Steroidobacter denitrificans gen. nov., sp. nov., a steroidal hormone-degrading gammaproteobacterium

Michael Fahrbach,¹ Jan Kuever,² Markko Remesch,² Birgit E. Huber,³
Peter Kämpfer,⁴ Wolfgang Dott⁵ and Juliane Hollender¹

Correspondence

Juliane Hollender
juliane.hollender@eawag.ch

¹Swiss Federal Institute of Aquatic Science and Technology, Eawag, Überlandstr. 133,
PO Box 611, CH-8600 Dübendorf, Switzerland

²Bremen Institute for Materials Testing, Foundation Institute for Materials Science,
Paul-Feller-Str. 1, D-28199 Bremen, Germany

³Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität Wien,
Veterinärplatz 1, A-1210 Vienna, Austria

⁴Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, Heinrich-Buff-Ring
26–32 (IFZ), D-35392 Giessen, Germany

⁵Institute of Hygiene and Environmental Medicine, RWTH Aachen University, Pauwelsstr. 30,
D-52074 Aachen, Germany

A denitrifying bacterium, designated strain FS^T, was isolated from anoxic digested sludge on oestradiol [17 β -oestra-1,3,5(10)-triene-3,17-diol] or testosterone (17 β -hydroxyandrost-4-en-3-one) as the sole source of carbon and energy with nitrate as the electron acceptor. Strain FS^T represents the first known bacterium to grow anaerobically on both oestradiol (C-18) and testosterone (C-19). Steroidal hormones were degraded completely by nitrate reduction to dinitrogen monoxide, which was further reduced to dinitrogen in stationary-phase cultures. Gram-negative cells were slightly curved rods, 0.3–0.5 $\times$ 0.6–1.6 μ m in size, motile, non-fermentative, non-spore-forming and catalase- and oxidase-positive, showing optimal growth at pH 7.0, 28 °C and 0.1 % (w/v) NaCl. Beside steroidal hormones, the bacterium utilized only a narrow range of organic substrates with nitrate as the electron acceptor, including several fatty acids and glutamate. No aerobic or anaerobic growth occurred on liquid or solid complex media. Phylogenetic analysis of the 16S rRNA gene sequence showed that strain FS^T has no known close relatives and represents a distinct lineage within the *Gammaproteobacteria*. Together with the genera *Nevskia*, *Hydrocarboniphaga*, *Solimonas* and *Sinobacter* (less than 88 % 16S rRNA gene sequence similarity to strain FS^T), it forms a phylogenetic cluster separated from the families *Chromatiaceae*, *Ectothiorhodospiraceae* and *Xanthomonadaceae*. The quinone system of strain FS^T consisted exclusively of ubiquinone Q-8. The dominant polar lipids were diphosphatidylglycerol and phosphatidylethanolamine. Spermidine in combination with putrescine and traces of *sym*-homospermidine were the basic polyamines. The major fatty acids detected in testosterone- or heptanoate-grown cells were C_{15:0} and C_{17:1 ω 8c}, minor hydroxylated fatty acids were C_{11:0} 3-OH and C_{12:0} 3-OH. The G + C content of the DNA was 61.9 mol%. Based on the high 16S rRNA gene sequence divergence and different phenotypic properties from previously described gammaproteobacteria in combination with chemotaxonomic data, strain FS^T is considered to represent a new genus and species, for which the name *Steroidobacter denitrificans* gen. nov., sp. nov. is proposed. The type strain of *Steroidobacter denitrificans* is FS^T (=DSM 18526^T =JCM 14622^T).

