Naxibacter varians sp. nov. and *Naxibacter haematophilus* sp. nov., and emended description of the genus *Naxibacter*

Peter Kämpfer,¹ Enevold Falsen² and Hans-Jürgen Busse³

Correspondence

Peter Kämpfer
peter.kaempfer@umwelt.
uni-giessen.de

¹Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany

²Culture Collection University Göteborg, Dept of Clinical Bacteriology, S-41346 Göteborg, Sweden

³Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

Six Gram-negative, rod-shaped, non-spore-forming bacteria isolated from clinical specimens and water samples were investigated for their taxonomic allocation. On the basis of 16S rRNA gene sequence similarities, these strains (CCUG 35299^T, CCUG 24677A, CCUG 48018, CCUG 49054, CCUG 48700A and CCUG 38318^T) were shown to belong to the *Betaproteobacteria*, closely related to *Naxibacter alkalitolerans* (97.9–98.8% sequence similarity to the type strain). Chemotaxonomic data (major ubiquinone Q-8; major polyamines hydroxyputrescine and putrescine; major polar lipids phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol; major fatty acids C_{16:1ω7c} and C_{16:0}, with 3-OH C_{10:0} and 2-OH C_{12:0} as hydroxylated fatty acids) supported the affiliation of the isolates to the genus *Naxibacter*. The results of DNA–DNA hybridization and physiological and biochemical tests allowed both genotypic and phenotypic differentiation of the isolates from the described *Naxibacter* species. Isolates CCUG 35299^T, CCUG 24677A, CCUG 48018, CCUG 49054 and CCUG 48700A were closely related on the basis of DNA–DNA reassociation experiments and therefore represent a single novel species, for which the name *Naxibacter varians* sp. nov. is proposed, with the type strain CCUG 35299^T (=CCM 7478^T). Strain CCUG 38318^T represents a second novel *Naxibacter* species, for which we propose the name *Naxibacter haematophilus* sp. nov. (type strain CCUG 38318^T =CCM 7480^T).

The betaproteobacterial genus *Naxibacter* was described by Xu *et al.* (2005) and at present the genus comprises only one species, *Naxibacter alkalitolerans*. Phylogenetically, it is placed in the vicinity of *Massilia*, *Telluria*, *Duganella* and *Janthinobacterium*. The single strain of this species was isolated from a soil sample and shown to produce polyketide antibiotics (Xu *et al.*, 2005). Another striking characteristic of this species was the detection of phosphatidylinositol mannosides in its polar lipid profile, which usually is a characteristic of Gram-positive bacteria; to the best of our knowledge, there is so far no other report of presence of this lipid in Gram-negative bacteria.

Strains CCUG 35299^T, CCUG 24677A, CCUG 48018, CCUG 49054, CCUG 48700A and CCUG 38318^T were

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CCUG 35299^T and CCUG 38318^T are respectively AM774587 and AM774589.

Results of two-dimensional TLC of the polar lipids of the type strains of the novel species and details of antibiotic susceptibility are available as supplementary material with the online version of this paper.

isolated from various sources. Strain CCUG 35299^T was isolated in 1996 from the eye of a 90-year-old man in Tromsø, Norway. Strains CCUG 48018 and CCUG 48700A were isolated from water in Sweden. Strain CCUG 24677A was isolated in 1989 in Stockholm, Sweden, from human blood. Strain CCUG 38318^T was also isolated from human blood, of a 23-year-old man with multiple health problems in 1997. All strains were presumptively identified as *Massilia*-like and all showed beige-coloured colonies on nutrient agar (Oxoid) at 37 °C. Subcultivation for further analyses was done on tryptone soy agar (TSA; Oxoid) at 28 °C for 48 h.

Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA. The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). Phylogenetic analysis was performed using the ARB software package (Ludwig *et al.*, 2004) and also the software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by

CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) were calculated and clustering with the neighbour-joining, maximum-parsimony and maximum-likelihood algorithms was performed by using bootstrap values based on 1000 replications. The almost-complete 16S rRNA gene sequences of all strains were compared by sequence similarity calculations after a neighbour-joining analysis. The results of these calculations indicated that the closest relative of strains CCUG 35299^T, CCUG 24677A, CCUG 48018, CCUG 49054 and CCUG 48700A was *N. alkalitolerans* (99.1–99.8% similarity to the type strain). Strain CCUG 38018^T showed 97.6% 16S rRNA gene sequence similarity to the type strain of *N. alkalitolerans* and 98.1% to CCUG 35299^T.

A phylogenetic tree is shown in Fig. 1. The branching pattern was confirmed by maximum-parsimony and maximum-likelihood analyses (data not shown).

For quinone, polar lipid and polyamine analyses, cells were grown on PYE medium (Busse *et al.*, 2005). Quinone systems and polar lipid profiles were analysed according to Tindall (1990a, b) and Altenburger *et al.* (1996) and polyamine patterns as described by Busse & Auling (1988) and Busse *et al.* (1997). The four strains CCUG 35299^T, CCUG 48700A, CCUG 24677A and CCUG 38318^T exhibited typical betaproteobacterial quinone systems, with the predominant compound ubiquinone Q-8 (96.6–98.6%) and minor amounts of ubiquinone Q-7 (1.4–3.4%).

The predominant compounds of the polar lipid profiles of the four strains were identical. They consisted of the major compounds phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. In CCUG 35299^T (see Supplementary Fig. S1a in IJSEM Online) and CCUG

24677A (results not shown), moderate amounts of an unknown polar lipid (L1) and an unknown phospholipid (PL1) and minor to trace amounts of two unknown aminolipids (AL1, AL2) and an unknown phospholipid (PL2) could be detected. Trace amounts of the unknown aminophospholipid APL1 (not indicated in Supplementary Fig. S1a) may be present as well but, due to its very low abundance, it is not visible on the chromatogram. The position is visible in Supplementary Fig. S1(b). Since strains CCUG 35299^T and CCUG 24677A belong to the same species, as demonstrated by the results of DNA–DNA hybridizations (see below), this similarity demonstrates their close relatedness. The next phylogenetic neighbour (Fig. 1), strain CCUG 48700A, also shared an identical polar lipid profile (not shown). Strain CCUG 38318^T, which is more distantly related to the other species of the *Naxibacter* clade (Fig. 1), exhibited some striking differences. In its polar lipid profile (Supplementary Fig. S1b), significant amounts of the unknown aminophospholipid APL1, unknown aminolipid AL3 and a yellow spot could be detected. None of the four strains exhibited the presence of phosphatidylinositol mannosides or any other glycolipid. This observation was surprising, because the close relative *N. alkalitolerans* was reported to contain phosphatidylinositol mannoside in its polar lipid profile (Xu *et al.*, 2005). Hence, we also analysed the profile of *N. alkalitolerans* (results not shown) and, in fact, we were not able to detect phosphatidylinositol mannosides or any other glycolipid in the profile of the type strain of this species. This result is in agreement with the present knowledge that this glycolipid is found in certain Gram-positive taxa but not in Gram-negative taxa.

Strains CCUG 48700A, CCUG 24677A and CCUG 38318^T showed polyamine patterns characteristic for betaproteobacteria, with the major compounds 2-hydroxyputrescine

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory database (accession numbers in parentheses) constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) were calculated and clustering with the neighbour-joining method was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.005 substitutions per nucleotide position.

and putrescine. Other polyamines such as spermidine and spermine were always detected in smaller amounts and 1,3-diaminopropane and cadaverine were detected in trace amounts in the pattern of CCUG 35299^T only. Similar polyamine patterns were detected in related type strains *Naxibacter alkalitolerans* CCUG 50882^T [$\mu\text{mol (g dry weight)}^{-1}$: 2-hydroxyputrescine, 39.4; putrescine, 60.1; spermidine, 11.5; spermine, 3.2], *Massilia timonae* CCUG 45783^T (2-hydroxyputrescine, 24.0; putrescine, 74.4; spermidine, 4.1; spermine, 1.4) and *Janthinobacterium agaricidamnosum* CCUG 43140^T (2-hydroxyputrescine, 37.3; putrescine, 137.5; cadaverine, 0.7; spermidine, 1.1; spermine, <1.0).

