Inhella fonticola sp. nov., isolated from spring water, and emended description of the genus *Inhella*

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A yellow-pigmented bacterial strain designated TNR-25^T was isolated from spring water in Taiwan and was characterized using a polyphasic taxonomic approach. Strain TNR-25^T was Gram-negative, obligately aerobic, rod-shaped, non-motile and non-spore-forming. Growth occurred at 15–40 °C (optimum, 25 °C), at pH 6.0–10.0 (optimum, pH 7.0) and with 0–0.5% NaCl (optimum, 0%). Phylogenetic analyses based on 16S rRNA gene sequences showed that strain TNR-25^T belonged to the genus *Inhella* and its closest neighbour was *Inhella inkyongensis* IMCC1713^T with 98.1% sequence similarity. The major fatty acids (>10%) of strain TNR-25^T were summed feature 3 (comprising C_{16:1}ω7c and/or C_{16:1}ω6c) and C_{16:0}. The major cellular hydroxy fatty acids were C_{10:0} 3-OH and C_{12:0} 3-OH. The isoprenoid quinone was Q-8 and the DNA G+C content was 69.6 mol%. The polar lipid profile consisted of a mixture of phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, diphosphatidylglycerol and several uncharacterized phospholipids. The DNA–DNA relatedness between strain TNR-25^T and *I. inkyongensis* IMCC1713^T was about 30.6–35.5%. On the basis of the genotypic and phenotypic data, strain TNR-25^T represents a novel species in the genus *Inhella*, for which the name *Inhella fonticola* sp. nov. is proposed; the type strain is TNR-25^T (=BCRC 80211^T=LMG 25721^T).

At the time of writing, the order *Burkholderiales* (Garrity *et al.*, 2005) in the class *Betaproteobacteria* encompassed four families, *Burkholderiaceae*, *Oxalobacteraceae*, *Alcaligenaceae* and *Comamonadaceae*, that have been circumscribed largely based on 16S rRNA gene sequence-based phylogeny. Although there has been a well-defined taxonomic hierarchy among the members of the order *Burkholderiales*, the positions of several genera closely related to the family *Comamonadaceae* have been known to be taxonomically uncertain and these genera have been classified as *incertae sedis* (Willems & Gillis, 2005). This phylogenetically uncertain group, called the *Sphaerotilus–Leptothrix* group (Spring, 2002), contains metabolically diverse genera such as *Aquabacterium*, *Roseateles*, *Rubrivivax*,

Leptothrix, *Sphaerotilus*, *Mitsuaria*, *Paucibacter*, *Methylibium* and *Aquicola*.

The genus *Inhella*, which belongs to the *Sphaerotilus–Leptothrix* group within the order *Burkholderiales*, class *Betaproteobacteria*, was proposed by Song *et al.* (2009). At the time of writing, this genus comprised only one recognized species, *Inhella inkyongensis*, which was isolated from a highly eutrophic artificial pond (Song *et al.*, 2009). Members of the genus *Inhella* are characterized as Gram-negative, flagellated, short to long rod-shaped, chemoheterotrophic, obligately aerobic, oxidase-positive and catalase-negative, and contain poly-β-hydroxybutyrate (PHB) granules. Chemotaxonomically, they have large amounts of summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c), C_{16:0} and C_{10:0} 3-OH, and Q-8 and Q-6 as respiratory quinones (Song *et al.*, 2009). The present study was carried out to clarify the taxonomic position of an *Inhella*-like bacterial strain (TNR-25^T) by using a polyphasic taxonomy approach.

During the characterization of micro-organisms from a freshwater spring in the Maolin Township (GPS location:

Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PHB, poly-β-hydroxybutyrate; PL, phospholipid; PS, phosphatidylserine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Inhella fonticola* TNR-25^T is HM013811.

A supplementary figure and a supplementary table are available with the online version of this paper.

22° 54' 12.5" N 120° 40' 59.9" E; pH 7.5, 25 °C) in Kaoshiung County, Taiwan, a yellow-coloured strain TNR-25^T was isolated and selected for detailed analysis. Strain TNR-25^T was isolated on R2A agar (BD Difco) plates after incubation at 25 °C for 3 days. Subcultivation was performed on R2A agar at 25 °C for 48–72 h. On this medium, strain TNR-25^T was able to grow at 15–40 °C. It was preserved at –80 °C in R2A broth with 20 % (v/v) glycerol or by lyophilization. *I. inkyongensis* strains NBRC 103252^T and CCUG 54308^T were obtained from the NITE Biological Research Center (NBRC) and the Culture Collection, University of Göteborg (CCUG), respectively. These two type strains were used as reference strains for phenotypic and genotypic tests.