During the last decade, the endocrine-disrupting activity of steroidal hormones has been recognized as a potential risk

to the fertility of wildlife populations in aquatic ecosystems (Jobling *et al.*, 1998; Sumpter & Johnson, 2005; Thomas *et al.*, 2002). Concerns over the potential negative ecological effects of steroidal hormones from human- and animal-derived wastes has resulted in increased

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain FS^T is EF605262.

interest in the biodegradation of these compounds in soil, sediment and manure and during wastewater treatment (Andersen *et al.*, 2003; Colucci *et al.*, 2001; Hanselman *et al.*, 2003; Jacobsen *et al.*, 2005; Jenkins *et al.*, 2003, 2004; Jürgens *et al.*, 2002; Layton *et al.*, 2000). Several aerobic bacteria are able to use steroidal hormones as growth substrates (Fujii *et al.*, 2003; Yoshimoto *et al.*, 2004; Sih & Whitlock, 1968; Talalay *et al.*, 1952). Anaerobic degradation of steroidal hormones is considered to be incomplete under different redox conditions, and would lead to an accumulation in anoxic habitats (Czajka & Londry, 2006; Jürgens *et al.*, 2002). It has been found that denitrification is an important treatment step in the sewage sludge process for removing natural steroidal hormones (Andersen *et al.*, 2003; Joss *et al.*, 2004). Recently, a novel denitrifying bacterium, *Denitratisoma oestradiolicum*, belonging to the betaproteobacterial family *Rhodocyclaceae*, which degrades oestradiol [17 β -oestra-1,3,5(10)-triene-3,17-diol] by nitrate reduction, has been isolated from activated sludge (Fahrbach *et al.*, 2006). In this study, we describe and classify a novel denitrifying gammaproteobacterium, strain FS^T, which was enriched and isolated from anoxic digested sludge using oestradiol or testosterone (17 β -hydroxyandrost-4-en-3-one) as the sole energy and carbon source and nitrate as the electron acceptor.

Anoxic cultivation techniques have been used in this study (Widdel & Bak, 1992). The composition and the preparation of dioxygen-free, steroidal hormone-containing mineral medium for routine cultivation conditions was described previously (Fahrbach *et al.*, 2006). Media used for quantitative experiments with strain FS^T contained freshly prepared sodium ascorbate (4 mM) as a reductant, which did not serve as a growth substrate. An enrichment culture with 1 mM oestradiol as the sole energy source and 5 mM nitrate as the electron acceptor was established by inoculating mineral medium with 5 ml anoxic digested sludge from a municipal wastewater-treatment plant (Aachen, Germany). A pure culture was obtained by repeated serial dilution series with either 1 mM oestradiol or 1 mM testosterone and 5 mM nitrate in combination with two testosterone agar dilution series prepared according to the protocol of Widdel & Bak (1992). The almost water-insoluble testosterone was added to the nitrate-containing agar medium from a sterile and anoxic stock solution (50 mM testosterone dissolved in DMSO) to obtain a final concentration of 1 mM. After 4–6 weeks, gas production started and small (approx. 1 mm in diameter) yellow–brown, disc-shaped colonies of strain FS^T appeared in the agar medium and were picked and transferred into liquid mineral medium. Purity was checked microscopically and by growth tests in medium containing 5 mM fumarate, 5 mM pyruvate, 0.05 % (w/v) yeast extract, 0.07 g peptone l⁻¹, oestradiol or testosterone. In addition, liquid R2A medium or R2A agar, CASO agar or CASO-Bouillon (all from Merck) or nutrient broth (Difco) were also used for purity tests. These complex media were employed either undiluted or as tenfold and hundredfold