The fatty acid profiles of all strains obtained with the method described by Kämpfer & Kroppenstedt (1996) are shown in Table 1. No pronounced differences in fatty acid profiles were found.

Results of the physiological characterization are given in the species description and in Table 2 (differential characters), obtained with methods described previously (Kämpfer *et al.*, 1991). Sensitivity towards antimicrobial compounds was examined according to the disc-diffusion plate method but, due to poor growth on Mueller–Hinton agar, the tests were done on PYE (peptone-yeast extract) agar and incubated for 4 days at 28 °C. The results are listed in the species descriptions and in Supplementary Table S1.

DNA–DNA hybridization experiments were performed with all strains and the type strain of *Naxibacter alkalitolerans*, CCUG 50882^T, using the method described by Ziemke *et al.* (1998) except that, for nick translation,

Table 1. Cellular fatty acid compositions (%) of *Naxibacter* strains

Strains: 1, *N. alkalitolerans* CCUG 50882^T; 2–6, strains of *N. varians* sp. nov. (2, CCUG 48018; 3, CCUG 35299^T; 4, CCUG 49054; 5, CCUG 24677A; 6, CCUG 48700A); 7, *N. haematophilus* sp. nov. CCUG 38318^T. –, Not detected.

Fatty acid	1	2	3	4	5	6	7
C _{10:0}	0.7	0.4	0.5	0.4	0.4	0.7	0.6
C _{10:0} 3-OH	6.2	3.6	4.0	4.5	4.1	5.3	4.7
C _{12:0}	6.3	3.0	3.4	3.4	3.6	4.0	4.5
C _{12:0} 2-OH	2.0	1.3	1.8	1.8	1.4	2.3	1.5
C _{14:0}	1.2	0.8	1.0	0.6	0.7	0.6	0.6
C _{15:0}	0.8	0.5	0.4	0.6	0.6	–	0.3
C _{16:1} ω 7c*	43.1	50.4	47.4	45.8	49.2	52.1	46.3
C _{16:0}	30.0	32.0	31.9	32.2	33.3	22.3	28.6
anteiso-C _{17:1} ω 9c	0.3	–	–	–	–	–	–
iso-C _{17:1} ω 9c	–	0.4	0.4	0.3	–	0.5	–
C _{17:0} cyclo	1.3	–	–	–	–	–	–
C _{18:1} ω 7c	6.9	7.5	8.4	9.2	6.5	12.2	12.7
C _{18:0}	0.4	0.2	0.6	0.5	–	–	0.5

*Included in summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH).

Table 2. Differential phenotypic properties of *Naxibacter* species

Strains: 1, *N. alkalitolerans* CCUG 50882^T; 2–6, *N. varians* strains (2, CCUG 48018; 3, CCUG 35299^T; 4, CCUG 49054; 5, CCUG 24677A; 6, CCUG 48700A); 7, *N. haematophilus* CCUG 38318^T. +, Positive; (+), weakly positive; –, negative; ONPG, *o*-nitrophenyl β -D-glucoside. All strains were positive for utilization of maltose, malate, fumarate and pyruvate and hydrolysis of *p*-nitrophenyl phosphate. All strains were negative for utilization of mannose, trehalose, mannitol, gluconate and sucrose.

