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TAXONOMIC DESCRIPTION
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Paenalcaligenes niemegkensis sp. nov., a novel species of the family *Alcaligenaceae* isolated from plastic waste

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Abstract

Strain NGK35^T is a motile, Gram-stain-negative, rod-shaped (1.0–2.1 µm long and 0.6–0.8 µm wide), aerobic bacterium that was isolated from plastic-polluted landfill soil. The strain grew at temperatures between 6 and 37 °C (optimum, 28 °C), in 0–10 % NaCl (optimum, 1 %) and at pH 6.0–9.5 (optimum, pH 7.5–8.5). It was positive for cytochrome c oxidase, catalase as well as H_2S production, and hydrolysed casein and urea. It used a variety of different carbon sources including citrate, lactate and pyruvate. The predominant membrane fatty acids were $C_{16:1}$ cis9 and $C_{16:0}$, followed by $C_{17:0}$ cyclo and $C_{18:1}$ cis11. The major polar lipids were phosphatidylglycerol and phosphatidylethanolamine, followed by diphosphatidyglycerol. The only quinone was ubiquinone Q-8. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain NGK35^T belongs to the genus *Paenalcaligenes* (family *Alcaligenaceae*), appearing most closely related to *Paenalcaligenes hominis* CCUG 53761A^T (96.90 %) and *Paenalcaligenes suwonensis* ABC02-12^T (96.94 %). The genomic DNA G+C content of strain NGK35^T was 52.1 mol%. Genome-based calculations (genome-to-genome distance, average nucleotide identity and DNA G+C content) clearly indicated that the isolate represents a novel species within the genus *Paenalcaligenes*. Based on phenotypic and molecular characterization, strain NGK35^T can clearly be differentiated from its phylogenetic neighbours establishing a novel species, for which the name *Paenalcaligenes niemegkensis* sp. nov. is proposed. The type strain is NGK35^T (=DSM 113270^T=NCCB 100854^T).

ISOLATION AND ECOLOGY

In the frame of a study targeting the culture-dependent microbial diversity of the terrestrial plastisphere, a set of microbial strains was isolated from plastic debris and plastic-polluted soil found on an abandoned landfill close to the small city of Niemegk in the Potsdam-Mittelmark district of Brandenburg, north-eastern Germany (52° 02′ 58.8″ N, 12° 39′ 34.8″ E). The investigation aimed at understanding the terrestrial plastisphere as a distinct microbial habitat and the potential of such plastic-associated micro-organisms to degrade polyethylene [1], one of the most common plastic types found in the terrestrial environment. The microbial isolates were enriched in slurries prepared with plastic debris from the landfill and mineral salt medium (MSM; 0.1% $(NH_4)_2SO_4$, 0.1% K_2HPO_4, 0.1% $NaNO_3$, 0.1% KCl, 0.02% $MgSO_4$ and 0.01% yeast extract) containing cycloheximide (0.01 M) but no additional carbon source, according to a protocol of Burd [2]. Slurries were incubated at 27 °C for 3–5 days before they were plated onto solid MSM containing 0.1% powdered polyethylene as an additional carbon source. In a second step, single colonies were isolated on solid MSM with 1% n-hexadecane as only carbon source, an alkane (C_{16}) whose basic chemical structure is identical to polyethylene and has been used earlier as a screening for polymer-degrading bacteria [3]. Purity of the microbial isolates was verified

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Keywords: *Paenalcaligenes niemegkensis*; plastic pollution; terrestrial plastisphere; bacteria.
Abbreviations: ANI, average nucledotide identity; dDDH, digital DNA–DNA hybridization; DPG, diphosphatidylglycerol; FAME, fatty acid methyl ester; IPL, intact polar lipid; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; MePA, methylphosphatidic acid; MSM, mineral salt medium; OHPE, hydroxyphosphatidylethanolamine; OL, ornithine lipids; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PG, phosphatidylglycerol; PLFA, phospholipid fatty acid.
The 16S rRNA gene sequence of strain NKG35^T as well as the Whole Genome Shotgun project have been deposited at DDBJ/ENA/GenBank under the accessions OV113552.1 and JAKGCT000000000. The genome version described in this paper is version JAKGCT000000000.1.
Two supplementary figures and three supplementary tables are available with the online version of this article.