dilutions. The latter media were also used to test for aerobic growth of strain FS^T. Furthermore, aerobic growth of strain FS^T was tested with 1 mM oestradiol or 1 mM testosterone in potassium phosphate-buffered mineral medium (20 mM, pH 7.2). For the determination of the temperature range/optimum under denitrifying conditions, 5 mM heptanoate was used as growth substrate in bicarbonate-buffered mineral medium containing 5 mM nitrate. The pH range/optimum was determined in either bicarbonate-buffered or HEPES-buffered (10 mM) mineral medium with 5 mM heptanoate and 5 mM nitrate. Gram type was determined according to Süßmuth *et al.* (1987) and with the KOH test described by Gregersen (1978). *Staphylococcus epidermidis* DSM 1798 and *Nitrosomonas europaea* ATCC 19718 were used as controls. Cytochrome-oxidase testing was determined with a Merck Bactident oxidase strip. Detection of catalase activity was carried out using a standard method (Gerhardt *et al.*, 1994). Cell morphology was observed by phase-contrast microscopy with a Leitz microscope and a Philips XL 30 ESEM FEG scanning electron microscope at 20 kV according to a modified method of Bruce *et al.* (1999). For maintenance, stock cultures grown on either oestradiol or testosterone and nitrate were stored at 4 °C and transferred every 4–8 weeks. Long-term storage of bacteria was achieved as stocks in glycerol (10 %, v/v) at –80 °C under oxic conditions.

Growth experiments were performed in butyl rubber-sealed 150 or 320 ml infusion bottles containing 100 or 200 ml mineral medium under a He/CO₂ (80:20, v/v) atmosphere in order to quantify dinitrogen monoxide and dinitrogen. Oestradiol (1 mM) was added in the form of crystals as the sole source of carbon and energy together with 5 mM nitrate as the electron acceptor. The medium was prereduced with 4 mM sodium ascorbate and inoculated (3 %, v/v) with an oestradiol-grown preculture. Nitrate and nitrite were analysed with a Dionex DX-100 ion chromatograph with conductivity detection, using an AS14 anion-exchange column (250 × 4 mm i.d.). The eluent was 1 mM NaHCO₃ and 3.5 mM Na₂CO₃ with a flow rate of 1.0 ml min⁻¹. Limits of quantification were at least 16 µM nitrate and 2 µM nitrite. Dinitrogen monoxide and dinitrogen in the headspace of cultures were quantified with a Perkin Elmer Autosystem gas chromatograph with thermal conductivity detector equipped with a Alltech CTR-1 column. The carrier gas was helium at a flow rate of 62.5 ml min⁻¹. The column temperature was set at 40 °C isothermal. Injector and detector temperatures were 65 and 140 °C, respectively. Gas samples of 100 µl volume were taken from the assays with a gas-tight syringe. Amounts of gas measured in the headspace were corrected by adding calculated amounts of dissolved gas, using the adapted Bunsen coefficient (D'Ans & Lax, 1983). After extraction with ethyl acetate, the steroidal hormones oestradiol, oestrone [3-hydroxyoestra-1,3,5(10)-triene-3-one], testosterone and 4-androstene-3,17-dione were analysed by reversed-phase HPLC using an Agilent HP1100 system

with UV-Vis diode array (210 nm for oestrogens, 244 nm for androgens) and fluorescence (excitation 220 nm and emission 315 nm only for oestrogens) detectors in series. Separation was achieved at 35 °C on a Thermo HyPurity C-18 column (150 × 2.1 mm i.d.; particle size 3 µm) with a flow rate of 0.2 ml min⁻¹. An acetonitrile–water gradient, from 20 to 95 % acetonitrile in 16 min, was applied. Growth could be assayed as increase in protein content assuming that 50 % of the dry cell weight is protein. Since the measurement of optical density was not possible in cultures growing on crystalline steroid hormones, increase in cell mass was monitored by protein determination as follows. Culture samples were centrifuged at 14 000 r.p.m. The pellet was resuspended in 0.5 M NaOH and boiled for 10 min. After centrifugation, an aliquot was analysed according to Bradford (1976) using the Bio-Rad protein assay. BSA standards were prepared in 0.5 M NaOH due to interference of the latter with the protein assay reagent. Obtained concentrations were corrected to account for a twofold higher response of the assay reagent to BSA in comparison with other proteins according to the manufacturer's instructions.

