Noviherbaspirillum denitrificans sp. nov., a denitrifying bacterium isolated from rice paddy soil and Noviherbaspirillum autotrophicum sp. nov., a denitrifying, facultatively autotrophic bacterium isolated from rice paddy soil and proposal to reclassify Herbaspirillum massiliense as Noviherbaspirillum massiliense comb. nov.

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Abstract

Thirty-nine denitrifying bacterial strains closely related to one another, represented by strains TSA40T and TSA66T, were isolated from rice paddy soils. Strains TSA40T and TSA66T were Gram-stain-negative, slightly curved rod-shaped, and motile by means of polar flagella. They were able to reduce nitrate, nitrite and nitrous oxide, but unable to fix atmospheric N2. While strain TSA661 was able to grow autotrophically by H2-dependent denitrification, strain TSA401 could not. Phylogenetic analysis suggested that they belong to the family Oxalobacteriae, the order Burkholderiales in the class Betaproteobacteria. Major components in the fatty acids (C16:0, C17:0 cyclo, C18:1ω7c and summed feature 3) and quinone (Q-8) also supported the affiliation of strains TSA401 and TSA66T to the family Oxalobacteriae. Based on 16S rRNA gene sequence comparisons, strains TSA40T and TSA66T showed the greatest degree of similarity to Herbaspirillum massiliense JC206T, Noviherbaspirillum malthae CC-AFH3T, Noviherbaspirillum humi U15T, Herbaspirillum seropedicae Z67T and Paucimonas lemoignei LMG 2207T, and lower similarities to the members of other genera. Average nucleotide identity values between the genomes of strain TSA40T, TSA66T and H. massiliense JC206T were 75–77%, which was lower than the threshold value for species discrimination (95–96%). Based on the 16S rRNA gene sequence analysis in combination with physiological, chemotaxonomic and genomic properties, strains TSA40T (=JCM 17722T=ATCC TSD-69T) and TSA66T (=JCM 17723T=DSM 25787T) are the type strains of two novel species within the genus Noviherbaspirillum, for which the names Noviherbaspirillum denitrificans sp. nov. and Noviherbaspirillum autotrophicum sp. nov. are proposed, respectively. We also propose the reclassification of Herbaspirillum massiliense as Noviherbaspirillum massiliense comb. nov.

We previously obtained 39 denitrifying strains by using a functional single cell isolation method [1] from rice paddy soils [2, 3]. Their 16S rRNA gene sequences were 98–100% similar to those obtained by culture-independent analyses [3–5], but were <98% similar to those of the type strains within the genus Herbaspirillum. Based on 16S rRNA gene sequence analysis using the MOTHUR program [6], the 39 strains were divided into two groups, each represented by strain TSA40T (=JCM 17722T=ATCC TSD-69T) and strain TSA66T (=JCM 17723T=DSM 25787T). The phylogenetic relatedness of the 39 strains based on the nearly full-length 16S rRNA gene sequences is shown in Fig.

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Keywords: denitrification; chemolithoautotroph; Oxalobacteriae; genome.

Abbreviations: ANI, average nucleotide identity; POCP, percentage of conserved proteins; RubisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase.

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the genome sequence of strain TSA40T are AB542397 and AB542397, respectively, and those for strain TSA66T are AB542418 and AB542419, respectively. Five supplementary figures and five supplementary tables are available with the online Supplementary Material.
Strains TSA40T and TSA66T were isolated from a rice paddy soil under denitrification-inducing conditions [2], while TSO23-1 was isolated from the same rice paddy soil under N₂O-reducing conditions [3]. In both cases, succinate was used as an electron donor. The culture was maintained on 100-fold diluted nutrient broth (DNB; Difco) supplemented with 3 mM nitrate and 4.4 mM succinate (DNBNS medium) or 1.5 % agar plates of the DNBNS medium (DNBNS agar medium) under anaerobic incubation at 30 °C as described previously [2].

