Vogesella perlucida sp. nov., a non-pigmented bacterium isolated from spring water

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A transparent, non-pigmented, Gram-negative, rod-shaped bacterium, designated strain DS-28^T, was isolated from water samples collected from a spring located in Tainan County, Taiwan. 16S rRNA gene sequence analysis indicated that the novel strain formed a monophyletic branch at the periphery of the evolutionary radiation occupied by the genus *Vogesella*; the only sole close neighbour of the novel strain was *Vogesella indigofera* ATCC 19706^T (97.4 % 16S rRNA gene sequence similarity). The isolate was distinguished from *V. indigofera* on the basis of genotypic data, several phenotypic properties and an inability to produce characteristic blue-pigmented colonies on peptone agar. The fatty acid profile was slightly different from that reported for *V. indigofera* ATCC 19706^T. It was evident from the genotypic and phenotypic data that strain DS-28^T represents a novel species of the genus *Vogesella*, for which the name *Vogesella perlucida* sp. nov. is proposed. The type strain is DS-28^T (=BCRC 17730^T=LMG 24214^T).

During the characterization of micro-organisms present in water samples collected from a spring located in Tainan County, Taiwan, several transparent or colourless bacterial colonies were isolated on nutrient agar (BD Difco). All isolates showing similar colony morphology were selected and a representative isolate, designated strain DS-28^T, was selected for detailed analysis. Strain DS-28^T was maintained on R2A agar (BD Difco) plates after incubation at 25 °C for 3 days. Subculturing was performed on R2A agar at 25 °C for between 48 and 72 h. On this medium, strain DS-28^T was able to grow at 15–45 °C, but not at 10 or 50 °C.

To ascertain the morphology of strain DS-28^T, cells in the lag, exponential and stationary phases of growth were observed under a phase-contrast microscope (DM 2000; Leica). The motility of the cells was tested by means of the hanging drop method. Staining of flagella was performed using the Spot Test Flagella Stain (BD Difco). A Gram stain kit (Set S; BD Difco) and the Ryu non-staining KOH method (Powers, 1995) were used to test the Gram staining reaction. Poly- β -hydroxybutyrate granule accumulation was observed under light microscopy after staining the cells with Sudan black. Colony morphology was observed on R2A agar by using a stereoscopic microscope (SMZ 800;

Nikon). Details of the morphology are given in the species description below.

The pH range for growth was determined by measuring the optical densities (at 600 nm) of cultures grown on nutrient broth (BD Difco) adjusted, prior to sterilization, to various pH values (pH 4–10, using increments of 0.5 pH units) using the appropriate biological buffers (Chung *et al.*, 1995). To investigate the tolerance of NaCl, nutrient broth was prepared according to the formula of the BD Difco medium but with NaCl at various concentrations (0, 0.5 and 1.0–10.0 %, w/v, using increments of 1.0 %). Anaerobic growth was determined after incubating strain DS-28^T in an Oxoid AnaeroGen system. Strain DS-28^T was investigated for a broad range of phenotypic properties. Catalase, oxidase, DNase, urease and lipase activities and hydrolysis of starch, casein, chitin, Tweens 20, 40, 60 and 80 were determined using standard methods (Gerhardt *et al.* 1994; Lányi, 1987; MacFaddin, 2000). Phenotypic characteristics, biochemical tests, carbon-source utilization (Biolog GN2) and API ZYM, API 20E and API 20NE (all from bioMérieux) reaction profiles were investigated.

The 16S rRNA gene sequence was analysed as described previously by Chen *et al.* (2001). Analysis of the sequence data was performed by using the BioEdit software package (Hall, 1999) and MEGA version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data using CLUSTAL_X (Thompson *et al.*, 1997). A distance matrix method (with distance options according to the Kimura two-parameter

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-28^T is EF626691.

Colonies, cells and genotypic/phenotypic characteristics of strain DS-28^T are compared with those of closely related phylogenetic neighbours in supplementary material available with the online version of this paper.

Fig. 1. Phylogenetic tree for strain DS-28^T and related strains, based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses), constructed after multiple alignments of data using CLUSTAL_X (Thompson *et al.*, 1997). Distances (with distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were performed using the MEGA software package version 3.1 (Kumar *et al.*, 2004). Numbers at the nodes are bootstrap percentages (>50 %) based on both neighbour-joining (above nodes) and maximum-parsimony (below nodes) tree-making algorithms. Filled circles indicate branches of the tree that were also recovered using the maximum-likelihood and maximum-parsimony tree-making algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.01 substitutions per nucleotide position.

