Dyadobacter ginsengisoli sp. nov., isolated from soil of a ginseng field

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A Gram-negative, aerobic, non-motile, non-spore-forming and rod-shaped bacterium, strain Gsoil 043^T, was isolated from soil from a ginseng field in Pocheon province, South Korea. The novel isolate was characterized in order to determine its taxonomic position. On the basis of 16S rRNA gene sequence similarity, strain Gsoil 043^T was shown to belong to the family 'Flexibacteraceae' and was related to *Dyadobacter fermentans* (96.7%), *Dyadobacter crusticola* (96.3%) and *Dyadobacter hamtensis* (95.8%). The 16S rRNA gene sequence similarity of the novel strain to other recognized species within the family 'Flexibacteraceae' was less than 87.0%. The G + C content of genomic DNA was 48 mol%. Phenotypic and chemotaxonomic data (major menaquinone, MK-7; major fatty acids, C_{16:1}ω7c, iso-C_{15:0} and C_{16:0}) supported the affiliation of strain Gsoil 043^T to the genus *Dyadobacter*. The results of physiological and biochemical tests enabled strain Gsoil 043^T to be differentiated genotypically and phenotypically from the three *Dyadobacter* species with validly published names. The novel isolate therefore represents a novel species for which the name *Dyadobacter ginsengisoli* sp. nov. is proposed, with the type strain Gsoil 043^T (=KCTC 12589^T=LMG 23409^T).

During a study of the culturable aerobic bacterial community in soil from a ginseng field in Pocheon province, South Korea, a large number of novel bacterial strains was isolated (Im *et al.*, 2005). One of these isolates, strain Gsoil 043^T, was found to be a member of the genus *Dyadobacter* (family 'Flexibacteraceae') and was subjected to a taxonomic investigation.

The genus *Dyadobacter* was described by Chelius & Triplett (2000) to accommodate Gram-negative, rod-shaped cells that are straight to curved, occur in pairs in young cultures and form chains of coccoid cells in old cultures and produce a flexirubin-like pigment (Reddy & Garcia-Pichel, 2005). At present, the genus comprises three species: *Dyadobacter fermentans* (Chelius & Triplett, 2000), *Dyadobacter crusticola* (Reddy & Garcia-Pichel, 2005) and *Dyadobacter hamtensis* (Chaturvedi *et al.*, 2005).

For the present study, we conducted phylogenetic (16S rRNA gene), phenotypic, genotypic and chemotaxonomic analyses to determine the precise taxonomic position of

strain Gsoil 043^T. On the basis of the results obtained, we propose that strain Gsoil 043^T should be placed in the genus *Dyadobacter* as the type strain of novel species.

Strain Gsoil 043^T was originally isolated from soil from a ginseng field in Pocheon province, South Korea. The soil sample was thoroughly suspended with 50 mM phosphate buffer (pH 7.0) and the suspension was spread on one fifth-strength modified R2A (0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g Casamino acids, 0.25 g soytone, 0.5 g glucose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K₂HPO₄, 0.05 g MgSO₄, 0.05 g CaCl₂ and 15 g agar 1 l⁻¹) agar plates after serial dilution with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30 °C for 1 month. Single colonies on the plates were purified by transferring them onto new plates and these were incubated again on modified R2A or half-strength modified R2A. Purified colonies were tentatively identified by partial sequences of the 16S rRNA gene (Im *et al.*, 2005). Strain Gsoil 043^T was one of the predominant isolates on modified R2A agar plates in aerobic conditions. Strain Gsoil 043^T was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20%, w/v) at -70 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 043^T is AB245369.

Gram-reaction testing was performed by the non-staining method as described by Buck (1982). Cell morphology was observed at $\times 1000$ magnification with a light microscope (Nikon) using cells grown for 3 days at 30°C on R2A agar. 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. For single carbon source assimilation studies, a defined liquid medium containing basal salt media was used composed of (g l^{-1}): 1.8 g K_2HPO_4 , 1.08 g KH_2PO_4 , 0.5 g NaNO_3 , 0.5 g NH_4Cl , 0.1 g KCl , 0.1 g MgSO_4 and 0.05 g CaCl_2 . A vitamin solution (Widdel & Bak, 1992), trace element solution SL-10 (Widdel *et al.*, 1983) and selenite/tungstate solution (Tschech & Pfennig, 1984) were added to this medium and the pH was adjusted to 6.8. This liquid medium was aliquoted into 96-well trays and filter-sterilized carbon sources were added to each well (individually at 0.1% w/v). Growth was visually examined on 96-well plates incubated at 30°C for up to 7 days. A negative control well, containing no carbon sources, was included. A positive control culture was grown in a well containing R2A broth.

