Emended description of *Janibacter terrae*, including ten dibenzofuran-degrading strains and *Janibacter brevis* as its later heterotypic synonym

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Ten Gram-positive strains that degraded dibenzofuran belonged to one homogeneous cluster with common biochemical and chemotaxonomic properties. The strains included the previously described strains [*Terrabacter* sp.] DPO 360 and DPO 1361 and were isolated from different locations in central Europe. They all shared menaquinone MK-8(H₄) and meso-diaminopimelic acid in the cell wall and showed the same cellular fatty acid, polar lipid and RiboPrint patterns. Chemotaxonomic properties, as well as DNA G + C content (72.8 mol%), were consistent with those described for the genus *Janibacter*. Analysis of 16S rDNA sequences suggested a close relationship to *Janibacter terrae* and *Janibacter brevis*. DNA–DNA hybridization of two dibenzofuran-degrading strains (DPO 360 and DPO 1361) and the type strains of *J. terrae* and *J. brevis* revealed that these strains belong to the same species. An emended description of the species *J. terrae* is given. *J. brevis* is a later heterotypic synonym of *J. terrae*.

Bacteria that degrade dibenzofuran were isolated from different sources and several geographical areas in Germany and France (Strubel *et al.*, 1989). Metabolism of dibenzofuran by these bacteria is initiated by angular dioxygenation of the compound (Engesser *et al.*, 1989). Three distinct extradiol dioxygenases are involved in further degradation of the resulting metabolite 2,2',3-trihydroxybiphenyl (Schmid *et al.*, 1997). In 1989, one of the dibenzofuran-degrading strains, DPO 1361, was assigned to the genus *Brevibacterium* on the basis of its cell-wall composition and biochemical properties (Strubel *et al.*, 1989). Based on the results of 16S rDNA analysis and DNA–DNA hybridization, Schmid *et al.* (1997) proposed that strains DPO 360 and DPO 1361 were representatives of the same novel species within the genus *Terrabacter*. In the same year, *Terrabacter* (Collins *et al.*, 1989) was grouped with the genus *Intrasporangium* (Kalakoutskii *et al.*, 1967) in the novel family *Intrasporangiaceae* (Stackebrandt *et al.*, 1997). Three novel genera were described within the family: *Janibacter*, with the sole species *Janibacter limosus* (Martin *et al.*, 1997), *Terracoccus* (Prauser *et al.*, 1997) and *Tetrasphaera* (Maszenan *et al.*, 2000). *Knoellia* (Groth *et al.*, 2002) is a

genus that is very close to the family *Intrasporangiaceae*. A soil isolate and two trichloroethylene-degrading strains isolated from groundwater were described, respectively, as the novel *Janibacter* species *Janibacter terrae* (Yoon *et al.*, 2000) and *Janibacter brevis* (Imamura *et al.*, 2000).

These recent developments in the taxonomy of bacteria closely related to [*Terrabacter* sp.] DPO 360 and DPO 1361 led us to reinvestigate the taxonomic position of the dibenzofuran-degrading isolates. Eight other dibenzofuran-degrading strains were included in the study. The 16S rRNA gene sequences of the strains showed highest similarity to those of *J. terrae* and *J. brevis*. Reassessment of the *J. terrae* and *J. brevis* sequences suggested that *J. brevis* may be a synonym of *J. terrae*.

Isolation sources and geographical areas of the dibenzofuran-degrading strains are shown in Table 1. Strains were isolated by enrichment cultures with dibenzofuran as the sole source of carbon and energy in the laboratory of K.-H. Engesser, University of Stuttgart. Type strains were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen). For morphological and physiological characterizations, standard methods were used. The API 50CH galleries (bioMérieux) were inoculated with AUX medium for testing utilization of substrates, and

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of *Janibacter brevis* DSM 13953^T is AJ310085.

Table 1. Dibenzofuran-degrading strains isolated from different locations in central Europe

DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany.