Flagellation and morphological characteristics were determined by transmission electron microscopy after negative staining of the cells, grown on DNBNS agar medium at 30 °C for 1 week, with 1 % (w/v) phosphotungstic acid. Flagellar staining of the cells, grown on DNBNS agar medium at 30 °C for 2 days and acetylene and ethylene contents were analysed by the acetylene block method using DNBNS medium as described previously [10]. N₂O-reducing ability was examined by measuring C14-labelled N₂O (99 at.%; Cambridge Isotope Laboratories) and GC/MS as described previously [3]. In addition, anaerobic growth under denitrification conditions with acetate, succinate or H₂ as an electron acceptor was tested using bicarbonate-buffered basal medium (2.5 g NaHCO₃, 1.5 g NH₄Cl, 0.6 g KH₂PO₄, 0.1 g KCl, 10 ml vitamin solutions and 10 ml trace element solutions per litre) [11]. The medium was dispensed into pressure tubes, sealed with a butyl rubber stopper and the air phase was replaced with N₂/CO₂ (80 : 20) gas [or N₂/H₂/CO₂ (80 : 10 : 10) gas when grown under autotrophic denitrification conditions].

Potential for metal respiration was tested using bicarbonate-buffered basal medium supplemented with 5 mM succinate and 20 mM Fe(III) citrate or Fe(III) pyrophosphate under N₂/CO₂ (80 : 20) atmosphere. Anaeromyxobacter dehalogens 2CP-C was used as a positive control for these tests.

For fatty acid and quinone analysis, cells were aerobically grown on R2A agar (Difco) and in R2A liquid medium, respectively, at 30 °C and harvested at exponential growth phase (after 24 h of incubation). Cellular fatty acids were saponified, methylated, extracted and analysed by GC (Hewlett Packard 6890) according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System. Polar lipids were extracted from the cells, separated by two-dimensional TLC and identified by spraying with the appropriate detection reagents as described elsewhere [12, 13]. Quinones were extracted from cells and analysed as described elsewhere [13], using reversed-phase HPLC. H. seropedicae JCM 21448T was used as a reference strain.

For the analysis of polyamines, strains were grown in liquid medium containing 1 % peptone, 0.2 % yeast extract and 0.1 % MgCl₂.7H₂O (pH 7.0) on a reciprocal shaker at 30 °C. Cells harvested in the late exponential growth phase were washed with saline and freeze-dried. Extraction, analysis and quantification of polyamines were carried out as described by Scherer and Kneifel [14] and Busse and Auling [15]. The detection of polyamines was carried out on a Triant C18 Plus column (250 by 4.6 mm; YMCA Co.) using a Shimadzu LC20A gradient liquid chromatograph equipped with a Hitachi model L-7485 fluorescence detector. The excitation wavelength was 280 nm and the emission wavelength was 451 nm. A linear gradient of 40–80 % acetonitrile/water was applied for 30 min (plus 30 min at the final concentration) with a flow rate of 1 ml min⁻¹ at 40 °C to elute the dansylated polyamines. Polyamine concentrations were calculated from peak areas using the method of internal standardization. The identity of the separated polyamines was confirmed by comparison with authentic standards.

Potential nitrogen fixation ability was examined by measuring acetylene reduction activity [8]. In brief, cells were grown on 5 ml semisolid modified Rennie medium [8] without out rice extract in a 10 ml serum vial. After 4 days of aerobic incubation at 30 °C, acetylene gas was injected to the vial at a final concentration of 10 %. The vial was further incubated for 2 days and acetylene and ethylene contents were analysed using a gas chromatograph equipped with flame ionization detector (GC9A; Shimadzu) and SHINCARBON-ST 50/80 column (Shinwa Chemical Industries). Furthermore, growth on nitrogen-free JNFb agar medium [9] was examined. H. seropedicae JCM 21448T was used as a positive control for these tests.
samples: putrescine, cadaverine, spermidine and 1,8-dia- 
minto-octane purchased from Sigma-Aldrich, and 2-hydroxy-
yputrescine obtained from Herbaspirillum frisingense NBRC 
102522^T, which was purchased from the National Biore-
source Center (NBRC), NITE, Japan.