Polar lipids were extracted and analysed by two-dimensional TLC according to Tindall (1990a, b) and Altenburger *et al.* (1996). Polyamines were extracted and analysed by HPLC as described by Busse & Auling (1988) and Stolz *et al.* (2007). Respiratory quinones were analysed according to Tindall (1990b) and Stolz *et al.* (2007). The fatty acid profile of strain FS^T (method according to Kämpfer & Kroppenstedt, 1996) was analysed from biomass obtained after growth on 1 mM testosterone or 2.5 mM heptanoate with nitrate as the electron acceptor. The G+C content of the DNA was analysed at the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) using the HPLC method and conditions as described by Mesbah *et al.* (1989) and Tamaoka & Komagata (1984). Purification and enzymic digestion of the DNA was performed according to Cashion *et al.* (1977) and Mesbah *et al.* (1989).

The 16S rRNA gene was amplified as reported previously (Kuever *et al.*, 2001). Sequencing was performed by AGOWA (Berlin, Germany). The 16S rRNA gene sequence of strain FS^T (1407 nt) was compared to the NCBI database (<http://www.ncbi.nlm.nih.gov>) using the BLAST tool (Altschul *et al.*, 1997). Sequences that were not included in the ARB database were loaded into the database and aligned using the ARB_ALIGN tool (Ludwig *et al.*, 2004). The alignment was inspected visually and corrected manually using the sequence editor ARB_EDIT. Phylogenetic analysis was done using the ARB software package (version 06.08.08; <http://www.arb-home.de>) in combination with the PHYL software package (Guindon & Gascuel, 2003). Tree topologies were evaluated by maximum-likelihood, parsimony and neighbour-joining analysis and were used for the construction of a consensus tree without using any

filters. Sequences of at least 1400 nt were used for the calculation of different trees.

Cells of strain FS^T were Gram-negative, slightly curved rods with rounded ends (0.3–0.5 × 0.6–1.6 µm) (Fig. 1). They occurred singly or in pairs and were motile by means of one polar flagellum. Further characteristics are listed in the species description. Anaerobic degradation of oestradiol, oestrone, testosterone or 4-androstene-3,17-dione with nitrate as the electron acceptor represents a hitherto-undescribed physiological property in bacteria. The oestradiol degradation time-course shown in Fig. 2 indicates that nitrate was reduced stoichiometrically to dinitrogen monoxide. Nitrite accumulation was not detected. Analysis of stationary-phase cultures of strain FS^T revealed that dinitrogen monoxide could be further reduced to dinitrogen after the nitrate was completely consumed. Since oestradiol is almost water-insoluble, its consumption could not be measured continuously by HPLC in time-course analyses (Fig. 2). Therefore, oestradiol oxidation by nitrate reduction was quantified in experiments with 100 ml cultures containing 500 µmol nitrate but different amounts of oestradiol (0–100 µmol). Cell dry mass production increased with the amount of steroid provided, and the amount of consumed nitrate was in good agreement with the amounts of dinitrogen monoxide formed. For example, synthesis of 51.0 mg cell dry mass l⁻¹ can account for the consumption of 53.3 µmol oestradiol and 449.3 µmol nitrate and the production of 219.0 µmol dinitrogen monoxide at the end of the exponential growth phase. The dissimilation equation of oestradiol is as follows:

Testosterone was also shown to be degraded completely by strain FS^T (data not shown). Interestingly, strain FS^T also grew aerobically on testosterone or 4-androstene-3,17-dione but not on oestradiol or oestrone, as is the case for the obligately aerobic betaproteobacterium *Comamonas testosteroni* (Levy & Talalay, 1959; Sih & Whitlock, 1968).

Fig. 1. Scanning electron micrograph of cells of strain FS^T grown on 1 mM testosterone and 5 mM nitrate. Original magnification, ×15 000. Bar, 2 µm.

