hydrolysis of aesculin and gelatin, glucose fermentation, arginine

dihydrolase, urease, β -galactosidase and assimilation of glucose, arabinose, mannose, maltose, *N*-acetylglucosamine, caprate and adipate. In API 20E tests, positive for acetoin production and negative for the ONPG test, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S production, tryptophan deaminase, indole production, gelatinase and acid production from glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In API ZYM tests, shows positive enzyme reactions for alkaline phosphatase, C4 esterase, C8 lipase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative reactions for C14 lipase, cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, α -mannosidase, *N*-acetyl- β -glucosaminidase and α -fucosidase. The following compounds are oxidized in the Biolog GN2 microtitre test system: glycogen, Tweens 40 and 80, methyl pyruvate, monomethyl succinate, acetic acid, formic acid, α -, β - and γ -hydroxybutyric acids, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactate, propionic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, D- and L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyrroglutamic acid and L-threonine. Cannot oxidize α -cyclodextrin, dextrin, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α -D-glucose, *myo*-inositol, α -D-lactose, lactulose, maltose, D-mannose, D-melibiose, methyl β -D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, *cis*-aconitic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, *p*-hydroxyphenylacetic acid, itaconic acid, malonic acid, quinic acid, D-saccharic acid, glucuronamide, alaninamide, L-histidine, D- or L-serine, DL-carnitine, γ -aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL- α -glycerol phosphate, α -D-glucose 1-phosphate or D-glucose 6-phosphate. Resistant to ampicillin, penicillin G and rifampicin and sensitive to chloramphenicol, gentamicin, kanamycin, nalidixic acid, novobiocin, streptomycin and tetracycline. The major fatty acids are 16:0 (33.6%), 18:1 ω 7c (16.2%) and summed feature 3 (16:1 ω 7c and/or 15:0 iso 2-OH; 33.9%). The DNA G+C content is 61.6 mol%.

The type strain Dant 3-8^T (= BCRC 17576^T = LMG 23579^T) was isolated from the gut of the termite *O. formosanus* (Shiraki) from southern Taiwan.

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