Strain	Isolation source	Place
DPO 210=DSM 11213	Wastewater treatment plant	Weil der Stadt, Germany
DPO 320=DSM 11345	Forest soil	Vosges, France
DPO 340=DSM 11346	Wastewater treatment plant	Stuttgart, Germany
DPO 360=DSM 11214	Contaminated soil from a coke oven	Northern Germany
DPO 400=DSM 11215	Contaminated soil from an industrial site	Southern Germany
DPO 410=DSM 11216	Contaminated soil from an industrial site	Southern Germany
DPO 420=DSM 11217	Contaminated soil from an industrial site	Southern Germany
DPO 430=DSM 11218	Contaminated soil from an industrial site	Southern Germany
DPO 440=DSM 11219	Contaminated soil from an industrial site	Southern Germany
DPO 1361=DSM 11220	Water of River Rhine	Leverkusen, Germany

with CHB medium (both media by bioMérieux) for testing acid production from carbohydrates. API 20NE galleries (bioMérieux) were read after 1 and 3 days and API 50CH after 1, 2, 3, 6 and 14 days incubation at 28 °C. Results were scored as positive when turbidity was visible after 6 days at the latest and was confirmed after 14 days. Utilization of carbon sources was tested in a mineral medium (Stanier *et al.*, 1966) with 2 g l⁻¹ of the respective carbon source. Cultures were read after incubation for up to 28 days.

Isoprenoid quinones were extracted and purified by using the small-scale integrated procedure of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1985). Polar lipids were extracted, examined by two-dimensional TLC and identified according to procedures described by Minnikin *et al.* (1984). For analysis of fatty acids, cells were grown on tryptic soy broth agar (TSBA) for 24 h. Fatty acid methyl esters were obtained from 40 mg (wet wt) cells by saponification, methylation and extraction, as described previously (Kämpfer & Kroppenstedt, 1996) and separated by a model 5898A gas chromatograph (Hewlett Packard). Peaks were integrated automatically and fatty acid names and percentages were determined by using the Microbial Identification standard software package (Sasser, 1990). Preparation of cell walls and determination of peptidoglycan structure were carried out by using methods described by Schleifer & Kandler (1972), with the modification that TLC on cellulose sheets was used instead of paper chromatography.

Riboprinting was done by the RiboPrinter Microbial Characterization system (Qualicon) according to the manufacturer's instructions, after cleavage with endonuclease *PvuII*.

DNA base composition was determined by HPLC. DNA was isolated after cell disruption with a French pressure cell and purified on hydroxyapatite, according to Cashion *et al.* (1977). P1 hydrolysis and nucleotide dephosphorylation with alkaline phosphatase were done as described by Mesbah *et al.* (1989). HPLC conditions (LKB equipment with Shimadzu CR-3A integrator) on a Nucleosil 100-5C18 column were chosen according to Tamaoka & Komagata

(1984). DNA-DNA hybridization was carried out at 66 °C as described by De Ley *et al.* (1970) with the modifications described by Huss *et al.* (1983), by using a GILFORD system model 2600 spectrophotometer equipped with a GILFORD model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992). After amplification of the 16S rDNA of DSM 13876^T, purified PCR products were sequenced and electrophoresed and the sequences were aligned as described previously (Rainey *et al.*, 1996). The following 16S rDNA sequences were used for alignments (Rainey *et al.*, 1996; Schmid *et al.*, 1997): strain [*Terrabacter* sp.] DPO 1361 (Y08853), *J. brevis* IAM 14781^T (AB016438), *J. terrae* KCCM 80001^T (AF176948) and *J. limosus* DSM 11140^T (Y08539).

All ten dibenzofuran-degrading strains formed one cluster with high similarities in their physiology and chemotaxonomically relevant cell components. On TSBA, the DPO strains formed opaque, yellow-whitish colonies with entire edges. Colony diameter varied between 0.1 and 0.4 mm after 3 days and between 0.6 and 1 mm after 6 days incubation at 28 °C. Cells were Gram-positive, non-motile, non-spore-forming cocci or rods. Cells of strain DPO 360 were irregular cocci of 0.6–1.4 µm that occurred singly or in pairs. Cells of strain DPO 1361 were egg-shaped rods of 0.7–0.8 × 1.4–2.3 µm. The strains grew aerobically at 28 and 35 °C. Growth at 37 °C on TSBA was poorer than at lower temperatures. Physiological properties, as determined in API 20NE and 50CH galleries, were highly similar for all ten strains. The strains reduced nitrate to nitrite, hydrolysed gelatin and utilized malate, fructose and turanose. Oxidase reaction of young cultures was negative and of 3-day-old cultures was delayed and weak. Inositol and sucrose (with one exception) and gluconate and trehalose (with two exceptions) were used. Variable results were achieved for the utilization of glucose, *N*-acetylglucosamine, maltose and adipate. All other tests in the API galleries were negative. No acid was produced from carbohydrates in the API 50CH system. The Biolog carbon source utilization test system was not applicable to the strains, as

they gave a relatively strong reaction without a carbon source (reference vial). Physiological properties of strains DPO 360 and DPO 1361, which were selected as reference strains, and of *J. brevis* DSM 13953^T, *J. terrae* DSM 13876^T and *J. limosus* strains are shown in Table 2.

