***Georgenia muralis* gen. nov., sp. nov., a novel actinobacterium isolated from a medieval wall painting**

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Two bacterial strains, designated 1A-C^T and 3A-1, were studied and, using these results and previously published data, taxonomically classified. Cells of the strains exhibited a rod-coccus cycle. The peptidoglycan determined for 1A-C^T was of type A4 α with lysine as the diagnostic cell-wall diamino acid and an interpeptide bridge of L-Lys $\leftarrow$ L-Glu. The menaquinone systems of the two strains contained MK-8(H₄) (82–94 %) and MK-7(H₄) (3–11 %). The polar lipid profiles consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannoside, two unidentified phospholipids and an unidentified glycolipid. The fatty acid profiles contained predominantly ai-C_{15:0} and significant amounts of i-C14:0 and i-C15:1 fatty acids. Genomic fingerprints clearly distinguished strains 1A-C^T and 3A-1 from each other. DNA–DNA relatedness between the two strains (92 %) demonstrated that they are members of a single species. Analyses of the 16S rDNA sequences of strains 1A-C^T and 3A-1, which were almost identical (99.6 % sequence similarity), and comparison with corresponding sequences demonstrated that they represent a novel lineage within the suborder *Micrococcineae*, most closely related to species of the genera *Beutenbergia*, *Bogoriella* and *Cellulomonas* (94.7–95.7 % sequence similarity). The results demonstrate that the two strains are members of a single new genus and a single novel species. Thus, the name *Georgenia muralis* gen. nov., sp. nov. is proposed. The type strain is strain 1A-C^T (= DSM 14418^T = CCM 4963^T). Another strain of the species is strain 3A-1 (= DSM 14419 = CCM 4964).

Keywords: *Georgenia muralis* gen. nov., sp. nov., 16S rDNA sequence, chemotaxonomy, phenotypic characteristics

INTRODUCTION

In a recent study, several bacterial strains isolated from a damaged medieval wall painting from a church in St Georgen, Styria (Austria), were characterized by means of physiological, biochemical and chemotaxonomic traits as well as analyses of partial 16S rDNA sequences (Altenburger *et al.*, 1996). These isolates were identified as members of the classes *Actinobacteria* and *Proteobacteria* and the aerobic, endospore-

forming bacteria (Altenburger *et al.*, 1996; Wieser *et al.*, 1999; H.-J. Busse, unpublished results). A pair of strains (originally designated G5 and G6) included in the former study could not be affiliated to any established genus. Due to the uniqueness of their chemotaxonomic characteristics and the distance from any established taxon indicated from partial 16S rDNA sequences, the two strains were re-examined in order to determine their taxonomic status.

In this paper, strains 1A-C^T (= G5^T) and 3A-1 (= G6) were characterized and classified. On the basis of our results, it is suggested that strains 1A-C^T and 3A-1 should be placed in a new genus as a novel species, for which the name *Georgenia muralis* gen. nov., sp. nov. is proposed.

Abbreviations: BOX-PCR, BOX A element-derived PCR; ERIC-PCR, enterobacterial repetitive intergenic consensus PCR; REP-PCR, repetitive extragenic palindromic PCR.

The EMBL accession numbers for the 16S rDNA sequences of strains 1A-C^T and 3A-1 are respectively X94155 and AJ308598.

METHODS

Bacterial strains and culture conditions. Strains 1A-C^T and 3A-1 were isolated from a medieval wall painting in the church of St Georgen, Styria, Austria, as described previously (Altenburger *et al.*, 1996). Isolate 3A-0 was detected as a colony variant of strain 3A-1. For morphological, physiological and chemotaxonomic studies, strains were cultivated in liquid casein mineral medium (CasMM) and on CasMM agar plates unless indicated otherwise (Altenburger *et al.*, 1996).

Morphological, physiological and biochemical characterization. Cell morphology was examined over 14 days by phase-contrast microscopy (Leitz DMRB). Gram behaviour was investigated by Gram staining, KOH test and aminopeptidase test (Moaledj, 1986). Growth on PYES agar (0.3% casein from peptone, 0.3% yeast extract, 0.23% disodium succinate, 1.5% agar, pH 7.2) and R2A agar (Oxoid) was tested at room temperature. Anaerobic growth was studied in an atmosphere of 95% N₂ and 5% CO₂ in an aerostat WA 2600 (Heraeus Instruments) at 28 and 37 °C on CasMM agar. Growth at different temperatures (4, 28, 37, 44 and 48 °C) was tested on CasMM agar. Tolerance of NaCl was tested on CasMM agar at NaCl concentrations of 1, 3, 5, 7, 10 and 15% (w/v). pH tolerance was examined on CasMM buffered as described by Nielsen *et al.* (1995). Carbon source utilization tests, sugar fermentation and qualitative enzyme tests were carried out in microtitre plates according to Kämpfer *et al.* (1991).

