Methylobacterium tarhaniae sp. nov., isolated from arid soil

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A reddish-orange-pigmented, Gram-stain-negative, aerobic, facultatively methylotrophic strain, N4211T, isolated from arid soil, collected from Abuja, Nigeria, was analysed by using a polyphasic approach. Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strain N4211T belonged to the genus Methylobacterium. Strain N4211T was most closely related to Methylobacterium aquaticum GR16T (98.56 %), Methylobacterium platani PMBO2T (97.95 %) and Methylobacterium variabile GR3T (97.2 %), and the phylogenetic similarities to all other species of the genus Methylobacterium with validly published names were less than 97.0 %. The major ubiquinones detected were Q-10. The major fatty acids were summed feature 7 (C18 : 1 cis11/9t6). The DNA G + C content was 67.3 mol%. DNA–DNA relatedness of strain N4211T and the most closely related strains M. aquaticum DSM 16371T and M. platani KCTC 12901T were 60.0 and 48.2 %, respectively. On the basis of phenotypic, phylogenetic and DNA–DNA hybridization data, strain N4211T is assigned to a novel species of the genus Methylobacterium for which the name Methylobacterium tarhaniae sp. nov. is proposed. The type strain is N4211T (=KCTC 23615T=DSM 25844T).

The genus Methylobacterium, proposed by Patt et al. (1976) with revised descriptions emended by Green & Bousfield (1983), belongs to the class Alphaproteobacteria and includes strictly aerobic, Gram-stain-negative, rod-shaped, pink-pigmented, facultatively methylotrophic (PPFM) bacteria, which can grow on single carbon compounds such as formate, formaldehyde, methanol and methylamine as sole sources of carbon and energy, as well as on a wide range of multi-carbon growth substrates (Green, 1992; Raja et al., 2008). The primary reservoirs of methylotrophic bacteria are mainly soil and water but these bacteria are also present in variety of natural and man-made environments, including dust, lake sediments, freshwater, seawater, phyllosphere, tree tissues, root nodules, rice grains, air, face-creams, fermented products, water supplies, bathrooms and air-conditioning systems (Austin et al., 1978; Yoshimura, 1982; Green & Bousfield, 1983; Corpe & Rheem, 1989; Green, 1992; Hiraishi et al., 1995; Trotsenko et al., 2001; Lidstrom & Chistoserdova, 2002; Van Aken et al., 2004; Anesti et al., 2004; Kang et al., 2007; Kato et al., 2008; Madhaiyan et al., 2009; 2012; Tani et al., 2012). Members of the genus Methylobacterium have been found to be a dominant component of bacterial phyllosphere communities (Delmotte et al., 2009). Species of the genus Methylobacterium are known to produce phytohormones, which can stimulate plant growth (Ivanova et al., 2001; Koegig et al., 2002), allow for fixation of atmospheric nitrogen (Sy et al., 2001) and help plants against pathogens (Holland & Polacco, 1994).

Strain N4211T was isolated on Streptomyces isolation medium starch casein agar (Küster, 1959), supplemented with filter-sterilized cycloheximide (50 μg ml⁻¹), nystatin (50 μg ml⁻¹) and rifampicin (0.5 μg ml⁻¹), after incubation at 28 ºC for 21 days following inoculation with a suspension of an arid soil collected from Abuja, Nigeria. Reddish-orange-pigmented colonies were selected and studied in more detail. The isolate was maintained on glucose-yeast extract (Gordon & Mihm, 1962) and yeast extract-malt extract [International Streptomycetes Project (ISP) medium 2; Shirling & Gottlieb, 1966] agar slopes at room temperature and as glycerol suspensions (20 %, v/v) at −20 ºC. Methylobacterium aquaticum DSM 16371T and Methylobacterium platani KCTC 12901T were used as reference strains for DNA–DNA hybridization studies and phenotypic analyses.

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product

Abbreviation: ISP, International Streptomyces Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Methylobacterium tarhaniae N4211T is JQ864432.

Four supplementary figures and a supplementary table are available with the online version of this paper.
were carried out following Chun & Goodfellow (1995). The almost complete (1417 bp) 16S rRNA gene sequence of strain N4211T was determined using an ABI PRISM 3730 XL automatic sequencer. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e
showed DNA–DNA relatedness values of 60.0 % to
M. variabile
and 48.2 % to
species were performed by using the program CLUSTAL W
Multiple alignments with sequences from closely related
members of the genus
Methylobacterium
were evaluated by bootstrap analysis (Felsenstein, 1985)
based on 1000 resamplings.