Table 2. Biochemical properties of strains DPO 360 and DPO 1361, *Janibacter brevis*, *Janibacter terrae* and *Janibacter limosus*

Taxa: 1, strain DPO 360 (=DSM 11214); 2, strain DPO 1361 (=DSM 11220); 3, *J. brevis* DSM 13953^T; 4, *J. terrae* DSM 13876^T; 5, *J. limosus* DSM 11140^T; 6, *J. limosus* DSM 11141. W, Weak reaction; D, delayed reaction; ND, not determined. The following tests showed positive results for all six strains: catalase, decomposition of casein and starch, growth in the presence of 10 % NaCl, reduction of nitrate to nitrite* and utilization of turanose and DL-malate. All strains were negative for oxidase, motility, indole from tryptophan, arginine dihydrolase, urease, decomposition of Tween 80 and aesculin, Voges–Proskauer, acid from glucose and β -galactosidase*. Strains DSM 11214, DSM 11220, DSM 11140^T and DSM 11141 did not utilize cellobiose, aconitate, *m*- or *p*-hydroxybenzoate, citrate, succinate, DL-tartrate, ethanolamine, histamine, sarcosine, spermine, β -alanine, L-arginine, L-leucine, L-ornithine or L-valine.

Property	1	2	3	4	5	6
Growth at 37 °C (on TSBA)	+	+	+	W	–	–
Decomposition of gelatin*	+	+	D	D	–	–
Production of H ₂ S from cysteine	W	W	–	–	+	+
Utilization of:						
D-Fructose†	+	+	+	+	+	–
D-Glucose†	–	+	+	+	+	+
Maltose†	–	+	D	D	+	+
D-Mannose†	–	W	–	+	W	–
Sucrose†	+	+	+	+	+	–
Trehalose†	–	+	+	D	+	+
Turanose†	+	+	+	D,W	+	+
Inositol†	W	+	+	+	W	+
N-Acetylglucosamine†	–	+	–	D	D	–
Gluconate†	+	+	D	+	+	–
Sucrose	+	+	ND	ND	+	+
Acetate	W	–	+	+	–	–
Aconitate	–	–	ND	ND	–	–
Benzoate	–	+	+	+	+	+
Formate	+	–	ND	ND	+	+
Gluconate	+	+	+	+	+	–
Hippurate	+	+	+	+	–	D
D-Malate	–	–	W	W	–	–
Putrescine	+	–	ND	ND	+	–
L-Glutamine	W	W	ND	ND	W	W
L-Histidine	W	+	ND	ND	W	+
L-Proline	W	W	ND	ND	W	W

*API 20NE gallery.

†API 50CH gallery.

Whole-cell fatty acid patterns of the ten DPO strains were highly similar. The main fatty acids were iso-branched (14-methylpentadecanoic acid) and *cis*-unsaturated fatty acids (*cis* 9-heptadecenoic and *cis* 9-octadecenoic acids), with minor amounts of iso-branched, methylated or straight-chain fatty acids (Table 3). The fatty acid pattern of the *Janibacter* strains tested was composed of the same main components, with the exception that *J. limosus* DSM 11140^T contained 15 % heptadecanoic acid as one main component. The type strain of *Terrabacter tumescens* differed in having only 3 % 14-methylpentadecanoic acid and high proportions (14 %) of hexadecanoic acid and *cis* 9-hexadecenoic acid (data not shown).

DPO strains 360, 340, 400, 430 and 1361 contained tetrahydrogenated menaquinone MK-8 [MK-8(H₄)] as the main quinone and the polar lipids diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. Purified cell walls of strains DPO 360 and DPO 1361 contained, besides muramic acid and glucosamine, the amino acids *meso*-diaminopimelic acid, glutamic acid and alanine in a molar ratio of 1:1:2, respectively. Fingerprints of the partial hydrolysate were compatible with the peptidoglycan type A1 γ *m*-Dpm-direct.