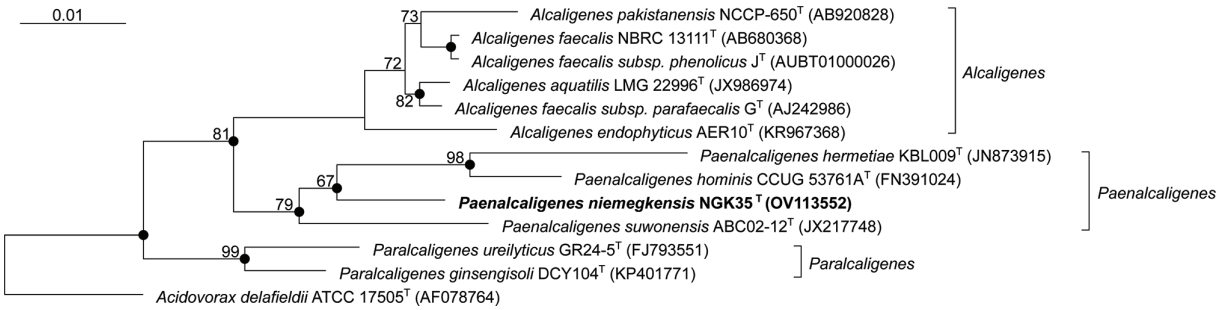

Fig. 1. Neighbourjoining phylogenetic tree based on the full-length 16S rRNA gene sequence of NGK35^T and type strains of species of the genera *Paenalcaligenes*, *Alcaligenes* and Paralcaligenes. *Acidovorax delafieldii* ATCC 17505^T (AF078764) was used as an outgroup. Filled circles at branch points indicate nodes that were also found in the maximum-likelihood (Fig. S1) and maximum-parsimony trees (Fig. S2). Bootstrap values (expressed as percentages of 1000 replicates) greater than 50% are shown at branch points. GenBank accession numbers are given in parentheses. Bar, 1% sequence divergence.

microscopically and by 16S rRNA gene sequencing. The isolate NGK35^T was classified as member of the genus *Paenalcaligenes* (family *Alcaligenaceae*) on the basis of 16S rRNA gene sequence divergence and was further investigated.

The genus *Paenalcaligenes*, which is closely related to the genus *Alcaligenes*, comprises three species with validly published names to date. The type species *Paenalcaligenes hominis* was isolated from human blood [4]. Two additional species were then proposed: *Paenalcaligenes hermetiae*, isolated from the larval gut of *Hermetia illucens* [5], and *Paenalcaligenes suwonensis*, isolated from spent mushroom compost [6]. So far, species within the genus are Gram-stain negative, short rods, catalase- and oxidase-positive, and mainly showing an aerobic respiratory metabolism. The quinone system is predominated by ubiquinone Q-8. The major polar lipids are composed of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE), accompanied with lower amounts of unidentified amino and polar lipids [4–6].The major fatty acids (>10 %) are $C_{16:0}$, $C_{17:0}$ cyclo, summed feature 2 (iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH) and summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), as well as summed feature 8 ($C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) [6].

Strain NGK35^T displays all these common characteristics. Together with our 16S rRNA gene sequencing results, this confirms that strain NGK35^T represents a new species of the genus *Paenalcaligenes*. In the following, the taxonomic position of this strain has been investigated using a polyphasic approach.

16S rRNA GENE PHYLOGENY

For a preliminary molecular characterization, the 16S rRNA gene was partially sequenced using gene fragments that were amplified with the bacterial primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) [7] and 907R (5′-CCGTCAATTCCTTTRAGTTT-3′) [8]. Sequencing of the purified PCR products (Hi Yield Gel/PCR DNA Fragment Extraction Kit; SLG, Gauting) was performed by Microsynth Seqlab

Table 1. Genome-based comparison of NGK35^T and other type strains of members of the genera *Paenalcaligenes*, *Alcaligenes* and *Paralcaligenes*

Reference strain	GGDC distance (DSMZ)*			(Jspecies)				G+C (mol%)
	Formula 1	Formula 2	Formula 3	ANIb (%)	Aligned (%)	ANIm (%)	Aligned (%)	(NGK35^T, 52.1%)
Paenalcaligenes suwonensis ABC02-12^T (NZ_JAANPV010000028.1)	13.7	19.3	14	70.27	41.69	84.01	3.18	50.4
Paenalcaligenes hominis CCUG 53761A^T (NZ_JAATIZ010000001.1)	13.3	19.2	13.6	68.35	34.73	83.99	1.64	48.3
Alcaligenes faecalis subsp. *phenolicus* J^T (AUBT01000001.1)	13.2	19.7	13.6	68.97	40.49	83.71	1.98	56.4
Alcaligenes pakistanensis NCCP-650^T (NZ_BMZN01000001.1)	13.2	19.0	13.5	68.64	39.52	83.36	1.70	55.4
Alcaligenes aquatilis LMG 22996^T (NZ_CP022390.1)	13.1	19.6	13.4	68.81	38.78	84.04	1.65	56.1
Alcaligenes faecalis NBRC 13111^T (NZ_CP023667.1)	13.1	20.2	13.5	68.82	39.94	83.57	1.76	56.6
Paralcaligenes ureilyticus GR24-5^T (NZ_SMAJ01000001.1)	12.9	19.9	13.2	68.18	32.93	85.28	0.82	57.8