Genomic DNA was extracted and PCR was performed using a 
Viriti 96-well thermal cycler (Applied Biosystems) to 
detect nitrite reductase genes ( nirK and nirS ), nitrous oxide 
reductase gene ( nosZ ), and nitrogenase gene ( nifH ) as 
described previously [1, 16]. The nearly full-length sequen-
ces of the 16S rRNA gene previously obtained [2, 3] were 
used to analyse the phyllogenetic positions of the strains 
relative to other Oxalobacteraeae strains. Sequences were 
divided with the reference sequences retrieved from 
the DDBJ/EMBL/GenBank databases by using CLUSTAL W 
[17]. Phylogenetic trees were reconstructed based on the 
neighbour-joining, maximum-likelihood and maximum-
parsimony methods by using MEGA v.6.06 [18]. Bootstrap 
analysis (n=1000) was performed to evaluate the topology 
of the phyllogenetic trees.

For genome analysis, DNA from strains TSA40^T 
and TSA66^T was extracted by using a PowerSoil DNA Isolation 
Kit (MoBio Laboratories). PCR-free libraries were prepared 
using a KAPA HyperPlus Kit (Kapa Biosystems) and Tru-
Seq DNA Sample Prep Kit (Illumina), for strains TSA40^T 
and TSA66^T, respectively, according to the manufacturers’ 
instruction. The genome of strain TSA40^T was analysed 
using the Illumina MiSeq with a 250 bp paired-end library, 
while the genome of strain TSA66^T was analysed using the 
Illumina HiSeq 2000 with a 101bp paired-end library. 
Resulting high-quality sequences were assembled using Vel-
et v.1.2.08 [19] to total lengths of 5 306 140 and 
5 384 605 bp, respectively, for the genome of strains TSA40^T 
and TSA66^T. Gene prediction and annotation were performed 
by the NCBI Prokaryotic Genomes Annotation Pipeline. Genome 
sequencing and annotation results are 
subsequently deposited in DDBJ/EMBL/ 
GenBank under accession numbers 
LSTO00000000 and 
JWJG00000000, respectively.

Average nucleotide identity (ANI) values between 
the genomes of strains TSA40^T, TSA66^T and 
H. massiliense JC206^T (GenBank accession no. CAHF00000000; [20]) were 
calculated using JSpecies [21]. Percentages of conserved 
proteins (POCP) were calculated [22] to assess the genome-
level relationships between strains TSA40^T, TSA66^T, 
H. massiliense JC206^T and H. seropedicae SmR1 (GenBank 
accession no. CP002039; [23]).

The morphological, cultural, physiological and biochemical 
characteristics of strains TSA40^T and TSA66^T are summarized 
in Table 1. Identical results were obtained from strains 
TSA66^T and TSO23-1. Cells of strains TSA40^T, TSA66^T 
and TSO23-1 were Gram-stain-negative, slightly-curved rods, 
0.4–0.6 µm wide and 1.4–2.2 µm long, with one or two polar 
flagella (Fig. S3). Colonies appeared white, circular and 
smooth when grown on DNBNS agar medium. Cells were 
able to grow aerobically on R2A agar and weakly on nutrient 
agar, but unable to grow in trypticase soy broth unlike other 
Herbaspirillum species. In DNBNS broth, strains TSA40^T, 
TSA66^T and TSO23-1 grew at 10–42 °C, with optimum 
growth temperature of 30 °C, and at pH 5.5–9.0. Cells 
could not grow in DNBNS medium supplemented with >1 % 
(w/v) NaCl. Cells were positive for catalase and oxidase 
activity, but negative for urease activity.