On the molecular side, unity of the DPO strains was confirmed by RiboPrinter patterns. All DPO strains and *J. brevis* DSM 13953^T showed two main fragments with molecular masses of about 7.5 and 10.5 kbp after cleavage with *Pvu*II. The patterns of *J. terrae* DSM 13876^T and *J. limosus* DSM 11140^T were also dominated by two fragments that, however, had different molecular masses.

The 16S rDNA sequence of DPO 1361 was > 99 % similar to the deposited sequences of *J. brevis* IAM 14781^T and *J. terrae* KCCM 80001^T. The deposited sequences of *J. brevis* IAM 14781^T and *J. terrae* KCCM 80001^T showed 99.8 % similarity to each other. Resequencing of the 16S rDNA of the type strain of *J. brevis* (AJ310085) gave a sequence that was 99.9 % similar to those of *J. terrae* and strain DPO 1361.

DNA–DNA reassociation of the type strains of *J. brevis* and *J. terrae* resulted in 80.6 % relatedness. Strain DPO 360 showed 82, 94.5, 78.9 and 86.2 % relatedness to DPO 1361, DPO 400, *J. brevis* DSM 13953^T and *J. terrae* DSM 13876^T, respectively. DNA–DNA relatedness between DPO 360 and *J. limosus* DSM 11140^T and DSM 11141 was 14.7 and 5.4 %, respectively. The DNA G + C content of strain DPO 360 was 72.8 mol%.

Whole-cell fatty acid analysis and riboprinting, together with Gram-staining, cell morphology and biochemical properties, revealed that all dibenzofuran-degrading strains belonged to one cluster. When fatty acid data were compared by Ward's method, fatty acid composition of the DPO strains was more similar within the group than to those of other bacteria included in this study or registered in the MIDI database. DPO 210 and DPO 360 showed the most atypical fatty acid patterns within the group; the fatty

Table 3. Fatty acid contents (%) of DPO strains and the most similar species in the genera *Janibacter* and *Nocardioides*

Taxa: 1, DPO strains ($n=10$); 2, DPO 360 (=DSM 11214); 3, *J. brevis* DSM 13953^T; 4, *J. terrae* DSM 13876^T; 5, *J. limosus* DSM 11140^T; 6, *J. limosus* DSM 11141; 7, *N. jensenii* DSM 20641^T; 8, *N. simplex* DSM 20130^T. –, Not detected.

Fatty acid	1	2	3	4	5	6	7	8
Saturated:								
C _{15:0}	–	–	0.66	0.20	0.92	0.68	1.07	–
C _{16:0}	0.2–0.9	0.2	1.55	0.55	2.12	0.92	2.00	1.13
C _{17:0}	0.8–1.6	0.8	2.18	0.52	17.79	4.51	5.81	–
C _{18:0}	–	–	0.78	0.35	3.94	0.91	1.90	0.77
C _{19:0}	–	–	–	–	1.90	–	–	–
Unsaturated:								
C _{15:1ω6c}	0–0.7	–	0.36	0.28	–	–	–	–
iso-C _{16:1} H	2–3	3.07	0.54	2.08	–	1.06	2.98	2.26
iso-C _{17:1ω9c}	3–6	5.66	2.06	3.51	–	–	1.14	–
anteiso-C _{17:1ω9c}	0–1.4	1.36	–	0.68	–	–	–	–
C _{17:1ω8c}	12–15	11.98	20.90	11.92	26.15	24.98	10.46	2.70
C _{17:1ω6c}	–	–	–	–	–	–	2.00	9.64
C _{18:1ω9c}	8–14	8.53	15.08	13.34	17.48	11.63	13.75	13.91
C _{18:1ω7c}	–	–	1.25	1.28	–	–	–	–
iso-C _{19:1}	–	–	–	0.30	–	–	–	1.55
Branched:								
iso-C _{4:0}	0.7–1.5	1.02	0.50	1.06	–	0.59	0.73	0.68
iso-C _{15:0}	4–5	4.43	3.72	2.38	0.56	–	6.62	0.49
anteiso-C _{15:0}	0–0.6	0.55	–	0.44	–	–	–	–
iso-C _{16:0}	36–44	41.07	30.45	38.10	12.21	34.82	30.25	42.09
10-Methyl C _{16:0}	–	–	–	–	–	–	–	1.34
iso-C _{17:0}	4–5	4.17	6.56	3.86	1.47	0.73	10.13	0.89
anteiso-C _{17:0}	3–6	5.89	1.97	5.67	–	–	2.22	–
10-Methyl C _{17:0}	4–5	5.16	2.68	5.97	2.84	8.32	1.42	6.04
iso-C _{18:0}	0–0.8	0.57	1.25	0.92	3.36	3.48	3.13	1.23
10-Methyl C _{18:0}	–	–	–	0.20	0.78	–	1.66	4.52
10-Methyl C _{19:0}	–	–	–	–	–	–	–	0.55
Feature 4*	3–5	2.75	3.85	3.85	1.08	2.41	1.78	4.85
Feature 6*	–	–	3.66	2.32	–	–	–	–
Feature 7*	0–1.6	1.36	–	–	–	–	–	3.69
Feature 8*	1.5–2.3	1.60	–	–	7.40	4.96	0.97	–