Decomposition of cellulose was tested on CasMM with the modification that casein was replaced by either 0.5% α -cellulose (Sigma) or 0.2% cellulose azur (Sigma).

Hydrolysis of starch, Tween 80 and casein was determined on CasMM agar supplemented with 1% potato starch, 0.5% Tween 80 or 5% casein. Tests for oxidase, catalase, nitrate reduction, H₂S production, indole production and urease activity were performed as described by Smibert & Krieg (1994).

Chemotaxonomic characterization. Isoprenoid quinones were extracted by the method of Tindall (1990) and analysed by HPLC as described by Altenburger *et al.* (1996). Extraction and analysis of polar lipids by two-dimensional TLC were performed according to Ventosa *et al.* (1993). Fatty acids were prepared as described previously (Osterhout *et al.*, 1991). Fatty acid methyl esters were analysed by GC using a Hewlett Packard HP 6890 Series GC system with an HP-5 capillary (30 m $\times$ 0.32 mm; film thickness, 0.25 μ m). Cell-wall preparation and analysis of the peptidoglycan type were done according to Groth *et al.* (1997a). Extraction and detection of polyamines were done as described by Busse & Auling (1988) and Altenburger *et al.* (1997). Whole-cell protein patterns were examined as described by Altenburger *et al.* (1996).

DNA base composition and DNA–DNA hybridization. DNA isolation and analysis of the G+C content were done according to Auling *et al.* (1986) and Kaneko *et al.* (1986). DNA–DNA hybridization was performed as described previously (Huß *et al.*, 1983; Jahnke, 1992).

16S rDNA sequence analysis. Extraction of DNA, amplification of the 16S rRNA genes and sequence analyses were done as described by Wieser *et al.* (1999). DNA sequences were aligned with the sequences currently available from the EMBL database using the program FASTA (Pearson & Lipman, 1988). A total of 1321 unambiguously determined

nucleotides between positions 41 and 1362 (*Escherichia coli* numbering; Brosius *et al.*, 1978) was used for comparative analyses. Pairwise evolutionary distances were calculated by the method of Jukes & Cantor (1969). A phylogenetic dendrogram was constructed by the neighbour-joining method (Saitou & Nei, 1987) with the program NEIGHBOR. The stability of the groupings was estimated by bootstrap analysis (1000 replications) using the programs of the PHYLIP package (Felsenstein, 1993). All software was taken from the Wisconsin Package (GCG, 1995).

rep-PCR. ERIC-PCR (enterobacterial repetitive intergenic consensus PCR), REP-PCR (repetitive extragenic palindromic PCR) and BOX-PCR (BOX A element-derived PCR) were carried out according to Louws *et al.* (1994) with the modifications described by Wieser & Busse (2000). REP-PCR was modified in that the primer annealing temperature was lowered to 40 °C.

RESULTS AND DISCUSSION

Morphological, biochemical and physiological characteristics

Observation of cell morphology revealed that cells of strains 1A-C^T and 3A-1 exhibited a rod–coccus cycle. Cells of exponentially growing cultures were short, coccoid rods with a width of about 1 μ m and a length of about 2 μ m. Stationary-phase cells appeared as cocci (approx. 1 μ m in diameter), occurring singly or in small clusters. Endospores and motile cells were not observed. Colonies observed after 6 days growth on CasMM agar were circular, convex, yellow and transparent. Maximum colony size (2 mm in diameter) was reached after 2 weeks incubation at room temperature on CasMM agar. Cells of the two strains stained Gram-positive. They were aminopeptidase-negative and displayed Gram-positive behaviour in the KOH test. Both strains were weakly catalase- and oxidase-positive. Good growth was observed on media with a low nutrient content such as CasMM agar, R2A agar and PYES agar. Aerobically, cells grew well at room temperature and at 28 and 37 °C. Weak growth was observed at 4 °C and no growth was observed at 44 or 48 °C. Both strains grew well under anaerobic conditions at 28 and 37 °C. The two strains grew well between 0 and 5% (w/v) NaCl and moderately at 7% (w/v) NaCl. No growth was observed at concentrations of 10 or 15% (w/v) NaCl. The pH range for growth was pH 6–9. Biochemically, strains 1A-C^T and 3A-1 could only be distinguished from each other by the assimilation of D-mannose, only observed in 1A-C^T. No characters were found to distinguish colony variant 3A-0 from strain 3A-1. Detailed physiological and biochemical properties are given in the species description below.