Bacterial cells were observed by phase-contrast microscopy (DM 2000; Leica) using cells grown on R2A agar at 25 °C for 6 h (lag growth phase), 18 h (exponential phase) and 36 h (stationary phase) to ascertain the morphology. Motility was tested by the hanging-drop method (Murray *et al.*, 1994). The Gram Stain Set S (BD Difco) kit and the Ryu non-staining KOH method (Powers, 1995) were used for testing the Gram reaction. PHB granule accumulation was observed under light microscopy after staining of the cells with Sudan black. Colony morphology was observed on R2A agar using a stereoscopic microscope (SMZ 800; Nikon).

The pH range for growth was determined by measuring the OD₆₀₀ of R2A broth cultures. The pH was adjusted prior to sterilization to pH 4–10 (at intervals of 1.0 pH unit) using appropriate biological buffers (Breznak & Costilow, 1994): citrate/Na₂HPO₄ buffer, pH range 4.0–5.0; phosphate buffer, pH range 6.0–7.0; Tris buffer, pH range 8.0–9.0; no buffer, pH 10.0. Verification of the pH values after autoclaving revealed only minor changes. The temperature range for growth was determined on R2A agar at 4–50 °C. To investigate NaCl tolerance, R2A broth was prepared according to the formula of the BD Difco medium with the NaCl concentration adjusted to 0 %, 0.5 % and 1.0–6.0 %, w/v (at intervals of 1.0 %). Growth under anaerobic conditions was determined after incubating the bacterial strains on R2A agar in the Oxoid AnaeroGen system. Bacterial strains were examined for a broad range of phenotypic properties. Activities of catalase, oxidase, DNase, urease and lipase (corn oil), and hydrolysis of starch, casein, gelatin, lecithin, and Tweens 20, 40, 60 and 80 were determined using standard methods (Smibert & Krieg, 1994). Additional, biochemical tests were performed using API ZYM, and API 20NE kits (bioMérieux) and carbon source utilization was evaluated using GN2 MicroPlates (Biolog). All commercial phenotypic tests were performed according to the manufacturers' recommendations.

For photosynthetic pigment analysis, bacterial strains were cultivated aerobically in the dark for 2 days at 25 °C in broth culture; *Roseivivax halodurans* JCM 10272^T and *Roseivivax halotolerans* JCM 10271^T were used as positive controls. The cell mass from 30 ml culture was extracted with 3 ml acetone/methanol (7 : 2, v/v) and the absorption spectrum of

the extract was recorded (Biebl *et al.*, 2005). The absorption spectrum of the pigments was examined on a Thermo Helios γ spectrophotometer. The presence of bacteriochlorophyll *a* in bacterial strains is typically indicated by maxima of 367 and 775 nm in the absorption spectrum.

Sensitivity of bacterial strains to antibiotics was tested by the disc-diffusion method after spreading cell suspensions (0.5 McFarland) on R2A agar (BD Difco) plates. Discs (Oxoid) contained the following antibiotics (μ g): ampicillin (10), chloramphenicol (30), gentamicin (10), kanamycin (30), nalidixic acid (30), novobiocin (30), rifampicin (5), penicillin G (10), streptomycin (10), sulfamethoxazole (23.75) plus trimethoprim (1.25), tetracycline (30), vancomycin (30) and erythromycin (15). The effect of antibiotics on cell growth was assessed after 2 days at 25 °C. The diameter of antibiotic disc was 8 mm and the strain was considered susceptible when the diameter of the inhibition zone was >13 mm, intermediate at 10–12 mm and resistant at <10 mm as described by Nokhal & Schlegel (1983).