Table 2. Phenotypic characteristics of strain NGK35^T and *P. hominis* CCUG 53761A^T

Unless otherwise indicated data were obtained in this study. Both strains tested in this study did not grow anaerobically. They were negative for indole production and acid formation from glucose but positive for H_2S production, catalase, urease, caseinase and cytochrome c oxidase, leucin arylamidase and naphthol-AS-BI-phosphohydrolase activity. They were negative for gelatinase, alkaline phosphatase, esterase lipase (C4), esterase lipase (C8), lipase (C14), valin 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. Both of them utilized the following as sole sources of carbon: D-ribose, L-rhamnose *N*-acetylglucosamine, amygdalin, arbutin, trehalose, turanose and DL-fucose. None of the strains tested in this study utilized: acetate, erythritol, L-xylose, D-adonitol, methy β-D-xylopyranoside, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, salicin, lactose, melibiose or L-arabitol. The full list of carbon sources and enzymes tested for strain NGK35^T, *P. hominis* CCUG 53761A^T, *P. suwonensis* ABC02-12^T and *P. hermetiae* KBL009^T can be found in Table S3. +, Positive; –, negative; W, weakly positive; ND, not determined.

Characteristic	NGK35^T	*P. hominis* CCUG 53761A^T
Colony colour*	Beige	Beige[a]
Cell size (µm)*	0.6–0.8×1.0–2.0	0.2–0.8×1.3–2.0[a]
Motility	+	+[a]
Growth temperature (°C):		
Range*	6–37	10–40[b]
Optimum*	28	25–30[a]
pH for growth:		
Range	6.0–9.5	ND
Optimum	7.5–8.5	ND
NaCl tolerance for growth (%, w/v):		
Range	0–10	ND
Optimum	1	0.5[b]
Assimilation of:		
Glycerol	W	–
D-Arabinose	+†	–
L-Arabinose	+	–
D-Xylose	+	–
D-Galactose	+	–
D-Glucose	+	–
D-Fructose	–	+
D-Mannose	–	+
L-Sorbose	–	+
Dulcitol	–	+
Inositol	–	+
D-Mannitol	–	+
D-Sorbitol	–	+
Aesculin	+	–
Cellobiose	+	–
Maltose	–	+
Sucrose*	W	–[b]
Inulin	–	+
Melezitose	–	+
Raffinose	–	+

Continued

Table 2. Continued

Characteristic	NGK35^T	*P. hominis* CCUG 53761A^T
Starch	–	+
Glycogen	–	+
Xylit	–	+
Gentiobiose	+	–
Turanose	w	+
D-Lyxose	–	+
D-Tagatose	–	+
L-Fucose	+†	+
D-Arabitol	–	+
L-Arabitol	–	–
Potassium gluconate	+	–
Potassium 2-ketogluconate	+	–
Potassium 5-ketogluconate	+	–
Citrate*	+	–[b]
DL-Lactate*	+	– (Lactic acid)[b]
Pyruvate	+	ND

*[a]Data obtained from Kämpfer *et al.* [4]. [b]Data obtained from Moon *et al.* [6].
†Indicates acid formation from carbon source.

(Göttingen, Germany). The sequences were compared with those available in the GenBank by Nucleotide BLAST analysis (Basic Local Alignment Search Tool; https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed September 2021). The partial 16S rRNA gene sequence was identical to the complete 16S rRNA gene sequence obtained from sequencing of the draft genome of strain NGK35^T (CAKKOW010000000.1). For a detailed phylogenetic placement of NGK35^T, the complete 16S rRNA gene sequence was aligned with the SILVA Incremental Aligner [9] and implemented into the 'All Species Living Tree Project' tree [10] using the ARB software package release 5.2 [11] and the SILVA database release 138.1 for analysis. Finally, the sequence alignment was verified manually. Pairwise sequence similarities were determined in ARB using the ARB neighbour-joining tool. Phylogenetic trees were reconstructed with the (i) maximum-likelihood method using RAxML [12] with general time reversible-GAMMA and rapid bootstrap analysis, (ii) the maximum-parsimony method using DNAPARS [13], and (iii) the neighbour-joining method using ARB neighbour-joining and the Felsenstein correction. Independent of the applied treeing method, NGK35^T was placed within the genus *Paenalcaligenes*, with the type strains *P. hominis* CCUG 53761A^T and *P. suwonensis* ABC02-12^T as its closest phylogenetic neighbours (Figs 1, S1 and S2, available in the online version of this article). Based on the BLAST analysis, the full-length 16S rRNA gene sequence of strain NGK35^T obtained from the draft genome had 96.9% similarity to *P. hominis* CCUG 53761A^T, 96.94% to *P. suwonensis* ABC02-12^T and 95.34% to *P. hermetiae* KBL009^T. Based on the analysis in ARB, the full-length 16S rRNA gene sequence had 97.1% sequence similarity to *P. suwonensis* and 97.0% to *P. hominis*, but only 95.5% to *P. hermetiae* KBL009^T based on the analysis in ARB (Table S1). *P. hominis*, the type species of this genus, is thus one of the phylogenetically closest relatives of strain NGK35^T.