Characteristic	1	2	3	4	5	6	7
Utilization as sole carbon source							
D-Glucose	+	–	–	–	+	–	–
L-Arabinose	+	–	–	–	–	+	–
Adipate	–	–	–	–	–	–	+
Lactate	–	–	+	+	+	+	–
<i>cis</i> -Aconitate	–	(+)	–	–	–	–	–
Citrate	+	–	–	–	–	–	–
DL-3-Hydroxybutyrate	+	+	+	+	+	+	–
2-Oxoglutarate	+	+	+	+	+	–	+
Pyruvate	+	+	+	+	+	+	+
Acetate	–	+	–	+	+	+	+
Acid formed from:							
Glucose	+	–	–	–	–	–	–
Maltose	+	–	–	–	–	–	–
Cellobiose	+	–	–	–	–	–	–
Aesculin hydrolysis	+	+	+	+	+	+	–
ONPG hydrolysis	+	+	+	+	+	–	–

2 μg DNA was labelled during a 3 h incubation at 15 °C. The results are shown in Table 3.

Examinations based on almost-entire 16S rRNA gene sequences showed affiliation of the six strains to the genus *Naxibacter*. They also shared the characteristics of the genus (Xu *et al.*, 2005), which are a fatty acid profile consisting of the major compounds C_{16:1} ω 7c and C_{16:0} and the

Table 3. DNA–DNA reassociation (%) between the novel strains and *N. alkalitolerans* CCUG 50882^T

Strain	1	2	3	4	5	6	7
1. <i>N. alkalitolerans</i> CCUG 50882 ^T	100	62	43	48	–	48	23
<i>N. varians</i>							
2. CCUG 48018	51	100	94	–	–	76	–
3. CCUG 35299 ^T	39	100	100	100	85	64	30
4. CCUG 49054	53	–	98	100	–	–	–
5. CCUG 24677A	–	–	100	–	100	–	–
6. CCUG 48700A	54	65	84	–	–	100	34
7. <i>N. haematophilus</i> CCUG 38318 ^T	28	–	–	–	–	19	100

–, Not done.

hydroxylated fatty acids C_{10:0} 3-OH and C_{12:0} 2-OH, the main quinone ubiquinone Q-8 (in congruence with that of *N. alkalitolerans*) and a polar lipid profile containing the major compounds phosphatidylglycerol and phosphatidylethanolamine. We could not detect the presence of phosphatidylinositol mannosides, reported to be another major compound in *N. alkalitolerans* (Xu *et al.*, 2005), in any of the strains classified in this study or in *N. alkalitolerans*. Hence, we conclude that Xu *et al.* (2005) erroneously identified this Gram-positive-characteristic lipid in *N. alkalitolerans*. From our point of view, this error makes an emended description of the genus *Naxibacter* necessary.

Relationship at the species level of strains CCUG 35299^T, CCUG 24677A, CCUG 48018, CCUG 49054 and CCUG 48700A, as indicated by high 16S rRNA gene sequence similarities, was confirmed by the results of DNA–DNA hybridizations (Table 3). Strain CCUG 35299^T showed only 97.6% sequence similarity to *N. alkalitolerans* CCUG 50882^T but 99% similarity to strain CCUG 38318^T. The results from DNA–DNA hybridizations, however, showed that these strains represent distinct species. On the basis of phenotypic traits (physiological properties) and the results of DNA–DNA hybridizations, we describe two novel *Naxibacter* species.

Description of *Naxibacter varians* sp. nov.

Naxibacter varians (va'ri.ans. L. part. adj. *varians* varying, pertaining to the variable results in biochemical tests).