Fermentative and oxidative acid production from carbohydrates was tested by growth in O-F basal medium with bromothymol blue (Atlas, 1993) supplemented with 1% carbohydrate [soft agar stabs with (fermentative) and without (oxidative) sterile mineral oil overlay]. The O-F medium tubes were incubated at 30°C for 5 days. Some physiological characteristics were determined with API 20E galleries according to the manufacturer's instructions (bioMérieux). Anaerobic growth was tested in serum bottles by adding 1 g thioglycolate l^{-1} to R2A broth and substituting the upper air layer with nitrogen gas. The anaerobic nitrate reduction test to determine the final electron acceptor was performed in serum bottles by adding 1 g thioglycolate l^{-1} and 10 mM KNO_3 to R2A broth under nitrogen gas. Aerobic nitrate reduction was later confirmed by inoculation into 12 ml R2A broth supplemented with 10 mM KNO_3 in three 25 ml serum bottles. The reduction of nitrate was monitored by an ion chromatograph (model 790 personal IC; Metrohm) equipped with a conductivity detector and an anion exchange column (Metrosep Anion Supp 4; Metrohm). Tests for the degradation of DNA (DNase agar Scharlau by flooding plates with 1 M HCl), casein, chitin, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 5 days. Growth at different temperatures (4, 15, 25, 30, 37 and 42°C) and various pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A agar supplemented with 1–10% (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar (TSA) and MacConkey agar at 30°C was also evaluated. Pigment was extracted according to a previously described method (Weeks, 1981) and a spectrum was obtained using a UV-visible spectrophotometer (Beckman). Additionally, the presence of flexirubin-type pigments was tested spectrophotometrically as described previously (Güde, 1980) using 20% KOH.

Extraction of genomic DNA was performed with a commercial genomic DNA extraction kit (Core Biosystem) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with CLUSTAL_X (Thompson *et al.*, 1997). Gaps were edited using BIOEDIT (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods using the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

For the measurement of the G + C content of chromosomal DNA, genomic DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995) and was enzymically degraded into nucleosides. DNA G + C content was determined as described by Mesbah *et al.* (1989) using a reverse-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and reextracted in *n*-hexane/water (1:1, v/v). The crude *n*-hexane-quinone solution was purified using Sep-Pak Vac cartridges silica (Waters) and subsequently analysed by HPLC as previously described (Hiraishi *et al.*, 1996). Cellular fatty acid profiles were determined for strains grown on TSA (Difco) for 2 days. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed by GC (6890; Hewlett Packard) were identified by the Microbial Identification software package (Sasser, 1990).

Strain Gsoil 043^T was aerobic, Gram-negative, non-motile and rod-shaped. Colonies grown on R2A agar plates for 5 days were smooth, circular, transparent, yellowish and 3–7 mm in diameter. The novel strain grew well on nutrient agar and TSA. On R2A agar medium, strain Gsoil 043^T was able to grow at 4– 30°C , but not 37°C . A flexirubin-like yellow-coloured pigment was produced. The pigment exhibited peaks at 412 and 448 nm when extracted in ethanol. The addition of alkali (20% KOH) changed the colour of the pigment to orange and also broadened the peak, thus confirming that it is a flexirubin-type pigment (Weeks, 1981). Other physiological characteristics of strain Gsoil 043^T are summarized in the species description and comparisons of selective characteristics with closely related type strains are shown in Table 1.