GENOME FEATURES

Sequencing of the draft genome of NGK35^T (ENA accession number CAKKOW010000000.1) allowed genomic analyses which clearly separated this strain from established species within the genus *Paenalcaligenes*. To reconstruct the NGK35^T draft genome, DNA extracted from *Paenalcaligenes* culture was sequenced using the MinION sequencing platform (Oxford Nanopore Technologies (ONT)). Reads were basecalled and quality checked with guppy version 4.4.2+9623 c1626 (ONT) and assembled with Flye version 2.8.2-b1689 [14, 15] using the parameters (--plasmid and --meta). Draft genome quality assessment was conducted using CheckM version 1.0.13 [16]. The resulting draft genome had a size of 3665035 bp, across seven contigs with a G+C content of 52.1 mol%, a completeness of 96.03% and a contamination of 0.71%. Reference genome sequences corresponding to type strains of phylogenetically closely related species of that genus according to 16S rRNA sequence analysis were obtained from the public NCBI database. Analysis of average nucleotide identity (ANIb based on BLAST+ and ANIm based on MUMmer) [17] between NGK35^T and reference genomes (Table 1) produced values well below the proposed 95–96% threshold for the species boundary [18]. Based

Table 3. Polar lipid profile as well as the quinone system of strain NGK35^T and other members of the genus *Paenalcaligenes*

Strains: 1, NGK35^T; 2, *P. hominis* CCUG 53761A^T; 3, *P. suwonensis* ABC02-12^T; 4, *P. hermetiae* KBL009^T. Intact polar lipids: +++, major amount; ++, moderate amount; +, minor amount. Ubiquinones: Q-n indicates that the side chain comprises *n* isoprenoid units. Unless otherwise indicated, data were obtained in this study.

Polar lipids	**1**	**2**	**3**	**4**
Methylphosphatidic acid	++	+	–	–
Phosphatidic acid	+	+	–	–
Ornithine lipids	+	–	–	–
Phosphatidylglycerol*	+++	+++ (++)[a]	+++[b]	+++[c]
Diphosphatidyglycerol*	++	++ (+++)[a]	+++[b]	+++[c]
Hydroxyphosphatidylethanolamine	+	+	–	–
Phosphatidylethanolamine*	+++	+++ (+++)[a]	+++[b]	+++[c]
Phosphatidylcholine	+	+	–	–
Lysophosphatidylglycerol	++	–	–	–
Lysophosphatidylethanolamine	++	+	–	–
Ubiquinones (%)				
Q-8*	100	100 (93)[a]	+++[b]	+++[c]
Q-7*	–	– (7)[a]	+[b]	+[c]

*[a]Data obtained from Kämpfer *et al.* [4]. [b]Data obtained from Moon *et al.* [6]. [c]Data obtained from Lee *et al.* [5].

on this algorithm, the most closely related species with a validly published name was *P. suwonensis* ABC02-12^T with an ANIb value of 70.27% and an ANIm value of 84.01%. Furthermore, as suggested by the 16S rRNA sequencing analysis, *P. hominis* CCUG 53761A^T was the next closest related species to NGK35^T with ANIb and ANIm values 68.35 and 83.99%. The digital DNA–DNA hybridization (dDDH) values were determined online at http://ggdc.dsmz.de/ggdc.php# using the Genome-to-Genome Distance Calculator version 3.0 [19] (Table 1). These calculations produced *in silico* DNA–DNA hybridization values well below the 70% threshold to delimit a bacterial species. Strain NGK35^T had a dDDH value of 19.3% (formula 2) with *P. suwonensis* and a dDDH value of 19.2 with *P. hominis* CCUG 53761A^T. Both values had a probability of 0% to be equal to or above 70% DDH. Formula 2 is recommended if incomplete genome sequences are submitted to the server [20]. The DNA G+C contents were calculated *in silico* in all cases. The G+C content of NGK35^T is 52.1 mol%. In comparison, the G+C content of the closest related type strains *P. suwonensis* and *P. hominis* are 50.4 and 48.3 mol%, respectively, while it is 56.1 mol% for *P. hermetiae* according to Lee *et al.* [5].