Strains TSA40^T, TSA66^T and TSO23-1 were able to grow 
with low-molecular-weight organic acids (e.g. acetate, buty-
rate, lactate, pyruvate, succinate, valerate) and their poly-
mers [e.g. Tween 40, Tween 80, poly(3-hydroxybutyric 
acid-co-3-hydroxyvaleric acid)], but not with many sugars 
(e.g. fructose, galactose, glucose, lactose, maltose, mannose, 
sorbitol, rhamnose, sorbitol, xylose), sugar acids (e.g. gluco-
nate), alcohols (e.g. butanediol, ethanol), or polysaccharides 
(e.g. starch, cellulose). This is in contrast to other 
Herbaspirillum species [9], but similar to H. massiliense 
DSM 25712^T, N. malthae JCM 18414^T and P. lenoignei 
LMG 2207^T [24]. Unlike H. massiliense DSM 25712^T and P. 
lenoignei LMG 2207^T, strains TSA40^T, TSA66^T and 
TSO23-1 utilized glycerol for growth.

Strains TSA40^T, TSA66^T and TSO23-1 were able to grow 
an aerobically using nitrate, nitrite and N₂O as an electron 
acceptor, whereas they did not reduce the Fe(III) substrates 
tested. Genes responsible for nitrate and N₂O reduction 
were previously identified [2, 3]. In addition, gene clusters 
for membrane-bound nitrate reductase (Nar), periplasmic 
nitrate reductase (Nap), cytochrome cd₁-containing nitrite 
reductase (Nir), nitric oxide reductase (Nort) and nitrous 
oxide reductase (Nos) were found in the genome of strains 
TSA40^T and TSA66^T. In contrast, H. massiliense JC206^T 
did not show nitrate-reducing activity [20]. In addition, genes 
associated with denitrification were not detected in the 
genome of H. massiliense JC206^T. Similarly, N. malthae 
JCM 18414^T did not show denitrification activity nor grew 
derittenitrification conditions in this study, although previous 
API 20E tests showed a positive result for nitrate 
reduction to N₂ gas [25]. In addition, denitrification func-
tional genes ( nirK, nirS and nosZ ) were not detected by PCR 
in N. malthae JCM 18414^T.

Strains TSA40^T, TSA66^T and TSO23-1 were able to use 
acetate, glycerol, succinate, pyruvate, butyrate, valerate 
and lactate as an electron donor for denitrification. Strains 
TSA66^T and TSO23-1 grew autotrophically using H₂ as an 
electron donor, nitrate as an electron acceptor and bicar-
bonate as a carbon source, whereas strain TSA40^T could 
not. The presence of genes encoding ribulose-1,5-bisphos-
phate carboxylase/oxygenase (RubisCO), phosphoribuloki-
nase and fructose-1,6-bisphosphatase/sedoheptulose-1,7-
bisphosphatase in the genome of TSA66^T also supports the 
autotrophic lifestyle of this strain. The RubisCO gene is 
present in the genomes of several Herbaspirillum species 
(but not in the genome of H. massiliense JC206^T, a strain 
closest to strain TSA66^T based on 16S rRNA gene sequence
not determined. All strains are motile by means of flagella. Strains 1, TSA40T; 2, TSA66T; 3, Noviherbaspirillum malthae JCM 18414T [25]; 4, Noviherbaspirillum humi [30]; 5, Noviherbaspirillum massiliense DSM 25712T [20]; 6, Herbaspirillum seropedicae JCM 21448T [9]; 7, Pauimonas lemoignei LMG 2207T [24]. *Determined in this study.

Table 1. Phenotypic and chemotaxonomic characteristics that separate strains TSA40T and TSA66T from closely related phylogenetic neighbours. Strains: 1, TSA40T; 2, TSA66T; 3, Noviherbaspirillum malthae JCM 18414T [25]; 4, Noviherbaspirillum humi [30]; 5, Noviherbaspirillum massiliense DSM 25712T [20]; 6, Herbaspirillum seropedicae JCM 21448T [9]; 7, Pauimonas lemoignei LMG 2207T [24]. +, Positive; −, negative; w, weakly positive; nd, not determined. All strains are motile by means of flagella.