Fig. 2. Growth of strain FS^T on 1 mM oestradiol with 5 mM nitrate in bicarbonate-buffered mineral medium (200 ml final volume). The increase in cell dry mass (▲) occurred simultaneously with dinitrogen monoxide formation (●) and resulted in complete depletion of nitrate (△) after 14 days. Nitrite was not formed during growth. Consumption of oestradiol could not be measured continuously in these experiments since this steroid is almost water-insoluble. Cell dry mass and dinitrogen monoxide and dinitrogen (○) concentrations are means of duplicate determinations.

Strain FS^T was isolated from anoxic digested sludge, but the physiological role of this isolate during anaerobic sludge treatment is far from clear. In addition, the temperature range for growth of strain FS^T is 20–38 °C (optimum at 28 °C), far below the average temperature of 38 °C that prevails in anoxic sludge digesters. Many bacteria, including strain FS^T, could be discharged into nitrate-free anoxic sludge digesters via excess sewage sludge from biological treatment.

Phylogenetic analysis revealed that strain FS^T represents a separate line of descent within the *Gammaproteobacteria*. All available 16S rRNA gene sequences of type species belonging to the gammaproteobacterial families *Chromatiaceae*, *Ectothiorhodospiraceae* and *Xanthomonadaceae* and the type species of several other genera were selected for this analysis (Fig. 3). A distant relationship of about 88 % 16S rRNA gene sequence similarity exists with the genera *Nevskia* (87.8 % 16S rRNA gene sequence similarity with the type strain of the type species), *Hydrocarboniphaga* (87.5 %), *Solimonas* (87.2 %) and *Sinobacter* (87.6 %), which cluster together with strain FS^T (Fig. 3). In addition to the high sequence divergence of the 16S rRNA gene, these genera possess different morphological and physiological characteristics (Table 1). No members of these genera are known to perform anaerobic metabolism of steroids while using nitrate as the electron acceptor which is then reduced to dinitrogen monoxide and further to dinitrogen. Denitrification is absent in these bacteria. Also, strain FS^T could not grow on liquid or solid complex

media. *Hydrocarboniphaga* strains showed an unusual appearance of colonies on various solid complex media and grow aerobically on aliphatic hydrocarbons (C₆–C₁₉) or other organic compounds (Palleroni *et al.*, 2004). Members of the genus *Hydrocarboniphaga* can be distinguished from strains of the genus *Nevskia* due to the property of the latter to form rosette-like colonies at air–water interfaces (Stürmeyer *et al.*, 1998). *Nevskia*, *Solimonas* and *Sinobacter* species are also strictly aerobic bacteria (Kim *et al.*, 2007; Zhou *et al.*, 2008), but members of the latter genera, together with strain FS^T, possessed narrow nutritional spectra. The major cellular fatty acids of testosterone- or heptanoate-grown cells of strain FS^T comprised C_{15:0} and C_{17:1}ω8c, whereas C_{18:1}ω7c, C_{16:0}, i-C_{16:0} and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) predominated in the genera *Nevskia*, *Hydrocarboniphaga*, *Solimonas* and *Sinobacter*. Larger amounts of C_{16:0} and summed feature 3 along with 10-methyl C_{16:0} were detected in testosterone-grown cells of strain FS^T. The complete fatty acid pattern of strain FS^T is listed in the species description. Ubiquinone Q-8 was the only quinone detected, which is a characteristic trait of many other gammaproteobacteria (Yokota *et al.*, 1992). Strain FS^T displayed a profile consisting of diphosphatidylglycerol and phosphatidylethanolamine as the major polar lipids, moderate amounts of phosphatidylglycerol, an unknown phospholipid (PL2) and an unknown aminophospholipid (APL) and minor to trace amounts of another unknown phospholipid (PL1), two unknown aminolipids and two unknown polar lipids (Fig. 4). The polyamine pattern of strain FS^T consisted of the predominant component spermidine [15.3 μmol (g dry weight)⁻¹], a minor amount of putrescine [2.8 μmol (g dry weight)⁻¹] and traces of *sym*-homospermidine [0.6 μmol (g dry weight)⁻¹], 1,3-diaminopropane [0.2 μmol (g dry weight)⁻¹] and spermine [0.09 μmol (g dry weight)⁻¹]. Among gammaproteobacteria, a polyamine pattern with the predominant compound spermidine has been reported for several genera such as *Alteromonas*, *Arenimonas*, *Arhodomonas*, *Aquamonas*, *Chromatium*, *Chromohalobacter*, *Cobetia*, *Ectothiorhodospira*, *Ferrimonas*, *Halomonas*, *Idiomarina*, *Marinobacter*, *Marinobacterium*, *Marinospirillum*, *Methylophaga*, *Morexella*, *Oceanobacter*, *Pseudoalteromonas*, *Pseudospirillum*, *Pseudoxanthomonas*, *Psychrobacter*, *Rhodanobacter*, *Aquimonas*, *Thioalkalivibrio*, *Thiomicrospira* and *Thiovirga*, but so far only a few taxa have been reported to contain minor to trace amounts of *sym*-homospermidine (*Legionella*, *Beggiatoa*, *Chromatium*) (Busse & Auling, 1988; Auling *et al.*, 1991; Hamana & Takeuchi, 1998; Hamana *et al.*, 2000, 2007; Young *et al.*, 2007), and the combination of the two has only been reported in *Allochrochromatium vinosum* (formerly *Chromatium vinosum*; Imhoff *et al.*, 1998).