Based on the highest similarity of the partial 16S rRNA gene to strain NGK35^T as presented above, *P. hominis* DSM 26613^T (CCUG 53761A) was obtained from the German Collection of Microorganisms and Cell Cultures GmbH and included in the comparative survey. In order to guarantee a comprehensive study, certain physiological, biochemical and chemotaxonomic key features of these strains were analysed, even if they had been performed in previous studies. A further comparison to the remaining members of the genus *Paenalcaligenes*, *P. suwonensis* and *P. hermetiae*, was performed with the data obtained by Moon *et al.* [6] and Lee *et al.* [5].

PHYSIOLOGY AND CHEMOTAXONOMY

The cell morphology in the exponential growth phase and the presence of flagella of cells grown in LB broth at 28 °C for 24 h agitated at 100 r.p.m. were investigated by scanning electron microscopy using a Thermo Fisher Scientific (former FEI) Quanta 3D Dual Beam instrument. The imaging was performed with a landing energy of electrons of 1 kV. Scanning transmission electron microscopy images were collected at 10 kV. Gram staining (using a Gram-staining set, Merck) and cell motility were determined according to the classical procedures described by Süßmuth *et al.* [21]. Cell motility was investigated along with the ability of H_2S formation in modified SIM agar (LB broth supplemented with (w/v) 0.02% ammonium ferric citrate, 0.02% sodium thiosulfate and 0.3% agar). The temperature and pH range for growth as well as salt tolerance were examined visually by checking turbidity and the formation of cell aggregates in liquid medium for 3–5 days. The growth capacity was determined in LB broth at 6, 12, 16, 20, 24, 28, 33, 37 and 40 °C. The NaCl and pH tolerance was examined at 28 °C with NaCl concentrations between 0–15%, with increments of 1 %, and pH values between pH 4.0 and 10, using increments of 0.5 pH units. NaCl tolerance was examined in LB broth, whereas pH tolerance was analysed in LB broth with 1% NaCl. The medium was buffered with glycine (pH 4.0–5.0 and pH 10.0), MES (pH 5.5–6.5), HEPES (7.0–8.0) and BTP (1.3-bistris(hydroxymethyl)methylamino)propane; pH 8.5–9.5). Anaerobic growth was tested on LB agar incubated at 28 °C for

Table 4. Fatty acid profile of strain NGK35^T and *Paenalcaligenes hominis*

Names are given in the IUPAC nomenclature with the *ω*-x nomenclature in parentheses. Numbers represent values of the cell membrane phospholipid fatty acid analysis, numbers in parentheses represent whole cell fatty acid methyl ester values. X:Y, number of carbon atoms:number of double bonds; iso, methyl branch at the penultimate carbon atom of the fatty acid; anteiso, methyl branch at the pre-penultimate carbon atom; cisX, carbon number position of the cis configurated double bond; X methyl, methyl branch at carbon number position X . A detailed list of fatty acid measurements can be found in table S2 including data of *Paenalcaligenes hominis*, *Paenalcaligenes suwonensis* and *Paenalcaligenes hermetiae* from Moon *et al.* [6].

Fatty acids (%)	**NGK35^T**	***P. hominis* CCUG 53761A^T**
$C_{12:0}$	(3.4)	(2.3)
$C_{14:0}$	0.1	0.11
$C_{15:1}$	0.05	–
$C_{15:0}$	0.05 (0.9)	–
$C_{14:0}$-3OH	(3.4)	(3.6)
$C_{14:1}$ trans-2 ($C_{14:1}$ *ω12t*)	(0.6)	–
$C_{16:1}$ cis-9 ($C_{16:1}$ *ω7c*)	28.4 (14.3)	29.0 (33.4)
$C_{16:1}$trans-9 ($C_{16:1}$ ω7t)	0.7	0.7
$C_{16:1}\Delta^{11}$ ($C_{16:1}$ *ω5*)	0.2	0.9
$C_{16:0}$	27.8 (32.3)	25.3 (30.0)
$C_{17:0}$ cyclo-10,11 (cy$C_{17:0}$ ω7,8)	19.7 (24.0)	14.0 (6.8)
$C_{17:0}$	0.5	0.02
$C_{18:2}\Delta^{9,12}$ ($C_{18:2}$ *ω6,9*)	–	0.07
$C_{18:1}\Delta^{9}$ ($C_{18:1}$ *ω9*)	–	0.2
$C_{18:1}$cis-11 ($C_{18:1}$ *ω7c*)	18.7 (17.4)	25.4 (20.5)
$C_{18:1}$trans-11 ($C_{18:1}$ *ω7t*)	–	0.3
$C_{18:0}$	3.77 (1.6)	3.5 (2.4)
$C_{19:1}$	0.03	0.08