Genomic DNA was isolated by a bacterial genomic DNA purification kit (DP02-150, GeneMark Technology) and the 16S rRNA gene sequence was analysed as described previously by Chen *et al.* (2001). Primers FD1 (5'-AGAG-TTGATCCTGGCTCAG-3') and RD1 (5'-AAGGAGGT-GATCCAGCC-3') were used for amplification of bacterial 16S rRNA genes by PCR. These primers correspond to nucleotide positions 8–27 and 1525–1541 of the *Escherichia coli* 16S rRNA gene, respectively, and can be used for amplifying the nearly full-length 16S rRNA gene. The PCR product was purified and direct sequencing was performed by using sequencing primers FD1, RD1, 520F and 800R (Weisburg *et al.*, 1991; Anzai *et al.*, 1997) with a DNA sequencer (ABI Prism 3730; Applied Biosystems). An almost-complete 16S rRNA gene sequence (1451 nt) of strain TNR-25^T was compared against 16S rRNA gene sequences available from the EzTaxon server (Chun *et al.*, 2007), the Ribosomal Database Project (Maidak *et al.*, 2001) and GenBank (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Analysis of the sequence data was performed by using the software package BioEdit (Hall, 1999) and MEGA version 3.1 (Kumar *et al.*, 2004), after multiple alignments of the data by CLUSTAL_X (Thompson *et al.*, 1997). The resulting multiple sequence alignment was corrected manually and gaps at the 5' and 3' ends of the alignment were omitted for further analyses. Distances (corrected according to Kimura's two-parameter model; Kimura, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). The maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were generated by using the treeing algorithms contained in the PHYLIP software package (Felsenstein, 1993). In each case, bootstrap values were calculated based on 1000 replications.

16S rRNA gene sequence analysis indicated that strain TNR-25^T belonged to the *Sphaerotilus–Leptothrix* group of the order *Burkholderiales* in the class *Betaproteobacteria*.

Strain TNR-25^T formed a distinct subline within the genus *Inhella* in the neighbour-joining tree (Fig. 1). The overall topologies of the phylogenetic trees obtained with the maximum-likelihood and maximum-parsimony methods were similar. Sequence similarity calculations (over 1400 bp) indicated that strain TNR-25^T was closely related to *I. inkyongensis* IMCC1713^T (98.1% 16S rRNA gene sequence similarity). Lower sequence similarities (<96.5%) were found with representative members of all other genera listed in Fig. 1.

DNA–DNA hybridization experiments were carried out by the method of Ezaki *et al.* (1989). The level of DNA–DNA relatedness of strain TNR-25^T with *I. inkyongensis* NBRC 103252^T was $30.6 \pm 2.5\%$ ($33.4 \pm 4.2\%$ in a reciprocal experiment). The level of DNA–DNA relatedness of strain TNR-25^T with *I. inkyongensis* CCUG 54308^T was $35.5 \pm 1.8\%$ ($32.6 \pm 2.3\%$ in a reciprocal experiment). Since the recommended DNA–DNA relatedness threshold for the definition of a species is 70% (Wayne *et al.*, 1987), these results indicate that strain TNR-25^T does not belong to the known species of the genus *Inhella*.

The fatty acid profiles of strain TNR-25^T, *I. inkyongensis* NBRC 103252^T and *I. inkyongensis* CCUG 54308^T were determined using cells grown on R2A agar at 25 °C for 2 days. The fatty acid methyl esters were prepared and separated according to the instructions of the Microbial Identification System (MIDI; Sasser, 1990) and identified by MIDI version 6.0 and the RTSBA6.00 database. The fatty

acid profile of strain TNR-25^T was similar to those of strains of *I. inkyongensis*, although there were differences in the proportions of some components (Table 1). The major fatty acids (>10%) of strain TNR-25^T and *I. inkyongensis* were summed feature 3 (comprising C_{16:1}ω7c and/or C_{16:1}ω6c) and C_{16:0}. The major cellular hydroxy fatty acids in strain TNR-25^T were C_{10:0} 3-OH and C_{12:0} 3-OH. Furthermore, the quantity of fatty acid C_{18:1}ω7c in strain TNR-25^T was higher than that of *I. inkyongensis*. In contrast to results reported previously (Song *et al.*, 2009), *I. inkyongensis* strains NBRC 103252^T and CCUG 54308^T contained the fatty acids C_{18:0}, anteiso-C_{11:0}, anteiso-C_{12:0} and C_{16:1}ω7c alcohol as minor components.