The phylogenetic tree based on the neighbour-joining
algorithm showed that strain N4211 T formed a cluster with
M. aquaticum GR16 T, M. platani PMBO2 T and
Methylobacterium variabile GR3 T among members of the
species were reconstructed with the neighbour-
Phylogenetic trees were reconstructed with the neighbourhood (Saitou & Nei, 1987), maximum-parsimony
(Kluge & Farris, 1969) and maximum-likelihood
(Felsenstein, 1981) algorithms in MEGA5 (Tamura et al., 2011).
Evolutionary distances were calculated using model
Jukes & Cantor (1969). Topologies of the resultant trees
were evaluated by bootstrap analysis (Felsenstein, 1985)
below the 70 % threshold recommended for the
levels of bootstrap support (%); only values ≥ 50 % are shown. GenBank accession numbers are given in parentheses. Bar,
0.01 substitutions per site.

DNA–DNA relatedness values between isolate N4211 T and its
closest phylogenetic neighbours Methylobacterium aquaticum
DSM 16371 T and Methylobacterium platani KCTC 12901 T,
were determined by the Identification Service at the Deutsche
Sammlung von Mikroorganismen und Zelkulturen (DSMZ)
Braunschweig, Germany. DNA was isolated using a French
pressure cell (Thermo Spectronic) and was purified by
cromatography on hydroxylapatite as described by Cashion
et al. (1977). DNA–DNA hybridization was carried out as
described by De Ley et al. (1970) following the modifications
described by Huss et al. (1983) using a model Cary 100 Bio
UV/VIS-spectrophotometer equipped with a Peltier-thermo-
stated 6 × 6 multicliff changer and a temperature controller with
in situ temperature probe (Varian).

The taxonomic position of the strain N4211 T was
supported by DNA–DNA relatedness data. Strain N4211 T
showed DNA–DNA relatedness values of 60.0 % to
M. aquaticum DSM 16371 T and 48.2 % to M. platani KCTC
12901 T (based on a mean of duplicate determinations), a
result well below the 70 % threshold recommended for the
delination of bacterial species by Wayne et al. (1987).

Biomass for chemotaxonomic studies was prepared by
growing strain N4211 T in ISP 2 broth cultures, at
160 r.p.m. for 10 days at 28 °C; cells were harvested by
centrifugation, washed twice in distilled water and freeze-
dried. Respiratory lipoquinones were extracted from
100 mg of freeze-dried cells based on the two-stage method
described by Tindall (1990a; b) and carried out by the
Identification Service of the DSMZ. Respiratory lipoqui-
none were separated into their different classes (mena-
quinones and ubiquinones) by thin layer chromatography
on silica gel (Macherey-Nagel art. no. 805 023), using
hexane : tert-butylmethylether (9 : 1, v/v) as the solvent.
UV-absorbing bands corresponding to menaquinones or
ubiquinones were removed from the plate and further
analysed by HPLC. This step was carried out on a LDC
Analytical HPLC (Thermo Separation Products) fitted
with a reverse phase column (Macherey-Nagel, 2 mm x
125 mm, 3 μm, RP18) using methanol as the eluant.
Respiratory lipoquinones were detected at 269 nm.

A starter culture for the fatty acid analyses was prepared in a
flask containing 20 ml trypticase soy broth (TSB; Difco)
which was shaken at 150 r.p.m. at 28 °C for 5 days. Five
millilitres of the resultant culture was used to inoculate
50 ml TSB which was incubated under the same conditions.
Biomass was harvested by cellulose filtration (pore size
0.45 μm) and the wet cells (200 mg) placed in an extraction
tube. Cellular fatty acids were extracted, methylated and
separated by gas chromatography according to the standard
protocol of the Sherlock Microbial Identification (MDI)
system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996), using
an Agilent Technologies 6890N instrument fitted with an
autosampler and a 6783 injector. Fatty acid methyl ester
peaks were quantified using TSBA 5.0 software. The DNA
G+C content of the isolate was determined following the

Predominant cellular fatty acids of strain N4211 T were
summed feature 7 comprising C18:1 cis11/t9/t6 (61.5 %),
summed feature 3 comprising C16:0 iso I/C14:0 3-
OH (9.2 %), C16:1 cis 9 (8.4 %), C16:0 (6.9 %), C15:0 3-
OH (5.3 %), iso-C18:0 10-methyl (3.8 %), C18:0 3-OH
(3.1 %) and C12:0 (1.7 %) (Table S1). The predominant
ubiquinone of strain N4211 T was Q-10 (72.0 %); an
unknown component (28.0 %) was also detected. The
G+C content of the DNA of the isolate was 67.3 %, which
is within the range expected for members of the genus
Methylobacterium (Green, 1992).