14 days in an anaerobic container with anaerobic gas generating sachets of Oxoid AnaeroGen (2.5 l, Thermo Fisher Scientific). Carbon source utilization and acid production from carbon sources as well as the enzymatic activity were determined by the API 50CH (26 d, 28 °C) and API ZYM (4 h, 28 °C) galleries (bioMérieux) according to the instructions of the manufacturer and the methods described earlier [22]. While API 50CH B/E medium was used to identify acid production from carbohydrates, a minimal medium (NH_4Cl 1 g l^{-1}, K_2HPO_4 1 g l^{-1}, KH_2PO_4 0.5 g l^{-1}, $MgSO_4{\cdot}7H_2O$ 0.2 g l^{-1}, $CaCl_2{\cdot}2H_2O$ 0.01 g l^{-1}, yeast extract 1 g l^{-1}) including trace elements (w/v; 2 mg l^{-1} $Fe(NH_4)_2(SO_4)_2{\cdot}H_2O$, 2 mg l^{-1} $Na_2SeO_3{\cdot}5H_2O$, 1 mg l^{-1} $CoCl_2{\cdot}6H_2O$, 1.1 mg l^{-1} $MnCl_2{\cdot}4H_2O$, 1 mg l^{-1} $Na_2MoO_4{\cdot}2H_2O$, 1 mg l^{-1} $Na_2WO_4{\cdot}2H_2O$, 0.2 mg l^{-1} $ZnSO_4{\cdot}7H_2O$, 0.25 mg l^{-1} $NiCl_2{\cdot}6H_2O$, 0.1 mg l^{-1} H_3BO_3, 0.02 mg l^{-1} $CuSO_4{\cdot}5H_2O$) was applied to test for the utilization of carbon compounds as sole carbon source. The utilization of additional carbon compounds not included in the API 50CH gallery (DL-lactate, acetate, pyruvate and citrate) was tested on minimal medium agar plates without yeast extract but with the addition of a vitamin solution (500×: 0.005% 4-aminobenzoic acid, 0.005% nicotinic acid, 0.005% calcium pantothenate, 0.005% pyridoxine hydrochloride B6, 0.005% riboflavin, 0.005% thiamine hydrochloride, 0.0025% biotin, 0.0025% folic acid, 0.0025% lipoic acid, 0.0025% vitamin B12) at 28 °C for 14 days. Catalase activity was determined by bubble formation in 1.5% (v/v) H_2O_2, while cytochrome c oxidase activity was analysed with *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine as a redox indicator as described by Kovács [23]. Susceptibility to lysozyme (1 mg ml^{-1}) was examined in a filter disc test by observing the formation of an inhibition aerola around the filter disc saturated with lysozyme. The production of indole from tryptophan was tested in a tryptophan medium (w/v; 1.5% trypticase peptone, 0.1% DL-tryptophan) by the addition of the Kovac's reagent for indoles (v/v; 10%). A glucose and urea containing medium (v/w; 1% trypticase peptone, 0.5% NaCl, 1% glucose-monohydrate and 1% urea) was used to determine urease activity by adding 1% ethanolic phenolphthalein solution as indicator. Calcium caseinate agar was used to determine the hydrolysis of casein, by observing clearing zones around colonies in the opaque medium. Hydrolysis of gelatin was examined using the nutrient gelatin stab method according to Dela Cruz and Torres [24].

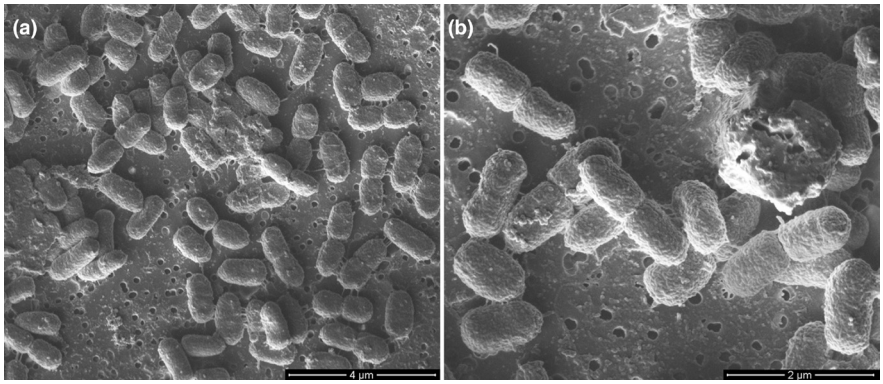

Fig. 2. Scanning electron microscopy images of strain NGK35^T cells in the early exponential growth phase grown in LB at 28 °C with agitation at 100 r.p.m.