16S rDNA sequence analyses

The almost complete 16S rDNA sequences of strains 1A-C^T and 3A-1 (1417 and 1429 nt, respectively) shared 99.6% similarity. Comparison with corresponding sequences of members of the class *Actinobacteria* demonstrated that strains 1A-C^T and 3A-1

Fig. 1. Phylogenetic tree based on 16S rRNA sequence comparisons indicating the phylogenetic position of strain 1A-C^T within the suborder *Micrococcineae*. *Nocardioidees plantarum* DSM 11054^T was included as an outgroup. Bar, 1 substitution per 100 nt.

Table 1. Patterns of 16S rRNA signature nucleotides that define strains 1A-C^T and 3A-1, *Beutenbergia cavernae* DSM 12333^T and the families *Bogoriellaceae* and *Cellulomonadaceae*

Positions are numbered according to the *E. coli* numbering. The following signature nucleotides were identical in all four taxa analysed: 41–401 (G–C), 45–396 (U–G), 99 (U), 142–221 (C–G), 248–276 (C–G), 258–268 (G–C), 379–384 (C–G), 502–543 (G–C), 586–755 (C–G), 591–648 (U–A), 610 (A), 602–636 (C–G), 615–625 (A–U), 616–624 (G–C), 630 (C), 660–745 (G–C), 670–736 (A–U), 1244–1293 (C–G), 1254–1283 (G–C) and 1310–1327 (G–C).

Position(s)	Strains 1A-C ^T and 3A-1	<i>B. cavernae</i> DSM 12333 ^T	<i>Bogoriellaceae</i>	<i>Cellulomonadaceae</i>
144–178	U–G	C–G	U–G	C–G
140–223	G–U	G–U	G–U	C–G
293–304	G–U	G–U	G–U	G–C
407–435	A–U	A–U	A–U	C–G
589–650	U–A	U–A	C–G	U–A
612–628	G–C	G–C	C–G	C–G
668–738	A–U	A–U	A–U	U–A
839–874	C–G	U–A	C–G	C–G
863	U	U	U	A
1133–1141	G–C	A–U	A–U	G–C
1134–1140	G–C	C–G	C–G	G–C
1263–1272	G–U	A–U	A–U	A–U
1414	C	U	C	U

clustered within the suborder *Micrococcineae*. Highest 16S rDNA sequence similarity values were obtained with members of the genus *Cellulomonas* (95.5–95.7%), *Beutenbergia cavernae* (95.5%) and *Bogoriella caseilytica* (94.7%); the data indicate that strains 1A-C^T and 3A-1 have a closer relationship to the genus *Cellulomonas*. Assessment of phylogenetic position placed 1A-C^T on the *Bogoriella caseilytica* lineage, close to the branching point with the *Cellulomonas* lineage (Fig. 1) within the radiation of representative taxa of the suborder *Micrococcineae* (Stackebrandt *et al.*, 1997). While the branching point between the *Bogoriella caseilytica* and *Cellulomonas* lineages received strong support from the calculated bootstrap value (935), the branching point of 1A-C^T did not appear to be very stable (538).

The presence of almost all of the signature nucleotides in the 16S rDNA sequences (positions 23–1461, *E. coli* numbering) of isolates 1A-C^T and 3A-1 confirmed their placement within the suborder *Micrococcineae* (Stackebrandt *et al.*, 1997) (Table 1). Only the A–A pair (positions 722–733) was replaced by a G–G pair in strains 1A-C^T and 3A-1. However, it has to be noted that *Beutenbergia cavernae* DSM 12333^T shares this characteristic signature nucleotide pair with strains 1A-C^T and 3A-1. Screening for 33 family-specific signatures (Stackebrandt *et al.*, 1997; Stackebrandt & Schumann, 2000) revealed highest agreement with the families *Bogoriellaceae* (84.8%) and *Cellulomonadaceae* (72.7%). Investigation of the corresponding signatures of *Beutenbergia cavernae* revealed an accordance of 81.8%. These values do not indicate