Cells are non-motile, non-spore-forming rods (approx. 2 µm long and 1 µm wide). Gram-negative and oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, PYE agar, nutrient agar and MacConkey agar at 25–30 °C. Growth is detected on TSA at 15–37 °C; no growth at 10 or 45 °C. Growth is positive in TS broth (TSB) at pH 5.5–10.5 (strain CCUG 48700A is even able to grow at pH 11.5). Beige, translucent and shiny colonies with entire edges are formed within 24 h, with a diameter of approximately 2 mm. The quinone system consists of the major compound ubiquinone Q-8 and small amounts of Q-7. The polyamine pattern contains the predominant compounds putrescine [44.8–69.0 µmol (g dry weight)⁻¹] and 2-hydroxyputrescine [26.3–46.3 µmol (g dry weight)⁻¹] and variable amounts of spermidine [1.4–16.8 µmol (g dry weight)⁻¹] and spermine [1.1–6.0 µmol (g dry weight)⁻¹]. Trace amounts [<1.0 µmol (g dry weight)⁻¹] of 1,3-diaminopropane and cadaverine may also be present. The major compounds in the polar lipid profile are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Moderate amounts of an unknown polar lipid and an unknown phospholipid and minor to trace amounts of two unknown aminolipids and an unknown phospholipid are present. The fatty acid profile is composed largely of C_{16:1ω7c} (45.8–50.4%) and C_{16:0} (31.9–33.3%). Carbon-source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all *Naxibacter* species) are indicated in

Table 2. Sensitive towards polymyxin B (300 U), penicillin G (10 IU), amoxicillin (25 µg), amoxicillin/clovalanate (30 µg), gentamicin (10 µg), trimethoprim (5 µg), ciprofloxacin (5 µg), rifampicin (5 µg) and colistin (25 µg).

The type strain CCUG 35299^T (=CCM 7478^T) was isolated from the eye of a 90-year-old man in Tromsø, Norway. Other strains of this species are CCUG 24677A, CCUG 48018, CCUG 49054 and CCUG 48700A.

Description of *Naxibacter haematophilus* sp. nov.

Naxibacter haematophilus (hae.ma.to.phi'lus. Gr. n. *haema* -atos blood; Gr. adj. *philos* loving; N.L. masc. adj. *haematophilus* loving blood, referring to the isolation of the type strain from blood).

Cells are non-motile, non-spore-forming rods (approx. 2 µm long and 1 µm wide). Gram-negative and oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, PYE agar and nutrient agar at 25–30 °C. Growth is detected on TSA at 15–37 °C; no growth at 10 or 45 °C. Growth is positive in TSB at pH 5.5–10.5. Colonies are beige, translucent and shiny with entire edges and form within 24 h, with a diameter of approximately 2 mm. The quinone system consists of the major compound ubiquinone Q-8 and small amounts of Q-7. The polyamine pattern contains the predominant compounds putrescine [95.9 µmol (g dry weight)⁻¹] and 2-hydroxyputrescine [21.0 µmol (g dry weight)⁻¹] and small amounts [<3 µmol (g dry weight)⁻¹] of spermidine and spermine. The major compounds in the aminolipid profile are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Moderate amounts of an unknown aminolipid and an unknown aminophospholipid and minor to trace amounts of one unknown aminolipid and three unknown phospholipids are present. The fatty acid profile is composed largely of C_{16:1ω7c} (52.1%) and C_{16:0} (22.3%). Carbon-source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all *Naxibacter* species) are indicated in Table 2. Sensitive towards polymyxin B (300 U), penicillin G (10 IU), amoxicillin (25 µg), amoxicillin/clovalanate (30 µg), gentamicin (10 µg), trimethoprim (5 µg), ciprofloxacin (5 µg), rifampicin (5 µg) and colistin (25 µg).

The type strain CCUG 38318^T (=CCM 7480^T) was isolated in 1997 from blood of a 23-year-old man with multiple health problems.

Emended description of the genus *Naxibacter* Xu *et al.* 2005

The description is that of Xu *et al.* (2005), with the exception that the polar lipid profile does not contain phosphatidylinositol mannosides. Another lipid present in major amounts is diphosphatidylglycerol. The polyamine pattern contains the predominant compounds 2-hydroxyputrescine and putrescine.

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