Morphological, physiological and biochemical characteristics of strain NGK35^T are given in the species description. Features that serve to differentiate strain NGK35^T from *P. hominis*, *P. suwonensis* and *P. hermetiae* are given in Tables 2 and 3.

For chemotaxonomic analyses (intact polar lipids, phospholipids fatty acids), biomass was grown in LB broth at 28 °C and harvested at the late exponential phase after 48 h. Quinones were analysed from biomass grown in tryptic soy broth (Merck) and whole cell fatty acids were analysed from biomass grown on tryptic soy agar (Merck), both grown at 30 °C for 48 h. The cell membrane was analysed in two ways: (i) to yield the whole cell fatty acid (total fatty acid methyl esters; FAME) inventory, and (ii) to obtain the intact membrane polar lipid (IPL) and the phospholipids fatty acid (PLFA) side chain composition. FAME extracts from biomass were prepared as described by Sasser [25] and analysed via gas chromatography (GC model 7890B, Agilent) with a flame ionization detector, as previously described by Derichs *et al.* [26]. Identification of fatty acids was verified by a gas chromatography–mass spectrometry system (model 8890 GC/5977B MSD, Agilent). The chromatographic conditions were used as previously described by Lipski and Altendorf [27]. For the IPL and PLFA analysis strain NGK35^T was extracted and the extract was chromatographically separated into fractions of different polarity yielding among others into a glycolipid and phospholipid fraction [28]. The glycolipid fraction did not contain any polar lipids. The phospholipid fraction was splitted into two halves. The first half was used to measure the intact membrane phospholipids using an ultra-high performance liquid chromatograph (Thermo Scientific Ultimate 3000 RS) coupled via an heated electrospray interface (H-ESI-II) to a Q-Exactive Plus Orbitrap mass spectrometer (MS; Thermo Scientific). HPLC conditions are described in Genderjahn *et al.* [28]. Orbitrap conditions were as follows: spray voltage −2.2 kV; capillary temperature 300 °C; nitrogen sheath gas at 49 and auxiliary gas at 12 arbitrary units at a temperature of 100 °C, S-Lens 60 V. The obtained data were acquired in negative ion mode with dependent MS/MS acquisition at ranges of m/z 405 to 2000. The full scan and fragment MS/MS spectra were collected at a resolution of 280000 and 70 000 (at m/z 200), respectively. The second half was used for membrane PLFA analysis using a saponification method described in [29]. The methylated PLFAs were measured on a Thermo Trace 1310 gas chromatograph (GC) equipped with a Thermo PTV injection system and a SGE BPX5 fused silica capillary column (50 m long, 0.22 mm ID, 0.25 µm film thickness) coupled to a Thermo TSQ9000 MS. GC-MS conditions are described in [28].

The major IPLs of strain NGK35^T are PG and PE. Moderate amounts of methylphosphatidic acid (MePA), DPG, lysophosphatidylglycerol (LPG) and lysophosphatidylethanolamine (LPE), as well as minor amounts of phosphatidic acid (PA), ornithine lipids (OLs), hydroxyphosphatidylethanolamine (OHPE) and phosphatidylcholine (PC) could be detected (Table 3). With this, the polar lipid profile is similar to that of *P. hominis*, which had a slightly lower abundance of MePA and LPE, but no OL and LPG. In general, the major polar lipids of strain NGK35^T are highly similar to the other type strains of this genus, with DPG being also one of the major polar lipids within the genus *Paenalcaligenes* [4–6]. It has to be noted, that due to their lower ionization ability in the ESI negative ion mode DPGs are slightly under-represented, which might explain the observed differences to the literature IPL data determined using thin-layer chromatography.

The percentages of the fatty acids were identified via PLFA and FAME analysis. The major fatty acids that are similar for both analytic methods (Table 4) were $C_{16:1}$ *ω7c* (28.4/14.3%), $C_{16:0}$ (27.8/32.2%), $C_{17:0}$ cyclo (19.7/24.0%) and $C_{18:1}$ *ω7c* (18.7/17.4%). With this, the fatty acid profile is similar to that of *P. hominis* showing the same major fatty acids and similarly lower amounts of $C_{18:0}$. Although grown at different conditions prior to analysis, the major fatty acids of strain NGK35^T are also similar to the other species of the genus *Paenalcaligenes* (Table S2) [6]. The quinone system was analysed by an Agilent 1260 infinity HPLC system as described by Wiertz *et al.* [30]. The only ubiquinone was Q-8. This is consistent with the quinone system of the other species of this genus which predominantly consisted of ubiquinone Q-8 with minor amounts of Q-7.