Isoprenoid quinones were extracted and purified according to the method of Collins (1985) and were analysed by HPLC. The respiratory quinones of strain TNR-25^T detected were Q-8 (92.9%) and Q-6 (7.1%), whereas the respiratory quinones of *I. inkyongensis* NBRC 103252^T detected were Q-8 (88%) and Q-6 (12%). The respiratory quinones of strain TNR-25^T were similar to those reported previously for *I. inkyongensis* (Song *et al.*, 2009), although there were differences in the proportions.

The DNA G+C content of strain TNR-25^T, determined by HPLC according to Mesbah *et al.* (1989), was 69.6 ± 1.0 mol%. The DNA G+C contents of *I. inkyongensis* strains NBRC 103252^T and CCUG 54308^T were 70.1 ± 1.0 and 68.7 ± 1.0 mol%, which are much higher than those reported previously (Song *et al.*, 2009). However, this result

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of *Inhella fonticola* TNR-25^T and related taxa in the order *Burkholderiales* of the class *Betaproteobacteria*. Numbers at nodes are bootstrap percentages >70% based on the 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. *Comamonas terrigena* IMI 359870 was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 1. Cellular fatty acid composition of *Inhella* species

Taxa: 1, *Inhella fonticola* TNR-25^T; 2, *Inhella inkyongensis* (data for strains NBRC 103252^T and CCUG 54308^T). For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; the *cis* isomer is indicated by the suffix *c*. All data are from this study. Bacterial strains were grown on R2A agar at 25 °C for 2 days. Values are percentages of the total fatty acids; fatty acids that make up <0.6% of the total are not shown or indicated by '—'.

Fatty acid	1	2
C _{12:0}	3.3	2.9–3.1
C _{14:0}	0.6	1.0–2.2
C _{16:0}	23.7	25.5–29.1
C _{17:0}	1.0	0.7–1.1
C _{18:0}	2.9	3.5–4.4
C _{10:0} 3-OH	5.4	6.6–7.4
C _{12:0} 3-OH	2.5	1.2–2.1
anteiso-C _{11:0}	1.4	1.3–2.0
anteiso-C _{12:0}	1.1	1.2–1.6
iso-C _{16:0}	—	0.8–1.1
C _{15:1} ω 6 <i>c</i>	—	0.7–0.9
C _{16:1} ω 7 <i>c</i> alcohol	1.1	0.6–0.9
C _{18:1} ω 7 <i>c</i>	7.1	0.9–1.7
C _{18:1} ω 9 <i>c</i>	1.4	0.6–1.0
Summed feature 3*	45.2	39.1–43.5

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 comprises C_{16:1} ω 7*c* and/or C_{16:1} ω 6*c*.

is close to those of other members of the *Sphaerotilus–Leptothrix* group: *Sphaerotilus*, 69 mol% (Spring, 2002; Willems & Gillis, 2005); *Leptothrix*, 68–71 mol% (Spring *et al.*, 1996; Willems & Gillis, 2005); *Roseateles*, 66 mol% (Suyama *et al.*, 1999; Gomila *et al.*, 2008); *Rubrivivax*, 70–74 mol% (Willems *et al.*, 1991; Ramana *et al.*, 2006); *Paucibacter*, 67 mol% (Rapala *et al.*, 2005); *Ideonella*, 68 mol% (Malmqvist *et al.*, 1994); *Aquabacterium*, 65–66 mol% (Kalmbach *et al.*, 1999).

Polar lipids were extracted and analysed by two-dimensional TLC according to Embley & Wait (1994). Molybdophosphoric acid was used for detection of all lipids, ninhydrin reagent was used for lipids containing free amino groups, Zinzadze reagent was used for phosphorus-containing lipids and α -naphthol reagent was used for glycolipids. Strain TNR-25^T exhibited a complex polar lipid profile consisting of phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), diphosphatidylglycerol (DPG) and several uncharacterized phospholipids (PL1–PL4) (see Fig. S1 available in IJSEM Online). Like its closest relative *I. inkyongensis* NBRC 103252^T, strain TNR-25^T exhibited a very similar polar lipid profile, with both strains possessing PE, PG, PS, DPG and PL2–PL4. However, an uncharacterized phospholipid (PL1) was present in strain TNR-25^T, but absent in *I.*

inkyongensis NBRC 103252^T. This result suggest that there are some differences in the polar lipid profiles between both strains, although they belong to the same genus and have very similar profiles.