The 16S rRNA gene sequence of strain Gsoil 043^T determined in this study was a continuous stretch of 1451 bp (base position 20–1505 with respect to the *Escherichia coli* numbering system). Sequence similarity calculations after a neighbour-joining analysis indicated that the closest relatives of strain Gsoil 043^T were *D. fermentans* NS 114^T (96.7% 16S rRNA gene sequence similarity), *D. crusticola*

Table 1. Physiological characteristics of *Dyadobacter ginsengisoli* sp. nov. Gsoil 043^T and related species of the genus *Dyadobacter*

Strains: 1, *D. ginsengisoli* Gsoil 043^T sp. nov. (data from this study); 2, *D. hamtensis* HHS 11^T; 3, *D. crusticola* C183-8^T; 4, *D. fermentans* NS 114^T. Data for strains 2–4 are from Chaturvedi *et al.* (2005). All strains are Gram-negative, non-motile, rod-shaped, test positive for catalase and oxidase activities and can tolerate 1% NaCl. All strains test positive for the utilization of D-glucose, D-cellobiose, sucrose, D-trehalose, D-raffinose, D-lactose and inulin as carbon sources and are resistant to penicillin (10 µg per disc). All strains are negative in tests for urease, gelatinase, starch hydrolysis, citrate utilization, H₂S production, indole production and arginine dihydrolase activity. Strains 1, 3 and 4 do not utilize alanine, asparagine, aspartic acid, cysteine, glutamic acid, histidine, isoleucine, leucine, phenylalanine, proline, serine, threonine, tyrosine and valine, whereas strain 2 is able to utilize all of these amino acids. +, Positive; –, negative; V, variable; W, weak reaction; R, resistant; S, sensitive; NA, data not available.

Characteristic	1	2	3	4
Colony size (mm)	3–7	2–3	NA	2–3
Colony colour	Yellow	Light yellow	Yellow	Orange
Cell shape	Rods	Long rods	Rods	Rods
Growth at 5 °C	+	–	+	–
Growth at 37 °C	–	+	–	+
Maximum growth temperature (°C)	30	37	25	37
pH range	5.5–8.5	6–8	6–8	6–8
Lipase activity	–	–	+	–
Aesculin hydrolysis	–	–	+	+
Fermentative metabolism	–	+	–	+
Nitrate reduction (aerobic)	+	–	–	–
DNA G+C content (mol%)	48	49	48	48
Acid production from				
D-Arabinose	–	+	–	+
D-Glucose	–	+	–	+
Sucrose	–	–	–	+
Utilization as a sole carbon source				
Acetate	–	+	–	+
D-Adonitol	+	+	–	–
D-Arabinose	–	+	–	+
L-Arabinose	+	+	–	+
Cellulose	–	+	–	+
Citrate	–	+	–	–
Dextran	–	+	–	–
Dulcitol	–	–	+	W
D-Fructose	+	–	–	+
Fumaric acid	–	+	–	+
D-Galactose	+	+	–	+
Glycerol	–	+	–	W
Inositol	–	–	+	+
Lactate	–	+	+	–
Malonate	–	+	–	+
D-Mannitol	–	–	V	+
D-Mannose	+	–	–	+
D-Ribose	–	–	+	–
D-Sorbitol	–	–	+	W
L-Sorbose	–	+	–	+
Tartaric acid	–	+	–	+
D-Xylose	+	–	+	+
Amino acids:				
Arginine	–	+	–	+
Glutamine	–	+	–	+
Glycine	–	+	–	+

Table 1. cont.

Characteristic	1	2	3	4
Lysine	—	+	—	+
Methionine	—	+	—	+
Tryptophan	—	+	—	+
Effect of antibiotics (µg per disc)				
Chloramphenicol (30)	R	S	R	R
Rifampicin (25)	R	S	S	R
Streptomycin (25)	R	S	R	R
Tetracycline (30)	R	S	S	S

C183-8^T (96.3 %) and *D. hamtensis* HHS 11^T (95.8 %). Lower sequence similarities (<87.0 %) were found with all other recognized species of the family 'Flexibacteraceae'. This relationship between strain Gsoil 043^T and other members of the genus *Dyadobacter* was also evident in the phylogenetic tree (Fig. 1). Strain Gsoil 043^T and the three species of the genus *Dyadobacter* mentioned above formed a monophyletic clade with a high bootstrap value (100 %) which was supported by both tree-making methods employed in this study.