*Unknown fatty acids; these compounds have no names listed in the Peak Library File of the MIDI system and therefore were not identified or included in percentage calculation.

acid patterns of the DPO strains were most similar to those of *J. brevis* DSM 13953^T, *J. terrae* DSM 13876^T, *J. limosus* DSM 11141, *Nocardioides jensenii* DSM 20641^T and *Nocardioides simplex* DSM 20130^T. Iso-hexadecanoic acid was the predominant fatty acid in these strains. The fatty acid pattern of the type strain of *J. limosus* was less similar, with a Euclidian distance of more than 30 units to the DPO cluster, and was dominated by straight-chain unsaturated acids. The group of *J. brevis*, *J. terrae* and DPO strains may be differentiated from *J. limosus* by the presence of iso-*cis* 8-heptadecenoic and anteiso-heptadecanoic acids, which are absent in *J. limosus*.

Like the fatty acid patterns, the carbohydrate pattern showed high homogeneity within the DPO group and between the

DPO group and the type strains of *J. brevis*, *J. terrae* and *J. limosus*. All utilized a limited range of the carbohydrates tested (Table 2). Forty substrates (mostly carbohydrates) that are not mentioned in Table 2 were tested in the API 50 CH and API 20NE galleries and gave no growth. Only fructose, turanose and inositol were metabolized by all strains. Additionally, almost all strains utilized gluconate and sucrose (with the exception of *J. limosus* DSM 11141) and trehalose (with the exception of strain DPO 360) (Table 2). Of the carbon acids and amines additionally tested, benzoate, DL-malate, L-glutamine, L-histidine and L-proline were utilized (Table 2). Strains of the DPO–*J. brevis*–*J. terrae* cluster may be differentiated from *J. limosus* by their ability to grow at 37 °C on TSBA and to hydrolyse gelatin.

RiboPrinter patterns are generally species-specific and, in some cases, specific even to the subspecies level. Therefore, the patterns can be used for identification at the species level and, for some taxa, for clustering of strains that belong to the same species. RiboPrinter patterns of the DPO strains homogeneously showed the same two main bands (Fig. 1). The pattern of *J. brevis* DSM 13953^T also showed the same main fragments. Only the patterns of DSM 11219 and *J. brevis* DSM 13953^T displayed minor differences when compared to the patterns of the other strains. Nevertheless, the RiboPrints of the strains mentioned were highly similar (similarity > 0.90, as computed by the RiboPrinter system) and belonged to one cluster. The patterns of *J. terrae* DSM 13876^T and *J. limosus* DSM 11140^T corresponded to the patterns of the DPO cluster in being dominated by only two fragments. One band of the DSM 13876^T pattern corresponded to the DPO pattern, but its second fragment was shifted towards a lower molecular mass. Neither of the bands of *J. limosus* DSM 11140^T corresponded to those of the DPO cluster. The RiboPrinter data suggest that the DPO strains and *J. brevis* DSM 13953^T form a homogeneous cluster. As physiological tests, 16S rDNA sequence analysis and DNA–DNA similarity data (see below) support the affiliation of the DPO strains and the type strains of *J. brevis* and *J. terrae* to the same species, the difference of one band in the RiboPrinter pattern of strain *J. terrae* DSM 13876^T should be considered as a strain-specific feature.