The physiological, biochemical and morphological characteristics of strain TNR-25^T are given in the species description, Table 2 and Table S1 (available in IJSEM Online). Phenotypic examination revealed many common traits between the novel strain and its closest relatives, *I. inkyongensis* strains NBRC 103252^T and CCUG 54308^T. However, strain TNR-25^T could be clearly differentiated from these two strains based on the following characteristics: colony pigmentation; cell motility; ability to hydrolyse starch and lecithin; ability to assimilate caprate; inability to grow at higher NaCl concentrations, at higher temperatures and under anaerobic conditions; inability to reduce nitrate; the absence of β -galactosidase and α -chymotrypsin activities; the inability to assimilate glucose, arabinose, mannose, mannitol, maltose and malate; and sensitivity to penicillin G, ampicillin, rifampicin and sulfamethoxazole/trimethoprim (Table 2). In addition, strain TNR-25^T could be also distinguished from *I. inkyongensis* NBRC 103252^T by its ability to utilize α -cyclodextrin, dextrin, glycogen, adonitol, sucrose, β -hydroxybutyric acid, sebacic acid, L-asparagine, L-histidine, L-ornithine, uridine and 2-aminoethanol as sole carbon sources (Table S1).

Strain TNR-25^T is Gram-negative, rod-shaped, chemoheterotrophic and oxidase-positive, and contains PHB granules. It has a large amount of summed feature 3 (comprising C_{16:1} ω 7*c* and/or C_{16:1} ω 6*c*) and C_{16:0}. Its major cellular hydroxy fatty acids are C_{10:0} 3-OH and C_{12:0} 3-OH, and it has the respiratory quinones Q-8 and Q-6. These characteristics of strain TNR-25^T are consistent with the description of the genus *Inhella* (Song *et al.*, 2009). On the basis of the data obtained from 16S rRNA gene sequence comparisons, strain TNR-25^T occupies a distinct position within the genus *Inhella*; this is supported by the unique combination of chemotaxonomic and biochemical characteristics of the isolate. It is clear from phylogenetic and phenotypic data that strain TNR-25^T constitutes a novel member of the genus *Inhella*. However, strain TNR-25^T could be differentiated from *I. inkyongensis* strains NBRC 103252^T and CCUG 54308^T by a combination of physiological and biochemical characteristics (Table 2 and Table S1). Hence, strain TNR-25^T represents a second species of the genus *Inhella* for which the name *Inhella fonticola* sp. nov. is proposed.

The abilities to grow under anaerobic conditions and reduce nitrate were not observed previously for *I. inkyongensis* by Song *et al.* (2009), who reported that *I. inkyongensis* was weakly positive for nitrate reduction and unable to grow under anaerobic conditions. Additionally, the DNA G+C contents of *Inhella* species differed from those reported previously (Song *et al.*, 2009). Hence, an emended description of the genus *Inhella* is proposed.

Table 2. Differential characteristics of *Inhella* species

Taxa: 1, *Inhella fonticola* TNR-25^T; 2, *Inhella inkyongensis* (data for strains NBRC 103252^T and CCUG 54308^T). All data are from this study except where marked. +, Positive; –, negative reaction; w, weakly positive; v, variable. All three strains are Gram-negative, rod-shaped, mesophilic, neutrophilic and non-spore-forming, contain PHB granules, lack bacteriochlorophyll *a* and are positive for oxidase, DNase, alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase activities, and positive for hydrolysis of Tweens 40 and 80. All three strains are negative for urease, arginine dihydrolase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, α -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities, indole production, glucose acidification, assimilation of *N*-acetylglucosamine, gluconate, adipate, citrate and phenylacetate, and hydrolysis of casein, corn oil, gelatin and Tweens 20 and 60. All three strains are sensitive to chloramphenicol, gentamicin, kanamycin, tetracycline, novobiocin, streptomycin, nalidixic acid, vancomycin and erythromycin.