Fig. 2. Genomic fingerprints of isolates 1A-C^T (lanes 2, 6 and 10), 3A-0 (lanes 3, 7 and 11) and 3A-1 (lanes 4, 8 and 12) generated by ERIC- (lanes 2-4), REP- (lanes 6-8) and BOX- (lanes 10-12) PCR. Lanes 1, 5, 9 and 13; lambda DNA BstEII digest.

affiliation of the two strains to any of these families. The phylogenetic distance and the low 16S rDNA sequence similarities of 1A-C^T and 3A-1, as well as the low congruence in signature nucleotides with established taxa, indicate that the two strains are at least members of a new genus.

Genomic fingerprinting

Several studies (Van Belkum *et al.*, 1992; Woods *et al.*, 1992; Versalovic *et al.*, 1993; Wieser & Busse, 2000; Kainz *et al.*, 2000) have demonstrated that genomic fingerprint techniques are suitable for the investigation of relationships at the species level. In order to investigate the relationship between the two strains, they were subjected to REP-PCR employing ERIC-, REP- and BOX-PCR. Strains 1A-C^T and 3A-1 each displayed a distinct band pattern (Fig. 2), indicating their genotypic heterogeneity. Almost identical genomic fingerprints were obtained for strain 3A-1 and its colony variant 3A-0, thus demonstrating their clonal relatedness.

Chemotaxonomic characteristics

The peptidoglycan of strains 1A-C^T and 3A-1 contained Ala, Glu and Lys in a molar ratio of 2:2:1. Enantiomeric analysis of cell-wall diamino acids resulted in the detection of D-Ala, L-Ala, D-Glu, L-Glu and L-Lys. According to these data, it was concluded that strains 1A-C^T and 3A-1 contain peptidoglycan of type A4 α (Schleifer & Kandler, 1972), murein type A11.54 (DSMZ, 1998). So far, this peptidoglycan type has only been found in *Arthrobacter sulfureus* (Stacke-

Fig. 3. Two-dimensional TLC of polar lipid extracts from strain 1A-C^T stained with molybdotetraphosphoric acid. DPG, Diphosphatidylglycerol; GL, unknown glycolipid; PG, diphosphatidylglycerol; PIM, phosphatidylinositol mannoside; PL, unknown phospholipid; ypi, yellow pigment.

ebbrandt *et al.*, 1983b) and *Beutenbergia cavernae* (Groth *et al.*, 1999b). In contrast, members of the phylogenetically closely related genus *Cellulomonas* are described as having peptidoglycan type A4 β , with the characteristic diamino acid ornithine (Rainey *et al.*, 1995). Analysis of the quinone systems of strains 1A-C^T and 3A-1 revealed MK-8(H₄) as the major compound (82–94%) and, in contrast to previous studies (Altenburger *et al.*, 1996), MK-7(H₄) as the minor compound (3–11%). Within the suborder *Micrococcineae*, a quinone system with the predominant menaquinone MK-8(H₄) has been reported for several genera including *Bogoriella* and *Beutenbergia* (Stackebrandt *et al.*, 1983a; Groth *et al.*, 1997a, b, 1999a, b; Martin *et al.*, 1997; Prauser *et al.*, 1997; Schumann *et al.*, 1997; Maszenan *et al.*, 2000). In contrast, *Cellulomonas* has been reported to have a menaquinone system with MK-9(H₄) as the major compound (Stackebrandt & Prauser, 1992). However, *Beutenbergia cavernae* is the only species that shares the combination of a predominant MK-8(H₄) and the rare murein type A11.54 with strains 1A-C^T and 3A-1.

The polar lipid patterns of the two isolates were qualitatively identical. As shown for strain 1A-C^T (Fig. 3), they were characterized by the occurrence of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol mannoside. In addition, two unidentified phospholipids and one unidentified glycolipid were detected. Lack of phosphatidylinositol and the occurrence of phosphatidylglycerol or phosphatidylinositol mannoside clearly distinguish strains 1A-C^T and 3A-1 from *Beutenbergia cavernae* and members of the genus *Cellulomonas* (Groth *et al.*, 1999b; Stackebrandt & Prauser, 1992).

Table 2. Cellular fatty acid composition of strains 1A-C^T and 3A-1

Values are percentages of total fatty acids.