Phenotypic characteristics of strain N4211 T, Methylo-
acterium aquaticum DSM 16371 T and Methylobacterium

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16 rRNA gene sequences (1417 nt) showing
the position of strain N4211 T amongst its phylogenetic neighbours. Methylobabdus multivorans DM13 T (GenBank accession
no. AF004845) was used as an outgroup. Asterisks indicate branches of the tree that were also recovered using the maximum-
likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Numbers at nodes indicate the
levels of bootstrap support (%); only values ≥ 50 % are shown. GenBank accession numbers are given in parentheses. Bar,
0.01 substitutions per site.
The morphological characteristics and physiological properties of strain N4211<sup>T</sup> were consistent with those of members of the genus *Methylobacterium* with cells being Gram-stain-negative, aerobic, rod-shaped and motile (Fig. 2). Cells of strain N4211<sup>T</sup> were 1.0–1.6 μm long and 0.3–1.2 μm wide, with cells being Gram-stain-negative, aerobic, rod-shaped and motile (Fig. 2). Cells of strain N4211<sup>T</sup> were 1.0–1.6 μm long and 0.3–1.2 μm wide, with cells being Gram-stain-negative, aerobic, rod-shaped and motile (Fig. 2).

The characteristics of strain N4211<sup>T</sup> were consistent with those of *M. platani* DSM 16371<sup>T</sup> and *M. aquaticum* DSM 16371<sup>T</sup>. All data were obtained in this study. All strains were positive for citrate utilization; catalase activity; ability to grow with L-arginine and glucose as sole carbon sources (1.0 %); ability to grow with L-methionine, L-serine, L-threonine as sole nitrogen sources (0.1 %); and alkaline phosphatase, leucine arylamidase and acid phosphatase activities. All strains were negative for hydrolysis of allantoic acid; H<sub>2</sub>S production; degradation of elastin (0.3 %), guanine phosphatase activities. All strains were negative for hydrolysis of allantoic acid; H<sub>2</sub>S production; degradation of elastin (0.3 %), guanine phosphatase activities. All strains were negative for hydrolysis of allantoic acid; H<sub>2</sub>S production; degradation of elastin (0.3 %), guanine phosphatase activities. All strains were negative for hydrolysis of allantoic acid; H<sub>2</sub>S production; degradation of elastin (0.3 %), guanine phosphatase activities.
on (+)-D-mannose, D-phenylalanine, L-phenylalanine and the activity of the enzyme naphthol-AS-BI-phosphohydrolase. Strain N4211T grew on formaldehyde (0.01 %, v/v) and methanol (1.0 %, v/v). The phenotypic characteristics that differentiate strain N4211T from its phylogenetically closest relatives are summarized in Table 1.

It is clear from these genotypic and phenotypic data that strain N4211T should be considered to represent a novel species in the genus *Methylobacterium*, for which the name *Methylobacterium tarhaniae* sp. nov. is proposed.

**Description of Methylobacterium tarhaniae* sp. nov.**

*Methylobacterium tarhaniae* (tar.han’i.ae. N.L. fem. gen. n. tarhaniae of Tarhan, named in honour of Leman Tarhan for her contributions to microbiol biotechnology).

Cells are Gram-stain-negative, aerobic, motile and rod-shaped (1.0–1.6 × 2.6–5.6 μm). Colonies are dark reddish-orange, smooth and translucent with an undulate margin. Growth occurs at 10–37°C (optimum, 25–28°C) and at pH 4.0–9.0 (optimum, pH 7.0). Positive result in tests for nitrate reduction, urea hydrolysis and catalase activity, but negative result for aesculin and arbutin hydrolysis, and indole and H2S production. Degrades starch and Tween 20, but not elatin, guanine, L-tyrosine, Tween 80, xanthine or xylan. Adonitol, amygdalin, cellobiose, formaldehyde, (+)-D-galactose, (-)-D-sorbitol, glucose, inositol, (+)-D-mannose, D-manitol, (+)-D-melezitose, dextrin, inulin, (+)-L-arabinose, lactose, maltose, methanol, (+)-L-rhamnose, sucrose and starch are utilized as sole carbon sources. DL-Phenylalanine, L-alanine, L-arginine, L-methionine, L-proline, L-serine and L-threonine are utilized as sole nitrogen sources. Does not utilize L-isoleucine, L-cysteine, glycine, L-hydroxyproline or L-valine as sole nitrogen sources. Positive for acid phosphatase, citrate, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, alkaline phosphatase, trypsin and urease activities, and negative for arginine dihydrolase, gelatinase, lysine decarboxylase, ornithine decarboxylase, α-galactosidase, α-glucosidase, β-glucosidase, esterase-lipase, N-acetyl-β-glucosaminidase, chymotrypsin, cystine arylamidase, esterase, lipase, α-fucosidase, α-mannosidase, β-galactosidase, β-glucuronidase, trypsin-like deaminase and valine arylamidase activities. The major isoprenoid quinone is Q-10. The major fatty acids are summed feature 7 (comprising C18:1 cis11/t9/t16, C18:1 trans 9/t6/c11 or C18:1 trans 6/t9/c11) and summed feature 3 (comprising C14:0 3-OH and/or C16:1 iso-I).

The type strain, N4211T (=KCTC 23615T=DSM 25844T), was isolated from arid soil collected from Abuja, Nigeria. The DNA G+C content of the type strain is 67.3 %.

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**References**