From the results presented, it is obvious that strain FS^T represents a novel taxon within the *Gammaproteobacteria*. Strain FS^T was therefore placed in a new genus *Steroidobacter* gen. nov. as the type strain of *Steroidobacter*

Fig. 3. Phylogenetic relationships based on 16S rRNA gene sequences of strain FS^T and type species of the gammaproteobacterial families Chromatiaceae, Ectothiorhodospiraceae and Xanthomonadaceae and of several other genera. No 16S rRNA gene sequences are available for the type species of the genera Thiospirillum, Thiopedia and Nitrosococcus. The tree is based on maximum-likelihood analysis (PHYML) using bootstrap values ($\geq 50\%$) based on 100 replications and the HKY model (Hasegawa *et al.*, 1985) and further additional information retrieved from neighbour-joining and parsimony tree topologies. Bar, 10 % sequence divergence.

denitrificans gen. nov., sp. nov. This conclusion is based on 16S rRNA gene sequence analysis in combination with chemotaxonomic and physiological data.

Description of *Steroidobacter* gen. nov.

Steroidobacter (Ste.roi'do.bac'ter. N.Gr. n. *steroides* a steroid; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Steroidobacter* rod-shaped bacterium that degrades steroids).

Gram-negative cells are mesophilic, neutrophilic, non-spore-forming, slightly curved rods. Chemo-organotrophs; metabolism is respiratory and non-fermentative. Exhibit reduction of nitrate to dinitrogen monoxide and further to

dinitrogen without any intermediate accumulation of nitrite. Utilize both oestradiol (C-18 steroid) and testosterone (C-19 steroid) as sole sources of carbon and energy with nitrate as the electron acceptor. Grow aerobically with testosterone but not with oestradiol. Grow on only a limited number of organic substrates. No aerobic or anaerobic growth on liquid or solid complex media. Nitrate, nitrite or dioxygen are used as electron acceptors, whereas sulfate, sulfite and (per)chlorate are not used. The polyamine pattern contains the major compound spermidine and the quinone system is exclusively ubiquinone Q-8. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and several unknown lipids are

Table 1. Phenotypic characteristics of strain FS^T in comparison with the closest related genera

Species of all genera listed are negative for Gram stain and are motile by a single flagellum of polar insertion with the exception of *Solimonas* and *Sinobacter*, which are non-motile. Data for reference genera were taken from Stürmeyer *et al.* (1998), Palleroni *et al.* (2004), Kim *et al.* (2007) and Zhou *et al.* (2008). It should be noted that type strain of *Nevskia ramosa* (Soe1^T = DSM 11499^T) has been designated by Stürmeyer *et al.* (1998) in accordance with Rule 18f of the Bacteriological Code; however, this has not yet been formally proposed. +, Positive; (+), weakly positive; –, negative; ND, not determined or no data available.