<table>
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<th>Characteristic</th>
<th>1</th>
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<td>Cell shape</td>
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<td>Slightly curved</td>
<td>Short rods</td>
<td>Short rods</td>
<td>Rods</td>
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<td>Cell size (µm)</td>
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<td>0.4–0.6×1.4–2.2</td>
<td>1.0–1.2×1.4–1.6</td>
<td>0.9–1.1×1.0–1.2</td>
<td>0.44</td>
<td>0.6–0.7×1.5–5.0</td>
<td>0.6–0.8×1.5–3.0</td>
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<td>Pigment</td>
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<td>–</td>
<td>Red-orange</td>
<td>Light brown</td>
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<td>−</td>
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<td>ND</td>
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<td>4*</td>
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<td>DNA G+C content (mol%)</td>
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* Determined in this study.
16S rRNA gene sequence similarity between strains TSA40T and TSA66T, and the type species of the genera *Herbaspirillum*, *Noviherbaspirillum*, *Herminimonas*, *Oxalibacteraceae*, *Collimonas*, *Paucimonas* and *Glacimonas* were 95.6–97.5, 96.4–96.9, 94.8–95.2, 94.4–96.5, 95.0–95.3, 95.6 and 95.5%, respectively. Based on the phylogenetic analysis, strains TSA40T and TSA66T were closely related to *H. massiliense* JC206T, *N. malthae* JCM 18414T, *Noviherbaspirillum humi* U15T and *P. lemoignei* LMG 2207T, by the neighbour-joining, maximum-likelihood and maximum-parsimony methods (Fig. 1). 16S rRNA gene sequence similarities were <97% between strains TSA40T, TSA66T, and *N. malthae* JCM 18414T, *H. humi* U15T and *P. lemoignei* LMG 2207T, however, those between between strains TSA40T, TSA66T and *H. massiliense* JC206T were 96.9–97.7%. The ANI values between strains TSA40T, TSA66T and *H. massiliense* JC206T were 75–77% (Table S2), which are below the cutoff value for species discrimination (95–96%; [21, 28]). The POCP values between strains TSA40T, TSA66T and *H. massiliense* JC206T were 48–54% (Table S3), which are around the proposed cutoff value (50%) for genus discrimination [22]. *H. seropedicae* SmR1 showed much lower POCP values (32–36%) to strains TSA40T, TSA66T and *H. massiliense* JC206T, suggesting that strains TSA40T, TSA66T and *H. massiliense* JC206T may belong to another genus different from *Herbaspirillum* based on the comparative genome analysis.
The DNA G+C contents of strains TSA40T and TSA66T were 61.8 and 59.9 mol% based on the draft genome sequencing. Strains TSA40T and TSA66T had Q-8 as the only ubiquinone, similar to other Noviherbaspirillum and Herbaspirillum species [25, 29]. The major components in the fatty acid profiles of strains TSA40T and TSA66T were C16:0, C17:0 cyclo, C18:1ω7c and summed feature 3 (Table S4), similar to Noviherbaspirillum species [25, 30]. Major polyamines were putrescine and 2-hydroxyputrescine (Fig. S4 and Table S5), similar to other species within the genus Noviherbaspirillum [25, 29, 30]. Phosphatidylethanolamine and phosphatidylglycerol were commonly detected major polar lipids in strains TSA40T, TSA66T and H. massiliense DSM 25712T (Fig. S5). Similar to the previous report [29], the presence of phosphatidylcholine varied between strains: it was only detected in strains TSA66T and TSO23-1. While unknown amino lipids and unknown lipids were commonly present in strains TSA66T and TSO23-1, diphosphatidylglycerol was only detected in strain TSO23-1. In addition, small amounts of phosphatidylinositol and an unknown aminophospholipid were detected only in H. massiliense DSM 25712T. Unknown glycolipids were detected only in strain TSA40T, whereas an unknown phospholipid was detected in strain TSA66T and H. massiliense DSM 25712T. Unknown amino phospholipids were detected in strain TSA40T and H. massiliense DSM 25712T.

