Nitratireductor basaltis sp. nov., isolated from black beach sand

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A Gram-negative, coccoid- or rod-shaped bacterium was isolated from black sand collected from Soesoggak beach, Jeju Island, Korea. The isolate, designated J3^T, grew at 15–45 °C, at pH 5.5–10.0 and in 0–8 % NaCl. It was oxidase- and catalase-positive. Strain J3^T reduced nitrate to nitrite, but did not reduce nitrite to nitrogen gas. Phylogenetic analysis of the 16S rRNA gene sequence showed that strain J3^T was closely related to *Nitratireductor aquibiodomus* NL21^T and belonged to the genus *Nitratireductor*. Major cellular fatty acids were $C_{18:1}\omega7c$ (82.0 %), $C_{19:0}\omega8c$ cyclo (4.3 %) and $C_{18:0}$ (4.0 %), a profile that is typical of members of the genus *Nitratireductor* and distinct from those of other genera in the family *Phyllobacteriaceae*. Differences in physiological characteristics and fatty acid profiles, as well as low DNA–DNA hybridization values, further established that strain J3^T was distinct from *N. aquibiodomus* NL21^T. Thus, strain J3^T (=KCTC 22119^T =JCM 14935^T) should be classified as the type strain of a novel species in the genus *Nitratireductor*, for which the name *Nitratireductor basaltis* sp. nov. is proposed.

The genus *Nitratireductor* was established by Labbé *et al.* (2004), who proposed *Nitratireductor aquibiodomus*, a novel species comprising a nitrate-reducing strain, NL21^T. This strain was isolated from a marine denitrification system fed with methanol (Labbé *et al.*, 2003). This strain differed from members of other genera of the family *Phyllobacteriaceae* (Mergaert & Swings, 2005, 2006) with respect to a relatively low DNA G+C content (57 vs 60–64 mol% for other representatives of the family) and in its fatty acid profile (Labbé *et al.*, 2004). Currently, only one species has been described in the genus *Nitratireductor*. In this study, strain J3^T, a *Nitratireductor*-like strain isolated from black sand from Soesoggak beach, Jeju Island, Korea, was characterized.

Strain J3^T was isolated from black sand by using the standard dilution plating method and was cultured routinely on marine agar 2216 (MA; Difco) at 30 or 37 °C. The Gram reaction was performed using the non-staining method described by Buck (1982). Cell morphology and motility were observed under a Nikon phase-contrast microscope at ×1000 magnification with cells grown for 3 days at 37 °C on MA. Growth on R2A (Difco) and trypticase soy agar (TSA; Difco) was also evaluated at 37 °C. Growth at different temperatures (4, 10, 15, 25, 30, 37, 41 and 45 °C) and pH (pH 4.0–13.0 at intervals of 0.5 pH units) were assessed on MA and marine broth (Difco), respectively. Salt tolerance was tested in trypticase soy broth containing 0.5–30 % (w/v) NaCl. Strain J3^T was Gram-negative and non-motile. Growth occurred on R2A, TSA and MA. After 3 days incubation on MA at 30 °C, colonies were circular, smooth, creamy and convex, 0.5–2.0 mm in diameter. Strain J3^T was able to grow at 15–45 °C, at pH 5.5–10.0 and in 0–8 % NaCl.

DNA was extracted using a commercial genomic DNA extraction kit (G-spin; iNtRON Biotechnology). PCR-mediated amplification using PCR Pre-Mix (Solgent) of the 16S rRNA gene and sequencing of the purified PCR product with a PCR purification kit (Cosmo genetech) were carried out according to Yoon *et al.* (1998). Phylogenetic analyses were carried out according to Kim *et al.* (2006). The 16S rRNA gene sequences of related taxa were aligned using the program CLUSTAL_X (Thompson *et al.*, 1997). The program MEGA3 (Kumar *et al.*, 2004) was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain J3^T is EU143347.

used for tree construction. The neighbour-joining method was used to construct phylogenetic trees (Saitou & Nei, 1987) and distance matrices were calculated using Kimura's two-parameter model (Kimura, 1980). Bootstrap analysis was performed based on 1000 replications (Felsenstein, 1985). DNA–DNA relatedness was determined using photobiotin-labelled DNA probes and microwell plates as described previously (Roh *et al.*, 2008).

Comparative 16S rRNA gene sequence analyses revealed that strain J3^T showed the highest 16S rRNA gene sequence similarity to *N. aquibiodomus* NL21^T (97.0 %) and it showed similarities of less than 96.3 % to the type strains of species of other genera in the family *Phyllobacteriaceae* of the *Alphaproteobacteria*. The phylogenetic tree also showed that strain J3^T formed a monophyletic group with a bootstrap value of 97 % with *N. aquibiodomus* NL21^T and could be distinguished clearly from members of related genera (Fig. 1). The DNA–DNA relatedness between J3^T and *N. aquibiodomus* NL21^T was less than 10 %. Low 16S rRNA gene sequence similarity and DNA–DNA relatedness confirmed that strain J3^T was distinct from *N. aquibiodomus* NL21^T at the species level.