DNA–DNA hybridization between strain Gsoil 043^T and its nearest phylogenetic neighbours was not attempted since strains differing by >3.0 % at the 16S rRNA gene sequence level are unlikely to exhibit >70 % relatedness at the whole genome level (Stackebrandt & Goebel, 1994).

The G + C content of the genomic DNA of strain Gsoil 043^T was 48 mol%. MK-7 was the predominant menaquinone. The fatty acid profile of strain Gsoil 043^T (Table 2) comprised mainly C_{16:1}ω7c (44.6 %), iso-C_{15:0} (20.3 %) and C_{16:0} (11.0 %). Significant differences in fatty acid profiles were found between strain Gsoil 043^T and the other species of the genus *Dyadobacter*. *D. hamtensis* and *D. fermentans* contained significantly higher amounts of iso-C_{17:0} 3-OH, whereas *D. crusticola* and strain Gsoil 043^T contained significantly higher amounts of C_{16:1}ω7c. However, strain Gsoil 043^T contained significantly lower amounts of C_{16:1}ω5c.

The species of the genus *Dyadobacter* could be differentiated from one another by the fatty acid profiles.

On the basis of the data and observations described above, strain Gsoil 043^T should be assigned to the genus *Dyadobacter* as the type strain of a novel species, for which the name *Dyadobacter ginsengisoli* sp. nov. is proposed.

Description of *Dyadobacter ginsengisoli* sp. nov.

Dyadobacter ginsengisoli (gin.sen.gi.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of the soil of a ginseng field, the source of the type strain).

Cells are Gram-negative, aerobic, non-motile and rod-shaped, 0.6–0.8 µm in diameter and 3.0–6.0 µm in length after 2 days culture on R2A agar. Colonies grown on R2A agar for 2 days are smooth, circular, transparent and yellowish. Grows well at 4–30 °C and at pH 5.5–8.5, but does not grow at 37 °C. Growth occurs in the absence of NaCl and in the presence of 1.0 % (w/v) NaCl, but not with 2 % (w/v) NaCl. Nitrate is reduced in aerobic conditions. Anaerobic growth does not occur. Does not degrade DNA, chitin or xylan. Substrate utilization, enzyme production, acid production and other physiological characteristics are given in Table 1. In addition, is able to utilize L-rhamnose, D-lyxose, N-acetylglucosamine, salicin, D-lactose, D-maltose,

Fig. 1. Neighbour-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of *Dyadobacter ginsengisoli* sp. nov. Gsoil 043^T with other related species. Filled circles at nodes indicate generic branches that were also recovered using maximum-parsimony algorithms. Bootstrap values (expressed as a percentage of 1000 replications) >70 % are shown at the branch points. Bar, 0.02 substitutions per nucleotide position.

Table 2. Cellular fatty acid profiles of *Dyadobacter ginsengisoli* sp. nov. Gsoil 043^T and related species of the genus *Dyadobacter*

Strains: 1, *D. ginsengisoli* Gsoil 043^T sp. nov. (TSA at 30 °C, data from this study); 2, *D. hamtensis* HHS 11^T (R2A medium at 25 °C); 3, *D. crusticola* C183-8^T (TSA at 25 °C); 4, *D. fermentans* NS 114^T (R2A medium at 25 °C). Data for strains 2–4 were taken from Chaturvedi *et al.* (2005). Major fatty acids are shown in bold type.

Fatty acids	1	2	3	4
C _{14:0}	—	1·2	1·4	—
iso-C _{15:1}	—	3·7	4·5	—
iso-C _{15:0}	20·3	24·7	13·4	22·9
C _{16:1ω5c}	7·9	19·8	21·4	14·3
C _{16:1ω7c}	44·6	14·8	41·2	28·6
C _{16:0}	11·0	8·6	12·4	5·7
iso-C _{15:0} 3-OH	3·9	—	2·4	2·9
Unknown	0·8	—	0·3	—
C _{16:0} 3-OH	2·3	2·5	2·2	2·9
C _{18:0}	—	—	0·2	—
C _{18:1}	—	2·5	0·8	—
iso-C _{17:0} 3-OH	7·6	22·2	2·9	22·9