Characteristic	Strain FS ^T	<i>Nevskia</i>	<i>Hydrocarboniphaga</i>	<i>Solimonas</i>	<i>Sinobacter</i>
Cell morphology	Slightly curved rods	Slightly curved rods	Rods	Rods	Long rods
Distinctive morphological features	No growth on agar plates	Rosette-like formation of microcolonies	Irregular-shaped colonies on agar media with swarming cells	Pale-yellow colonies on R2A agar	Circular, convex and smooth colonies. Pale-yellow on LB and YT agar
Cell width × length (μm)*	0.3–0.5 × 0.6–1.6	0.7–1.1 × 1.5–2.3	0.75–0.85 × 1.5–2.0	0.3–0.5 × 0.2–0.4	0.3–0.4 × 2.4–2.6
DNA G + C content (mol%)*	61.9	67.8	60.0	40.5	65.1
Catalase	+	(+)	ND	+	–
Cytochrome oxidase	+	–	ND	–	+
Major quinone system	Q-8	ND	ND	Q-8	Q-8†
Major cellular fatty acids	C _{15:0} , C _{17:1} ω8c	C _{18:1} ω7c, C _{16:0} , i-C _{16:0}	C _{18:1} ω7c, i-C _{16:0} , summed feature 3	C _{16:0} , C _{18:1} , summed feature 3‡	C _{18:1} ω7c, C _{16:0} , summed feature 3
Hydroxylated cellular fatty acids	C _{11:0} 3-OH, C _{12:0} 3-OH	C _{12:0} 3-OH, C _{14:0} 2-OH, C _{16:0} 2-OH, C _{16:0} 3-OH§	C _{12:0} 3-OH, C _{14:0} 2-OH, C _{16:0} 2-OH, C _{16:0} 3-OH§	C _{12:0} 2-OH, C _{16:0} 2-OH, C _{16:0} 3-OH	C _{12:0} 2-OH
Denitrification	+	–	–	–	–
Growth on complex media	–	+	+	+	+
Nutritional spectrum of organic or inorganic substrates	Narrow	Wide	Wide	Narrow	Narrow
Utilization of:					
Acetate	+	+	(+)	+II	–
Benzoate	–	+	–	ND	–
Ethanol	–	+	+	–	–
DL-Lactate	–	+	+	–	–
D-Glucose	–	+	–	+II	+
Glutamate	+	+	ND	ND	ND

*Values for the type strains of the type species.

†Minor components are Q-9, MK-7 and MK-6.

‡Kim *et al.* (2007) described summed feature 3 as C_{16:1} iso I and/or C_{14:0} 3-OH; otherwise it represents C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

§Detailed fatty acid profiles of *Nevskia ramosa* and *Hydrocarboniphaga effusa* were obtained from W.-J. Li (personal communication).

||Assimilated only in the presence of LB broth as growth factor.

present in the polar lipid profile. The major fatty acids of testosterone- or heptanoate-grown cells are C_{15:0} and C_{17:1}ω8c. The DNA G + C content of the type strain of the type species is 61.9 mol%. The type species is *Steroidobacter denitrificans*.

Description of *Steroidobacter denitrificans* sp. nov.

Steroidobacter denitrificans (de.ni.tri'fi.cans. N.L. v. *denitrifico* to denitrify; N.L. part. adj. *denitrificans* denitrifying).