In 1997, strains DPO 360 and DPO 1361 were assigned to the genus *Terrabacter* by Schmid *et al.* (1997), as their 16S rDNA sequences were most related (95.9%) to the sequence of *T. tumescens* at that time. Because of their DNA–DNA reassociation results, the authors stated that

Fig. 1. RiboPrinter patterns of dibenzofuran-degrading strains and of *J. limosus* DSM 11140^T, *J. terrae* DSM 13876^T and *J. brevis* DSM 13953^T, after digestion of DNA with endonuclease *PvuII*.

the two strains belonged to one novel species that was different from *T. tumescens*. It is only since then that the genus *Janibacter* (Martin *et al.*, 1997) and the family *Intrasporangiaceae* (Stackebrandt *et al.*, 1997) have been described. *Sanguibacter* (Fernández-Garayzábal *et al.*, 1995) and *Ornithinicoccus* (Groth *et al.*, 1999) are related closely to the family *Intrasporangiaceae*, but now are reclassified as a family on its own or as a genus separate from the *Intrasporangiaceae*, respectively (Stackebrandt & Schumann, 2000). The results of our polyphasic approach consistently suggest that the DPO strains belong to the genus *Janibacter* and are indistinguishable from the species *J. brevis* and *J. terrae*. These two species and the DPO strains showed the same biochemical and chemotaxonomic features [directly cross-linked *meso*-diaminopimelic acid as the peptidoglycan type, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol as polar lipids and menaquinone MK-8(H₄)]. Correspondence of chemotaxonomic and biochemical data was confirmed by the high similarity of 16S rDNA sequences and DNA–DNA reassociation values > 78% between DPO 360, DPO 1361 and the type strains of *J. terrae* and *J. brevis*.

The species *J. brevis* and *J. terrae* were described at the same time by different working groups (Imamura *et al.*, 2000; Yoon *et al.*, 2000). Both were isolated from environmental samples. Our hybridization of the DPO strains and the type strains of the two species revealed > 78% relatedness to each of them. This prompted us to hybridize the DNA of the type strains of *J. brevis* DSM 13953^T and *J. terrae* DSM 13876^T. These two were also about 80% similar. After resequencing the 16S rDNA of *J. terrae* DSM 13876^T, the 16S rDNA sequence similarity between *J. brevis* IAM 14781^T and *J. terrae* DSM 13876^T was 99.9%. Therefore, we suggest that *J. brevis* and *J. terrae* are synonyms for one species. The effective description of *J. terrae* was published on pp. 1821–1827 of volume 50 of the *International Journal of Systematic and Evolutionary Microbiology*, whereas *J. brevis* was published on pp. 1899–1904 of the same volume (Imamura *et al.*, 2000; Yoon *et al.*, 2000). According to Rule 24b of the International Code of Nomenclature of Bacteria, the name *J. terrae* has priority and *J. brevis* should be recognized as the later synonym of the species.

Emended description of *Janibacter terrae* Yoon, Lee, Kang, Kho, Kang and Park 2000 (syn. *Janibacter brevis* Imamura, Ikeda, Yoshida and Kuraishi 2000)

Cells are Gram-positive, non-motile, non-spore-forming cocci or short rods that occur singly, in short chains or in clumps. Colonies on complex media are white, cream or yellowish, opaque, glistening and convex with entire margins. Optimal growth temperature is 25–30 °C. Maximum growth temperature on solid TSBA medium is 37 °C and in liquid R medium is 40 °C. Young cultures are oxidase-negative; a weakly positive reaction may occur with older cultures or on special media. Catalase-positive. Decomposes casein, gelatin and starch. H₂S may be

produced from cysteine. Grows in brain heart infusion in the presence of 10% NaCl. Reduces nitrate to nitrite. Negative results for indole production from tryptophan, acetoin production, arginine dihydrolase, urease, β -galactosidase and decomposition of Tween 80 and aesculin. No acid is produced from glucose or other carbohydrates. Variable results with glucose, mannose, maltose, *N*-acetylglucosamine, benzoate, acetate, adipate and putrescine as substrate. Most or all strains utilize fructose, turanose, sucrose, trehalose, inositol, malate and gluconate. Chemotaxonomic characteristics are as described by Yoon *et al.* (2000) and Imamura *et al.* (2000). DNA G + C content is 69.0–72.8 mol%.

The type strain is CS12^T (= KCCM 80001^T = JCM 10705^T = DSM 13876^T). All strains are isolated from environmental samples such as soil and water. Many strains are able to degrade environmental pollutants.

Acknowledgements

We thank Petra Hobeck, Gabriele Pötter and Anika Vester for excellent technical assistance. We are grateful to K.-H. Engesser for letting us have the DPO strains.

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