model; Kimura, 1983), including clustering by neighbour-joining (Saitou & Nei, 1987) (Fig. 1) and a discrete character-based maximum-parsimony (Kluge & Farris, 1969) method, was used. In each case, bootstrap percentages (based on 1000 replications) were calculated. The 16S rRNA gene sequence of strain DS-28^T was a continuous stretch of 1414 bp. The 16S rRNA gene sequence analysis indicated that strain DS-28^T belonged to the class *Betaproteobacteria*, forming a distinct subline within the genus *Vogesella*. Bootstrap resampling showed that the association between strain DS-28^T and the type strain of the only recognized member of the genus *Vogesella*, *Vogesella indigofera* ATCC 19706^T, was statistically significant. The 16S rRNA gene sequence similarities (over 1350 bp) calculated for DS-28^T indicated that the strain was closely related to *V. indigofera* ATCC 19706^T (97.4%), *Gulbenkiania mobilis* E4FC31^T (94.6%), *Aquitalea magnusonii* TRO-001DR8^T (93.1%), *Chromobacterium subtsugae* PRAA4-1^T (93.1%), *Chromobacterium violaceum* ATCC 12472^T (92.9%) and *Laribacter hongkongensis* DSM 14985^T (92.6%). Lower sequence similarities (<92.0 %) were found with respect to representative members of the other genera shown in Fig. 1. The overall topologies of the phylogenetic trees obtained using neighbour joining, maximum likelihood or maximum parsimony were similar.

Fatty acid methyl esters were prepared, separated and identified according to the instructions of the Microbial Identification System (Microbial ID; Sasser, 1990). The predominant fatty acid constituents of strain DS-28^T were

summed feature 3 (*C*_{16:1} ω 7*c* or iso-*C*_{15:0} 2-OH) and *C*_{16:0}. The fatty acid profile of strain DS-28^T was generally comparable with that of *V. indigofera* ATCC 19706^T, but differed in the proportions of certain fatty acids (Table 1).

The DNA G+C content of strain DS-28^T was estimated (*n*=2) as described previously by Mesbah *et al.* (1989). The nucleoside mixture was separated by means of HPLC. The DNA G+C content of strain DS-28^T was found to be 61.8±1.0 mol%.

Table 1. Comparison of long-chain fatty acid compositions (%) of strain DS-28^T and *V. indigofera* ATCC 19706^T

Data for *V. indigofera* were taken from the MIDI database. Both strains were grown on tryptic soy broth agar medium at 25 °C.

Fatty acid	Strain DS-28 ^T	<i>V. indigofera</i> ATCC 19706 ^T
<i>C</i> _{10:0}	2.40	—*
<i>C</i> _{10:0} 3-OH	4.75	5.25
<i>C</i> _{12:0}	2.93	2.86
<i>C</i> _{14:0}	1.17	0.73
<i>C</i> _{16:0}	18.10	26.62
<i>C</i> _{18:1} ω 7 <i>c</i>	3.83	3.92
Summed feature 3†	61.68	56.88

*Less than 0.5 %.

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

DNA–DNA hybridization experiments were performed with *V. indigofera* ATCC 19706^T by using the method described by Ezaki *et al.* (1989). The results indicated low levels of DNA relatedness between strain DS-28^T and its closest phylogenetic neighbour, *V. indigofera* ATCC 19706^T (7.5 ± 1.8 %). These values are clearly below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987).

The sensitivity of strain DS-28^T to different antibiotics was tested after spreading cells (0.5 McFarland) on Mueller–Hinton agar (BD Difco) plates. The antibiotic discs (Oxoid) contained following antibiotics: ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), rifampicin (5 µg), penicillin G (10 µg), streptomycin (10 µg), sulfamethoxazole (23.75 µg) plus trimethoprim (1.25 µg) and tetracycline (30 µg). The effects of the antibiotics on cell growth were assessed after 3 days.

Detailed results from the phenotypic and biochemical characterization of strain DS-28^T are provided in Table 2 and in the species description. Phenotypic characteristics that serve to differentiate strain DS-28^T from *V. indigofera* ATCC 19706^T and other closely related strains are presented in Table 2 and in Supplementary Table S1 (available in IJSEM Online). Strain DS-28^T was unable to produce the blue-pigmented colonies on peptone agar or R2A agar that are characteristic of *V. indigofera* ATCC 19706^T (see Supplementary Fig. S1 in IJSEM Online). Strain DS-28^T produced acid from a few carbohydrates, such as mannitol, inositol and sucrose. Carbon-substrate utilization tests with various sugars and organic acids produced some positive results. In API 20NE tests, strain DS-28^T showed positive reactions for nitrate reduction, indole production and assimilation of glucose, mannitol, *N*-acetylglucosamine, maltose and malate; negative results were obtained for glucose acidification, arginine dihydrolyase, urease, β -glucosidase, protease, β -galactosidase and for the assimilation of arabinose, mannose, adipate, phenylacetate, gluconate, caprate and citrate. Strain DS-28^T showed positive enzyme reactions (API ZYM) for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α -glucosidase, but was negative for cystine arylamidase, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase.