Characteristic	1	2
Colony pigmentation	Yellow	White
Motility	–	+
NaCl range for growth (%)	0–0.5	0–1.0 (0–0.7)*
Temperature for optimal growth (°C)	25	30 (25)*
Growth at 37 °C	+	+ (–)*
Nitrate reduction	–	+ (w)*
Hydrolysis of:		
Starch	+	–
Lecithin	+	–
Aesculin	–	– (w)*
Gelatin	–	– (w)*
Enzymic activities		
Catalase	+	–
β -Galactosidase	–	v†
α -Chymotrypsin	–	+
Leucine arylamidase	+	+ (–)*
Assimilation of:		
Glucose	–	+
Arabinose	–	+
Mannose	–	+
Mannitol	–	+
Maltose	–	+
Caprate	+	–
Malate	–	+
Susceptibility to:‡		
Penicillin G	S	R
Ampicillin	S	R
Rifampicin	S	R
Sulfamethoxazole/trimethoprim	S	R
DNA G + C content (mol%)	69.6	68.7–70.1 (57.4)*

*Results in parentheses obtained from Song *et al.* (2009).

†Negative in API ZYM kit, but positive in API 20NE kit.

‡S, Sensitive; R, resistant.

Emended description of the genus *Inhella* Song *et al.* 2009

The formal description given by Song *et al.* (2009) remains correct with the exception of the following: some species are non-motile or motile by means of a single polar flagellum; catalase activity is variable; obligately aerobic or facultatively anaerobic; and the DNA G + C content is 68.7–70.1 mol%. The predominant polar lipids are PE, PG, PS, DPG and several uncharacterized PLs.

Description of *Inhella fonticola* sp. nov.

Inhella fonticola (fon.ti'co.la. L. masc. n. *fons fontis* a spring, fountain; L. suff. *-cola* from L. masc. or fem. n. *incola* an inhabitant of a place, a resident; N.L. n. *fonticola* an inhabitant of a fountain or spring).

Cells are Gram-negative, obligately aerobic, non-motile, non-spore-forming, rod-shaped and chemo-heterotrophic. PHB accumulation is observed. Bacteriochlorophyll *a* is absent. After 24 h of incubation on R2A agar at 25 °C, the mean cell size is approximately 0.3–0.5 μ m in diameter and 1.0–1.2 μ m in length. Colonies are yellow, convex, round and smooth with entire edges. The colony size is approximately 0.9–1.1 mm in diameter on R2A agar after 48 h of incubation at 25 °C. Growth occurs at 15–40 °C (optimum, 25 °C), at pH 6.0–10.0 (optimum, pH 7.0) and with 0–0.5 % NaCl (optimum, 0 %). Positive for oxidase, catalase and DNase activities, and hydrolysis of starch, lecithin, and Tweens 40 and 80. Negative for urease activity and hydrolysis of casein, corn oil, and Tweens 20 and 60. In API 20NE tests (bioMérieux), positive for assimilation of caprate and negative for nitrate reduction, indole production, glucose acidification, aesculin and gelatin hydrolysis, arginine dihydrolase, urease and β -galactosidase activities, and assimilation of glucose, arabinose, mannose, mannitol, *N*-acetylglucosamine, maltose, gluconate, adipate, malate, citrate and phenylacetate. In the API ZYM kit (bioMérieux) alkaline phosphatase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase activities are present and C4 esterase, C8 esterase lipase, C14 lipase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities are absent. The following compounds are utilized as sole carbon sources in the GN2 MicroPlate (Biolog): α -cyclodextrin, dextrin, glycogen, Tweens 40 and 80, adonitol, sucrose, β -hydroxybutyric acid, sebacic acid, L-asparagine, L-histidine, L-ornithine, uridine and 2-aminoethanol. None of the other substrates in the GN2 MicroPlate are utilized. Sensitive to penicillin G, ampicillin, chloramphenicol, gentamicin, rifampicin, kanamycin, tetracycline, novobiocin, streptomycin, sulfamethoxazole plus trimethoprim, nalidixic acid, vancomycin and erythromycin. The major fatty acids (>10 %) are summed feature 3 (comprising C_{16:1} ω 7c and/or C_{16:1} ω 6c) and C_{16:0}. The respiratory quinones are Q-8 and Q-6. Exhibits a

complex polar lipid profile consisting of PE, PG, PS, DPG and four uncharacterized PLs.

The type strain is TNR-25^T (=BCRC 80211^T=LMG 25721^T), isolated from a freshwater spring, Maolin Township, Kaoshiung County, Taiwan. The DNA G+C content of the type strain is 69.6 mol%.

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