D-melibiose and inulin as sole carbon sources. Does not utilize D-fucose, ethanol, L-xylose, pyruvic acid, formic acid, propionate, 3-hydroxybutyrate, valerate, caprate, maleic acid, phenylacetate, benzoic acid, 3-hydroxybenzoate, 4-hydroxybenzoate, malate, succinic acid, glutaric acid, itaconate, adipate, suberate, oxalic acid, gluconate, xylitol, amygdalin, methanol or glycogen as sole carbon sources. Resistant to (μg per disc) tetracycline (100), ampicillin (100), kanamycin (100), rifampicin (100), chloramphenicol (100), carbenicillin (100), hygromycin (100) and streptomycin (50), but sensitive to streptomycin (100). The predominant menaquinone is MK-7. Major cellular fatty acids are C_{16:1ω7c}, iso-C_{15:0}, and C_{16:0}. The DNA G+C content of the type strain is 48 mol% (determined by HPLC).

The type strain, Gsoil 043^T (=KCTC 12589^T=LMG 23409^T), was isolated from soil of a ginseng field in Pocheon province, South Korea.

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References

- Atlas, R. M. (1993).** *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Buck, J. D. (1982).** Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.

Chaturvedi, P., Reddy, G. S. N. & Shivaji, S. (2005). *Dyadobacter hamtensis* sp. nov., from Hamta glacier, located in the Himalayas, India. *Int J Syst Evol Microbiol* **55**, 2113–2117.

Chelius, M. K. & Triplett, E. W. (2000). *Dyadobacter fermentans* gen. nov., sp. nov., a novel Gram-negative bacterium isolated from surface-sterilized *Zea mays* stems. *Int J Syst Evol Microbiol* **50**, 751–758.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Güde, H. (1980). Occurrence of cytophagas in sewage plants. *Appl Environ Microbiol* **39**, 756–763.

Hall, T. A. (1999). BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

Hiraishi, A., Ueda, Y., Ishihara, J. & Mori, T. (1996). Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* **42**, 457–469.

Im, W.-T., Jung, H.-M., Cui, Y.-S., Liu, Q.-M., Zhang, S.-L. & Lee, S.-T. (2005). Cultivation of the three hundreds of bacterial species from the soil of the ginseng field and mining the novel lineage bacteria. In *Proceedings of the International Meeting of the Federation of Korean Microbiological Societies*, abstract A035, p. 169. Seoul: Federation of Korean Microbiological Societies.

Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005). *Sphingopyxis granuli* sp. nov., a β-glucosidase-producing bacterium in the family *Sphingomonadaceae* in α-4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.

Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.

Kouker, G. & Jaeger, K.-E. (1987). Specific and sensitive plate assay for bacterial lipases. *Appl Environ Microbiol* **53**, 211–213.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Moore, D. D. & Dowhan, D. (1995). Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, pp. 2–11. Edited by Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. New York: Wiley.

Reddy, G. S. N. & Garcia-Pichel, F. (2005). *Dyadobacter crusticola* sp. nov., from biological soil crusts in the Colorado Plateau, USA, and an emended description of the genus *Dyadobacter* Chelius and Triplett 2000. *Int J Syst Evol Microbiol* **55**, 1295–1299.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*. MIDI Technical Note 101. Newark, DE: MIDI Inc.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M.-S. & Lee, S.-T. (2004). Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Tschech, A. & Pfennig, N. (1984). Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. *Arch Microbiol* **137**, 163–167.

Weeks, O. B. (1981). Preliminary studies of the pigments of *Flavobacterium breve* NCTC 11099 and *Flavobacterium odoratum* NCTC 11036. In *The Flavobacterium-Cytophaga Group*, pp. 109–114. Edited

by H. Reichenbach & O. B. Weeks. Weinheim: Gesellschaft Für Biotechnologische Forschung.

Widdel, F. & Bak, F. (1992). Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*, 2nd edn, pp. 3352–3378. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Widdel, F., Kohring, G. & Mayer, F. (1983). Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov., sp. nov., and *Desulfonema magnum* sp. nov. *Arch Microbiol* **134**, 286–294.