Cellular fatty acids were analysed from cells of J3^T and *N. aquibiodomus* NL21^T grown on TSA (Difco) for 3 days at 30 °C. Cellular fatty acids were analysed according to the protocol of the Sherlock Microbial Identification system (Sasser, 1990) using GC. The fatty acid pattern of strain J3^T was similar to that of *N. aquibiodomus* NL21^T (Table 1), and the strain could therefore be affiliated to the genus *Nitratireductor*; however, the fatty acid profiles of members of the genus *Nitratireductor* differed from those of members of related genera in the family *Phyllobacteriaceae*, as clarified previously (Labbé *et al.*, 2004). However, some differences in fatty acid content were observed between strain J3^T and *N. aquibiodomus* NL21^T; strain J3^T had a smaller amount of $C_{18:0}$ and a larger amount of summed feature 3 compared with *N. aquibiodomus* NL21^T. The absence or presence of certain minor fatty acids also enabled the strains to be differentiated from each other (Table 1).

Catalase activity was determined by bubble production in 3 % (v/v) H_2O_2 and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. The G+C content of the chromosomal DNA was determined by the thermal denaturation method as described previously (Gonzalez & Saiz-Jimenez, 2002). Physiological characteristics of strain J3^T, including nitrate-reducing ability, were determined with API 20NE, API ZYM and API 50CH galleries, according to the instructions of the manufacturer (bioMérieux). Differential physiological characteristics of strain J3^T and *N. aquibiodomus*, the type species of the genus *Nitratireductor*, are listed in Table 2. Both species reduced nitrate to nitrite, but did not reduce nitrite to nitrogen gas. Both species were negative for urease, unlike most members of related genera. The DNA G+C content of strain J3^T was 56.7 ± 1.3 mol%, which is similar to that for *N. aquibiodomus* NL21^T. In contrast to *N. aquibiodomus* NL21^T, strain J3^T assimilated gluconate, mannitol and maltose, but did not assimilate citrate. Strain J3^T showed α- and β-galactosidase activities, which are absent in most members of the family *Phyllobacteriaceae* and in *N. aquibiodomus* NL21^T (Labbé *et al.*, 2004).

Phenotypic, chemotaxonomic and phylogenetic analyses established that the isolate could be affiliated to the genus *Nitratireductor* and suggest that it represents a novel species of the genus, for which the name *Nitratireductor basaltis* sp. nov. is proposed.

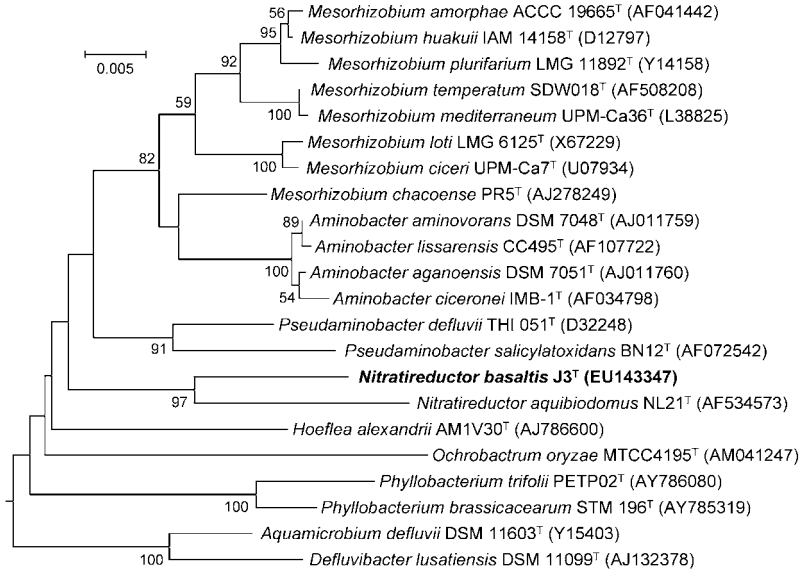

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain J3^T, *N. aquibiodomus* NL21^T and representatives of related genera. The tree was constructed based on the neighbour-joining algorithm. Numbers indicate bootstrap values (%) after 1000 resamplings; only values >50 % are shown. GenBank accession numbers are given in parentheses. Bar, 0.005 substitutions per site.