Exhibits the following properties in addition to those given in the genus description. Cells are motile, 0.3–0.5 μm wide

and 0.6–1.6 μm long, with rounded ends, occurring singly or in pairs. Colonies in dioxygen-free agar dilution series with testosterone and nitrate are small (diameter approx. 1 mm), yellow–brown and disc-shaped. Temperature and pH ranges for growth are 20–38 °C and pH 6.1–7.8. Optimal growth conditions are 28–30 °C and pH 7.0. Only a few electron donors can be used with nitrate as the electron acceptor: oestradiol, oestrone, testosterone, 4-androstene-3,17-dione, acetate, propionate, valerate, caproate, heptanoate and glutamate. Does not utilize the following electron donors with nitrate as the electron acceptor: cholesterol, acetone, ascorbate, primary aliphatic

Fig. 4. Polar lipid profile of strain FS^T following two-dimensional TLC. AL1, AL2, Unidentified aminolipids; APL, unidentified aminophospholipid; DPG, diphosphatidylglycerol; L1, L2, unidentified polar lipids; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL1, PL2, unidentified phospholipids.

alcohols (C₁–C₄), cyclohexanol, formate, butyrate, palmitate, stearate, isobutyrate, crotonate, adipate, DL-lactate, succinate, pyruvate, fumarate, citrate, glycerol, pentane, benzoate, (+)-D-glucose, (+)-D-galactose, sucrose, yeast extract, DMSO, ammonium and thiosulfate. No aerobic or anaerobic growth on R2A or CASO agar or on CASO-Bouillon or nutrient broth. Aerobic growth with testosterone or 4-androstene-3,17-dione but not with oestradiol or oestrone in phosphate-buffered liquid mineral medium. The polyamine pattern contains the major compound spermidine and minor to trace amounts of putrescine, 1,3-diaminopropane, *sym*-homospermidine and spermine. The polar lipid profile is characterized by the major compounds diphosphatidylglycerol and phosphatidylethanolamine, moderate amounts of phosphatidylglycerol and an unknown phospholipid and an unknown aminophospholipid and minor to trace amounts of another unknown phospholipid, two unknown aminolipids and two unknown polar lipids. The fatty acid profile is as follows: in testosterone-grown cells, C_{9:0} (0.6 %), C_{10:0} (1.3 %), C_{11:0} (2.7 %), C_{12:0} (1.8 %), C_{13:0} (4.2 %), C_{14:0} (0.9 %), C_{15:0} (16.5 %), C_{16:0} (7.0 %), C_{17:0} (6.2 %), C_{15:1}ω8c (2.5 %), C_{16:1}ω9c (0.9 %), C_{17:1}ω8c (27.1 %), C_{17:1}ω6c (3.0 %), C_{18:1}ω7c (4.2 %), C_{11:0} 3-OH (1.2 %), C_{12:0} 3-OH (1.3 %), summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 8.3 %), C_{16:1} iso H (0.8 %), 10-methyl C_{16:0} (7.0 %) and 10-methyl C_{17:0} (2.4 %); in heptanoate-grown cells, C_{9:0} (0.8 %), C_{10:0} (0.2 %), C_{11:0} (4.2 %), C_{12:0} (0.2 %), C_{13:0} (6.6 %), C_{15:0} (19.5 %), C_{16:0} (0.7 %), C_{17:0} (5.4 %),

C_{15:1}ω8c (4.0 %), C_{15:1}ω6c (0.3 %), C_{17:1}ω8c (40.6 %), C_{17:1}ω6c (3.9 %), C_{18:1}ω7c (0.5 %), C_{11:0} 3-OH (1.6 %), C_{12:0} 3-OH (0.2 %), summed feature 3 (0.7 %), summed feature 1 (C_{15:1} iso H and/or C_{13:0} 3-OH; 0.6 %), C_{16:1} iso H (1.5 %), 10-methyl C_{16:0} (1.5 %) and 10-methyl C_{17:0} (2.7 %).

The type strain, FS^T (=DSM 18526^T =JCM 14622^T), was isolated from an enrichment culture inoculated with anoxic digested sludge from a municipal wastewater-treatment plant in Aachen (Germany).

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