Strain NGK35^T shows molecular and phenotypic characteristics that are typical for species of the genus *Paenalcaligenes*. However, it can be clearly differentiated from other species of this genus by a number of significant characteristics. At the molecular level, the

results of the 16S rRNA phylogenetic analysis and the different genome-based indices indicated that strain NGK35^T represents a novel species and that *P. suwonensis* and *P. hominis* are the most closely related species within that genus. In addition, all the three members of the genus *Paenalcaligenes* can be distinguished from NGK35^T with regard to several phenotypic and chemotaxonomic features (Tables 2 and 3). Although being similar with respect to H_2S production, catalase, urease and cytochrome c oxidase activity as well as the major polar lipids, fatty acids and major ubiquinone (Table 3), the strains presented could be differentiated by contrasting aspects such as anaerobic growth, casein hydrolysis, assimilation of and acid formation from carbon sources as well as the exact polar lipid and fatty acid profiles (Tables 2 and 3). On this basis, strain NGK35^T represents a novel species of the genus *Paenalcaligenes* for which the name *Paenalcaligenes niemegkensis* sp. nov. is proposed.

EMENDED DESCRIPTION OF THE GENUS *PAENALCALIGENES*

The description of the genus *Paenalcaligenes* is given by Kämpfer *et al.* [4] and Lee *et al.* [5] with the following addition: minor to moderate amounts of the polar lipids MePA, PA, OHPE, PC and LPE. Also, OL and LPG in low abundance. Distinct fatty acids of summed features can be identified. A component of summed feature 2 is $C_{14:0}$ 3-OH. The components of summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$) and 8 (comprising $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) are $C_{16:1}\omega7c$ and $C_{18:1}\omega7c$. The major fatty acids are thus $C_{16:1}\omega7c$ and $C_{18:1}\omega7c$ in addition to $C_{16:0}$ and $C_{17:0}$ cyclo.

DESCRIPTION OF *PAENALCALIGENES NIEMEGKENSIS* SP. NOV.

Paenalcaligenes niemegkensis (nie.meg.ken'sis. N.L. masc. adj. *niemegkensis* referring to Niemegk, the city in Germany where the type strain was isolated from).

Cells are Gram-stain-negative, aerobic and motile. When grown on LB agar at 28 °C for 2 days colonies are beige, with a smooth surface. Cells are rod-shaped (Fig. 2) with a cell length of 1.0–2.0 µm and width of 0.6–0.8 µm. Growth is possible at temperatures between 6 and 37 °C, but not at 40 °C (optimum at 28 °C), in a pH range of pH 6.5–9.5 (optimum pH 7.5–8.5) and at NaCl concentrations of up to 10% (optimum 1%). Catalase, urease, caseinase, cytochrome c oxidase, (API ZYM) esterase (C 4), leucin arylamidase (weak) and naphthol-AS-BI-phosphohydrolase activities are positive. Strain NGK35^T is able to produce H_2S. It is not susceptible to lysozyme and no indole production occurs. The strain is able to use the following carbon sources: aesculin, *N*-acetylglucosamine, amygdalin, D- and L-arabinose, arbutin, cellobiose, citrate, D- and L-fucose, D-galactose, gentiobiose, D-glucose, glycerol (weak), DL-lactate, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, pyruvate, L-rhamnose, D-ribose, sucrose (weak), turanose (weak), trehalose and D-xylose. Acid production occurs from D-arabinose and L-fucose, but not from glucose. The cellular fatty acids are predominated by $C_{16:1}$ cis9 ($C_{16:1}\omega7c$), $C_{16:0}$, $C_{17:0}$ cyclo ($cyC_{17:0}\omega7,8$) and $C_{18:1}$ cis11 ($C_{18:1}\omega7c$). The quinone system is composed of the single ubiquinone Q-8. The polar lipid profile contains the major polar lipids PG and PE, followed by DPG, MePA, LPG and LPE, and minor amounts of PA, OLs, OHPE and PC. The DNA G+C content is 52.1 mol%.

The type strain is NGK35^T (=DSM 113270^T=NCCB 100854^T) and was isolated from plastic debris and plastic-polluted soil sampled at an abandoned landfill site in Niemegk, Brandenburg, Germany.

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Author contributions
J.M., D.W. and S.L. formulated the study design. S.L. and J.M.L. conducted the sampling. J.M.L. isolated the described bacterial species. J.M. performed the morphological and physiological analyses. D.L. and J.M. sequenced the draft genome. D.L. performed the quality control and A.B. the genome assembly. K.M. extracted and analysed the PLFAs and IPLs, while A.L. extracted and analysed the FAMEs and quinones. V.R. performed SEM imaging. J.M. visualized the data and prepared the original draft. All authors contributed to the writing of the paper.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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