Fatty acid	1A-C ^T	3A-1
i-C14:1	0.6	2.3
i-C14:0	6.0	6.4
C14:0	0.5	2.9
i-C15:1	4.1	4.2
i-C15:0	4.9	2.8
ai-C15:0	66.6	62.1
i-C16:1	2.4	2.4
i-C16:0	1.4	—
C16:0	0.6	—
Unknown 1	2.0	—
Unknown 2	8.2	17.1
Unknown 3	2.7	—

Fatty acid profiles of 1A-C^T and 3A-1 were dominated by branched acids of the iso- and anteiso-type with the predominant acid ai-C15:0, along with significant amounts of i-C14:0 and i-C15:1 and an unknown compound (Table 2). Strains 1A-C^T and 3A-1 could be distinguished from *Beutenbergia cavernae* DSM 12333^T (Groth *et al.*, 1999b) by quantitative differences in i-C14:0, i-C15:0 and C16:0 fatty acids. The fatty acid profiles of 1A-C^T and 3A-1 also discriminate the two strains from members of the genus *Cellulomonas* (Funke *et al.*, 1995). In addition to the major compound ai-C15:0, species of the genus *Cellulomonas* are reported to contain high concentrations of the unbranched fatty acid C16:0, which was detected in only small amounts in strain 1A-C^T.

Analysis of the polyamines of strain 1A-C revealed a pattern with relatively low concentrations of polyamines. Its pattern consisted of the major compounds spermidine [0.26 µmol (g dry wt)⁻¹] and spermine

[0.18 µmol (g dry wt)⁻¹] and small amounts of cadaverine [0.02 µmol (g dry wt)⁻¹]. Similar polyamine patterns have already been reported for other *Actinobacteria* such as members of the genera *Corynebacterium* and *Agromyces* (Altenburger *et al.*, 1997). However, this feature clearly differentiates strains 1A-C^T and 3A-1 from other Gram-positive genera that contain MK-8(H₄) as the predominant menaquinone and which were shown to contain significantly higher concentrations of polyamines (5- to 10-fold), such as *Nocardioidea*, *Terrabacter*, *Terracoccus* and *Sanguibacter* (Busse & Schumann, 1999).

A quinone system consisting of menaquinone MK-8(H₄) and the occurrence of L-Lys in the peptidoglycan have also been reported for the genera *Demetria* and *Bogoriella*, but the polar lipid patterns and the fatty acid profiles of these genera clearly differ from those of strains 1A-C^T and 3A-1 (Groth *et al.*, 1997a, b; Table 3) confirming the distant relationships of the two isolates indicated by analysis of 16S rDNA sequences.

DNA base composition and DNA relatedness

The G+C content of strain 1A-C^T was 69.7 mol%. The DNA–DNA relatedness between strains 1A-C^T and 3A-1 was 92%.

Taxonomic considerations

Both the levels of 16S rDNA sequence similarity to members of the genera *Cellulomonas*, *Bogoriella* and *Beutenbergia* and differences in signature nucleotides (Table 1) prevent assignment of strains 1A-C^T and 3A-1 to any of these three genera. Although the two strains share some chemotaxonomic characteristics with related genera, they can be distinguished from members of *Cellulosimicrobium*, *Cellulomonas*, *Bogoriella* and *Beutenbergia* on the basis of peptidoglycan type, fatty acid composition, polar lipid profile and/or quinone system (Table 3).

Table 3. Differential characteristics of *Georgenia* gen. nov. and related taxa

Data were taken from this study and from Groth *et al.* (1999b) (*Beutenbergia*), Groth *et al.* (1997a) (*Bogoriella*), Groth *et al.* (1997b) (*Demetria*), Schumann *et al.* (2001) (*Cellulosimicrobium*) and Stackebrandt & Prauser (1992) and Funke *et al.* (1995) (*Cellulomonas*).

Characteristic	<i>Georgenia</i>	<i>Beutenbergia</i>	<i>Bogoriella</i>	<i>Demetria</i>	<i>Cellulosimicrobium</i>	<i>Cellulomonas</i>
Cell morphology	Rod-coccus cycle	Rod-coccus cycle	Irregular rods, coccoid	Coccoid, short rods	Primary mycelium, rod, coccoid	Branched rods
Major fatty acids	ai-C15:0, ai-C15:1, i-C14:0	i-C15:0, ai-C15:0	ai-C15:0	C18:1, C18:0, C17:0, ai-C17:0, C16:0	ai-C15:0, i-C16:0, C16:0, i-C15:0	ai-C15:0, C16:0
Major menaquinone	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-9(H ₄)	MK-9(H ₄)
Polar lipid composition*	DPG, PG, PIM, 2PL, GL	DPG, PI, 3PL	DPG, PG, PI, 1PL	DPG, PG, PI, PE, 2PL	Unknown	DPG, PG, PGL
Interpeptide bridge	L-Lys ← L-Glu	L-Lys ← L-Glu	L-Lys ← L-Ala ← L-Ala ← L-Glu	L-Lys ← L-Ser ← D-Asp	L-Lys ← L-Ser ← D-Asp	L-Orn ← D-Asp/L-Orn ← D-Glu
G+C content (mol%)	70	71	70	66	74	72–76

* DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unknown phospholipid (number of unknown phospholipids indicated); GL, unknown glycolipid.

Although isolates 1A-C^T and 3A-1 could be clearly distinguished from each other by their genomic fingerprints, they shared almost identical protein profiles obtained after separation by SDS-PAGE (results not shown). The degree of DNA relatedness clearly demonstrated that strains 1A-C^T and 3A-1 are members of a single species. The novel species *Georgenia muralis* gen. nov., sp. nov. is proposed, with strain 1A-C^T (= DSM 14418^T = CCM 4963^T) as the type strain. A second strain of the species is strain 3A-1 (= DSM 14419 = CCM 4964).

Description of *Georgenia* gen. nov.

Georgenia (Ge.or.gen'i.a. suff. -ia to denote a locality; N.L. fem. n. *Georgenia* referring to the village St Georgen in Styria, where strains 1A-C^T and 3A-1 were isolated).

Cells exhibit a rod-coccus cycle. Rods and cocci occur singly or in small clusters. Cocci are 1 µm in diameter, rods are 2 µm in length and 1 µm in width. Gram-positive, non-sporulating, non-motile. Growth occurs under both aerobic and anaerobic conditions. Oxidase and catalase-positive. Peptidoglycan type is A4α with L-Lys ← L-Glu as the interpeptide bridge (murein type A11.54). Menaquinone type is MK-8 (H₄). Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol mannoside, two unidentified phospholipids and one unknown glycolipid. The predominant fatty acid is ai-C15:0; significant amounts of ai-C15:1 and i-C14:0 are present. Polyamine content is low. Major polyamines are spermidine and spermine. Phylogenetically, this genus is a member of the suborder *Micrococcineae*, order *Actinomycetales*. The type species is *Georgenia muralis*.

Description of *Georgenia muralis* sp. nov.

Georgenia muralis (mu.ra'lis. L. adj. *muralis*, -le pertaining or belonging to walls).

Morphological, chemotaxonomic and general characteristics are as described for the genus. An unidentified compound is present in the fatty acid profile in significant amounts. Colonies reach a maximum diameter of 2 mm. They are yellow-pigmented, transparent, circular and convex. *N*-Acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-maltose, α-D-melibiose, sucrose, salicin, D-trehalose, D-xylose and D-mannitol are assimilated. L-Rhamnose, D-ribose, adonitol, *i*-inositol, maltitol, D-sorbitol, putrescine, acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, L-malate, mesaconate, oxoglutarate, pyruvate, suberate, L-alanine, β-alanine, L-aspartate, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate are not assimilated. Assimilation of D-mannose is variable. *p*-Nitrophenyl

(pNP) β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, bis-pNP phosphate, pNP phenylphosphonate, L-alanine *p*-nitroanilide (pNA) and L-proline pNA are hydrolysed. pNP Phosphorylcholine, 2-deoxythymidine-5'-pNP phosphate and L-glutamate γ-3-carboxy-pNA are not hydrolysed. Aesculin, cellulose azur and potato starch are hydrolysed. α-Cellulose, casein and Tween 80 are not decomposed. Nitrate is reduced to nitrite. Negative for H₂S production, indole production and urease activity. Good growth occurs at 28 and 37 °C, weak growth at 4 °C and no growth at 44 °C. Good growth occurs at NaCl concentrations up to 5% (w/v), with weak growth at 7% (w/v) NaCl. Growth is observed between pH 6 and 9. The genomic DNA G+C content of strain 1A-C^T is 70 mol%. Isolated from a medieval wall painting in the church of St Georgen in Styria, Austria.

The type strain is strain 1A-C^T (= DSM 14418^T = CCM 4963^T); a second strain of this species is 3A-1 (= DSM 14419 = CCM 4964).

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