On the basis of the data obtained from 16S rRNA gene sequence comparisons, strain DS-28^T occupies a distinct position within the genus *Vogesella*. This genotypic insight was supported by the unique combination of chemotaxonomic characteristics (Tables 1 and 2) and biochemical traits (Table 2 and Supplementary Table S1) of the novel strain. It is clear from the genotypic and phenotypic data that strain DS-28^T represents a second species of the genus *Vogesella*, for which the name *Vogesella perlucida* sp. nov. is proposed.

Table 2. Some genotypic and phenotypic characteristics that can be used to distinguish strain DS-28^T from its closely related phylogenetic neighbour *V. indigofera* ATCC 19706^T

Data for the isolation source and the DNA G+C content for *V. indigofera* ATCC 19706^T were taken from Grimes *et al.* (1997); all other data listed were obtained in this study. Both strains were positive for nitrate reduction, indole production, acetoin production, inositol fermentation, oxidase, catalase, alkaline phosphatase, esterase (C4), lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and for assimilation of *N*-acetylglucosamine, glucose and malic acid. Both strains were negative for the following: glucose fermentation, gelatin and aesculin hydrolysis, H₂S production, urease, α -chymotrypsin, cystine arylamidase, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase, lysine decarboxylase, ornithine decarboxylase and for assimilation of arabinose, mannose, capric acid, adipic acid, trisodium citrate and phenylacetic acid.

Characteristic	Strain DS-28 ^T	<i>V. indigofera</i> ATCC 19706 ^T
Isolation source	Spring water	Pond sediment
Colony colour	Colourless to white	Blue
Lipase (C14)	+	–
α -Glucosidase	+	–
Fermentation of (API 20E):		
Mannitol	+	–
Sucrose	+	–
Amygdalin	–	+
Assimilation of (API 20NE):		
Mannitol	+	–
Maltose	+	–
Potassium gluconate	–	+
Oxidation of (Biolog):		
Sucrose	+	–
Inositol	–	+
DL-Lactic acid	–	+
Propionic acid	–	+
Urocanic acid	+	–
L-Alanine	–	+
L-Asparagine	–	+
L-Ornithine	–	+
DNA G+C content (mol%)	61.8	65.4

Description of *Vogesella perlucida* sp. nov.

Vogesella perlucida (per.lu'ci.da. L. fem. adj. *perlucida* transparent, referring to the transparent and colourless colonies, which distinguish it from the type species *V. indigofera*, which forms blue-pigmented colonies).

Cells are aerobic, Gram-negative, non-spore-forming and rod-shaped. Cells are motile by means of single polar flagellum. Poly- β -hydroxybutyrate granules are accumulated. After 48 h growth on R2A agar at 25 °C, the mean cell size is approximately 0.5 × 1.0–2.0 µm. Visible, round, entire, convex and colourless colonies are formed. The

colony diameter is approximately 1.0–2.5 mm on R2A agar after 48 h incubation at 25 °C. Growth occurs at 4, 10, 15, 25, 30, 37, 42 and 45 °C, with 0–2 % NaCl and at pH 7–9. Optimal growth occurs at 30 °C, 0.5 % NaCl and pH 7.5. Catalase-positive. Positive for cytochrome oxidase, catalase, lipase (corn oil) and hydrolysis of Tweens 20, 40, 60 and 80. Negative for DNase and for hydrolysis of starch, chitin and casein. The major fatty acids are summed feature 3 (C_{16:1}ω7c or iso-C_{15:0} 2-OH) and C_{16:0}. Additional phenotypic properties are listed in Table 2. With the Biolog GN2 test system, the following compounds are utilized as sole carbon sources: D-fructose, sucrose, N-acetyl-D-glucosamine, D-psicose, Tweens 40 and 80, hydroxy-L-proline, L-proline, L-glutamic acid, β-hydroxybutyric acid, γ-aminobutyric acid and urocanic acid. Unable to oxidize α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, adonitol, trehalose, turanose, L-arabinose, D-arabitol, cellobiose, i-erythritol, L-fucose, D-galactose, gentiobiose, myo-inositol, α-D-lactose, lactulose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, raffinose, L-rhamnose, D-sorbitol, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, formic acid, D-gluconic acid, DL-lactic acid, quinic acid, succinic acid, bromosuccinic acid, acetic acid, cis-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, D-saccharic acid, sebacic acid, succinamic acid, L-alanine, L-asparagine, L-aspartic acid, L-histidine, L-leucine, L-ornithine, DL-carnitine, glucuronamide, L-alaninamide, D-alanine, L-alanyl glycine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-phenylalanine, L-pyroglyutamic acid, D-serine, L-serine, L-threonine, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol-phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate. Strain DS-28^T is sensitive to tetracycline, kanamycin, rifampicin, gentamicin, penicillin G, chloramphenicol, nalidixic acid, novobiocin, streptomycin, ampicillin and sulfamethoxazole plus trimethoprim.

The type strain, DS-28^T (=BCRC 17730^T=LMG 24214^T), was isolated from a spring in southern Taiwan. The DNA G+C content of the type strain 61.8 mol%.

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