Table 1. Fatty acid profiles of type strains of species of the genus *Nitratireductor*

Data for *N. aquibiodomus* NL21^T were taken from this study and Labbé *et al.* (2004). Values are percentages of total fatty acids. −, Not detected.

Fatty acid	*N. basaltis* sp. nov. J3^T	*N. aquibiodomus* NL21^T
Saturated		
$C_{16:0}$	2.48	0.96–2.16
$C_{17:0}$	0.88	0.33–1.72
$C_{18:0}$	3.95	3.15–4.23
$C_{20:0}$	0.17	−
Unsaturated		
$C_{17:1}\omega 8c$	0.37	0–0.59
$C_{17:1}\omega 6c$	0.37	0–0.54
$C_{18:1}\omega 7c$	81.93	75.01–76.47
$C_{18:1}\omega 9c$	−	0–0.61
$C_{20:1}\omega 7c$	0.27	0.48–0.63
Branched		
iso-$C_{13:0}$	0.28	−
iso-$C_{15:0}$	0.31	−
iso-$C_{17:0}$	2.86	1.93–3.25
Hydroxy		
iso-$C_{15:0}$ 3-OH	0.31	0.99–1.86
$C_{18:0}$ 3-OH	−	0.31–0.52
Cyclopropane acids		
$C_{19:0}\omega 8c$ cyclo	4.25	9.35–12.14
Other		
Summed feature 3*	1.58	0.56–0.63
Summed feature 5†	−	0–0.42
Unknown	−	1–1.12

*Summed feature 3 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7c$.
†Summed feature 5 comprises anteiso-$C_{18:0}$ and/or $C_{18:2}\omega 6,9c$.

Table 2. Physiological characteristics of type strains of species of the genus *Nitratireductor*

Data for *N. aquibiodomus* NL21^T were taken from Labbé *et al.* (2004). Both strains are positive for nitrate reduction to nitrite, assimilation of D-glucose, and the presence of oxidase, catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. Both strains are negative for nitrite reduction to nitrogen gas, indole production, gelatin hydrolysis, assimilation of caprate, erythritol, inulin, phenylacetate, L-rhamnose, salicin, trehalose and turanose and the presence of α-chymotrypsin, α-fucosidase, β-glucosidase, lipase (C14), α-mannosidase and urease. +, Positive; W, weakly positive; −, negative.

Characteristic	*N. basaltis* J3^T	*N. aquibiodomus* NL21^T
Morphology	Coccoid, rods	Rods
Motility	−	+
NaCl tolerance (%)	0–8	0–5
Assimilation of:		
Gluconate	+	−
Adipate	−	W
Malate	−	W
Citrate	−	+
Sucrose	+	−
D-Arabinose	−	+
D-Fructose	−	+
Activity of:		
Cystine arylamidase	−	+
Trypsin	W	+
Acid phosphatase	−	+
α-Galactosidase	+	−
β-Galactosidase	+	−
β-Glucuronidase	W	−
α-Glucosidase	−	+
N-Acetyl-β-glucosaminidase	−	+
DNA G+C content (mol%)	56.7	57

Description of *Nitratireductor basaltis* sp. nov.

Nitratireductor basaltis (ba.sal′tis. L. masc. gen. n. *basaltis* of basalt, pertaining to the source of isolation of the type strain).

Cells are Gram-negative, non-motile, coccoid or rod-shaped (0.6–0.7 μm wide and 0.6–2.0 μm long). Colonies are circular, smooth, creamy, convex and measure 0.5–2.0 mm in diameter after 3 days incubation on MA at 30 °C. Cells grow at 15–45 °C, at pH 5.5–10.0 and in 0–8 % NaCl. Growth occurs on R2A, TSA and MA. Positive for catalase, oxidase and valine arylamidase. Positive for the assimilation of *N*-acetylglucosamine, L-arabinose, cellobiose, D- and L-fucose, gentiobiose, glycerol, gluconate, maltose, D-mannitol, melibiose, D-ribose, D-sorbitol and D-xylose; weakly positive for assimilation of 2-ketogluconate. Negative for assimilation of D-adonitol, starch, amygdalin, arbutin, D- and L-arabitol, dulcitol, aesculin ferric citrate, D-galactose, glycogen, inositol, 5-ketogluconate, D-lactose, D-xylose, D-mannose, melezitose, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, methyl β-D-xylose, raffinose, L-sorbose, D-tagatose, xylitol and L-xylose. Major fatty acids are $C_{18:1}\omega 7c$, $C_{19:0}\omega 8c$ cyclo and $C_{18:0}$. Other physiological and biochemical characteristics are shown in Tables 1 and 2.

The type strain is J3^T (=KCTC 22119^T =JCM 14935^T), isolated from black sand collected from Soesoggak beach, Jeju Island, Korea.

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