Based on the 16S rRNA gene sequence analysis in combination with physiological, chemotaxonomic and genomic properties, strains TSA40T (=JCM 17722T=ATCC TSD-69T) and TSA66T (=JCM 17723T=DSM 2587T) are the type strains of two novel species within the genus Noviherbaspirillum, for which we propose the names Noviherbaspirillum denitrificans sp. nov. and Noviherbaspirillum autotrophicum sp. nov., respectively. In addition, based on the 16S rRNA gene sequence analysis as well as physiological, chemotaxonomic and comparative genome analysis, we propose to reclassify Herbaspirillum massiliense as Noviherbaspirillum massiliense comb. nov.

**DESCRIPTION OF NOVIHERBASPIRILLUM AUTOTROPHICUM SP. NOV.**

Noviherbaspirillum autotrophicum (au.toтроphi.cum. Gr. pronoun autos self; Gr. adj. trophikos nursing, tending, or feeding; N.L. adj. autotrophicus self-nursing or self-feeding, i.e. able to grow with carbon dioxide as a sole carbon source).

Cells are Gram-stain-negative, facultatively anaerobic, motile, slightly curved rods. Cells have one or two polar flagella. Colonies formed on R2A agar medium are smooth, circular, white and convex. Cells are 0.4–0.6 μm in diameter and 1.4–2.2 μm in length. Cells are positive for catalase and oxidase activity, but negative for urease activity. Cells can grow at 10–42 °C (optimum 30 °C) and at pH 5.5–9.0. No growth occurs in the presence of 1% (w/v) NaCl on 100-fold diluted nutrient broth supplemented with 3 mM nitrate and 4.4 mM succinate. Cells can grow both aerobically and anaerobically with nitrate as an electron acceptor. Preferred organic sources are low-molecular-weight organic acids such as acetate, butyrate, lactate, pyruvate, succinate and valerate and their polymers such as Tween 40, Tween 80 and poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid). Sugars such as fructose, galactose, glucose, lactose, maltose, mannose, sorbitol, rhamnose, sorbitol and xylose, sugar acids such as gluconate, alcohols such as butanediol and ethanol, and polysaccharides such as cellulose and starch do not support growth. The major cellular fatty acids (>10% of total fatty acids) are C16:0, C17:0 cyclo, C18:1ω7c and summed feature 3 (C16:1ω7c/iso-C15 2-OH). The predominant ubiquinone is Q-8. Major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. Small amounts of an unknown aminophospholipid, an unknown phospholipid and unknown glycolipids are detected.

The type strain, TSA40T (=JCM 17722T=ATCC TSD-69T), was isolated from rice paddy soil originally collected from Tokyo, Japan. The DNA G+C content of the type strain is 61.8% (as determined by draft genome sequencing).

**DESCRIPTION OF NOVIHERBASPIRILLUM DENITRIFICANS SP. NOV.**

Herbaspirillum denitrificans (de.ni.trifィ.can.s. N.L. part. adj. denitrificans denitrifying).

Cells are Gram-stain-negative, facultatively anaerobic, motile, slightly curved rods. Cells have one or two polar flagella. Colonies formed on R2A agar medium are smooth, circular, white and convex. Cells are 0.4–0.6 μm in diameter and 1.4–2.2 μm in length. Cells are positive for catalase and oxidase activity, but negative for urease activity. Cells can grow at 10–42 °C (optimum 30 °C) and at pH 5.5–9.0. No growth occurs in the presence of 1% (w/v) NaCl on 100-fold diluted nutrient broth supplemented with 3 mM nitrate and 4.4 mM succinate. Cells can grow both aerobically and anaerobically with nitrate as an electron acceptor. Preferred organic sources are low-molecular-weight organic acids such as acetate, butyrate, lactate, pyruvate, succinate and valerate and their polymers such as Tween 40, Tween 80 and poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid). Sugars such as fructose, galactose, glucose, lactose, maltose, mannose, sorbitol, rhamnose, sorbitol and xylose, sugar acids such as gluconate, alcohols such as butanediol and ethanol, and polysaccharides such as cellulose and starch do not support growth. The major cellular fatty acids (>10% of total fatty acids) are C16:0, C17:0 cyclo, C18:1ω7c and summed feature 3 (C16:1ω7c/iso-C15 2-OH). The predominant ubiquinone is Q-8. Major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. Small amounts of phosphatidylcholine, an unknown aminolipid and unknown lipids are detected. The presence of diphosphatidylglycerol and an unknown phospholipid varies by strains.
The type strain, TSA66T (=JCM 17723T=DSM 25787T), was isolated from rice paddy soil originally collected from Tokyo, Japan. The DNA G+C content of the type strain is 59.9 % (as determined by draft genome sequencing).

**DESCRIPTION OF NOVIHERBASPIRILLUM MASSILIENSE COMB. NOV.**

*Noviherbaspirillum massiliense* (mas.si.li.en’es. N.L. neut. adj. *massiliense* of Massilia, the Latin name of Marseille where the type strain was cultivated).


Cells are rod-shaped with a mean diameter of 0.44 µm. Motile with tufts of polar flagella. Optimal growth occurs under aerobic conditions. Weak growth is observed under microaerophilic conditions and with 5 % CO₂. No growth is observed under anaerobic conditions. Growth occurs at 30–37 °C, with optimal growth at 37 °C. Catalase, oxidase and arginine dihydrolase activities, as well as aesculin hydrolysis are present. Nitrate reduction and indole production are absent. Preferred organic sources are low-molecular-weight organic acids such as acetate, butyrate and succinate, but not pyruvate. Sugars such as fructose, galactose, glucose, lactose, maltose, mannose, sorbitol, rhamnose, sorbitol and xylose, sugar acids such as glucuronate, and alcohols such as butanediol, ethanol and glycerol do not support growth. Cells are susceptible to ticarcillin, imipenem, trimethoprim/sulfamethoxazole, gentamicin, amikacin and colimycin. Preferred organic sources are low-molecular-weight organic acids. Nitrate reduction and indole production are observed under anaerobic conditions. Growth occurs at 30–37 °C, with optimal growth at 37 °C. Catalase, oxidase and arginine dihydrolase activities, as well as aesculin hydrolysis are present. Nitrate reduction and indole production are absent. Preferred organic sources are low-molecular-weight organic acids such as acetate, butyrate and succinate, but not pyruvate. Sugars such as fructose, galactose, glucose, lactose, maltose, mannose, sorbitol, rhamnose, sorbitol and xylose, sugar acids such as glucuronate, and alcohols such as butanediol, ethanol and glycerol do not support growth. Cells are susceptible to ticarcillin, imipenem, trimethoprim/sulfamethoxazole, gentamicin, amikacin and colimycin. Major polar lipids are phosphatidylethanolamine and phosphatidyglycerol. Small amounts of phosphatidylglycerol, an unknown aminophospholipid and an unknown phosphatidylglycerol. Small amounts of phosphatidylinositol, an unknown aminophospholipid and an unknown phospholipid are detected.

The type strain, JC206T (=CSUR P159T=DSM 25712T), was isolated from the faecal flora of a healthy patient in Senegal. The DNA G+C content of the type strain is 59.73 % (as determined by draft genome sequencing).

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Ethical statement**

No experiment was conducted with human or animal subjects.

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