Six novel *Arthrobacter* species isolated from deteriorated mural paintings

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A group of 21 bacterial strains was isolated from samples of biofilm formation in the Servilia tomb (necropolis of Carmona, Spain) and the Saint-Catherine chapel (castle at Herberstein, Austria). A polyphasic taxonomic study of these isolates, including morphological, biochemical and chemotaxonomic characterization, rep-PCR fingerprinting, 16S rRNA gene sequence analysis, DNA base ratio and DNA–DNA relatedness studies, allocated them to the genus *Arthrobacter*. The isolates represent six novel species, for which the names *Arthrobacter castelli* sp. nov., *Arthrobacter monumenti* sp. nov., *Arthrobacter parietis* sp. nov., *Arthrobacter pigmenti* sp. nov., *Arthrobacter tecti* sp. nov. and *Arthrobacter tumbae* sp. nov. are proposed. The respective type strains are LMG 22283^T (=DSM 16402^T), LMG 19502^T (=DSM 16405^T), LMG 22281^T (=DSM 16404^T), LMG 22284^T (=DSM 16403^T), LMG 22282^T (=DSM 16407^T) and LMG 19501^T (=DSM 16406^T).

It is now well recognized that wall paintings may be deteriorated by micro-organisms, as established by several studies that linked the observed damage with the microbial community present, as reviewed by Ciferri (1999). Despite this knowledge, a reliable characterization of bacteria associated with such damage is often lacking (Heyrman & Swings, 2001). To obtain greater insight into the bacterial community involved in biodeterioration of mural paintings, three sites (from the necropolis at Carmona, Spain, and the Saint-Catherine chapel of the castle at Herberstein, Austria) were sampled. In previous studies from these sites, 385 isolates were analysed by fatty acid methyl ester GC (Heyrman *et al.*, 1999) and representatives of different fatty acid clusters were further studied by 16S rRNA gene sequencing (Heyrman & Swings, 2001). Several of these representative strains showed the highest sequence similarity with members of the genus *Arthrobacter*. This paper presents the results of further investigation of 21 strains

belonging to the fatty acid clusters that were attributed to *Arthrobacter* based on the previous studies. A polyphasic taxonomic examination on these isolates resulted in the description of six novel species. At the time of writing, there are 44 recognized species of the genus *Arthrobacter* (<http://www.bacterio.cict.fr/bacterio/a/arthrobacter.html>). Of these established species, three have an uncertain position according to Keddie *et al.* (1986): *Arthrobacter duodecadis*, *Arthrobacter mysorens* and *Arthrobacter viscosus*.

The 21 strains studied originate from three samples taken at two different mural painting sites (Fig. 1). Two samples were taken at the Servilia tomb of the Roman necropolis at Carmona (Spain), one from the wall (C1) and one from the ceiling (C2) at the right side of the entrance to the tomb. The third sample (H) was a small piece of a rosy biofilm that almost completely covered the north wall of the chancel of the Saint-Catherine chapel in the castle of Herberstein (Austria). The strains were isolated as described by Heyrman *et al.* (1999). All isolates were further subcultured on nutrient agar (NA) at 28 °C.

Total genomic DNA was purified for 16S rRNA gene sequencing and rep-PCR using a slight modification of the method of Pitcher *et al.* (1989), as described by Heyndrickx *et al.* (1996). rep-PCR genomic fingerprinting was performed with the REP- and (GTG)₅ primers (Versalovic *et al.*, 1994) using the PCR conditions described previously by Rademaker & de Bruijn (1997). Electrophoresis and pattern analysis were further performed as described by Heyrman

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *A. castelli* LMG 22283^T, *A. pigmenti* LMG 22284^T, *A. tecti* LMG 22285, *A. tecti* LMG 22282^T, *A. parietis* LMG 22281^T, *A. mysorens* LMG 16219^T, *A. viscosus* LMG 16473^T, *A. tumbae* LMG 19501^T and *A. monumenti* LMG 19502^T are AJ639826–AJ639832, AJ315069 and AJ315070, respectively.

A figure showing phylogenetic positions of representative novel isolates and *Arthrobacter* species based on neighbour-joining of 16S rRNA gene sequences and details of DNA–DNA relatedness values and fatty acid profiles of the novel taxa are available as supplementary material in IJSEM Online.

Fig. 1. Grouping of combined normalized (GTG)₅ and REP-PCR patterns of 21 *Arthrobacter* isolates from damaged murals in a dendrogram based on UPGMA clustering of Pearson's correlation similarity coefficients. The origin of the strains is denoted C for Carmona (samples 1 and 2) and H for Herberstein.

et al. (2003). 16S rRNA gene sequencing and phylogenetic analysis were performed as described by Heyrman & Swings (2001). Phylogenetic trees were constructed using the BioNumerics software (Applied Maths) by applying the neighbour-joining and maximum-parsimony methods on a multiple alignment similarity matrix. The stability of relationships was assessed by a bootstrap analysis of 1000 datasets. For determination of the G + C content and levels of DNA–DNA hybridization, approximately 1 g biomass was harvested from agar plates. DNA was purified by using a combination of the protocols of Marmur (1961) and Pitcher *et al.* (1989), as described by Logan *et al.* (2000). The G + C content of the DNA was determined by HPLC (Mesbah *et al.*, 1989) using the further specifications given by Logan *et al.* (2000). DNA–DNA hybridization experiments were performed using a modification of the microplate method of Ezaki *et al.* (1989), as described by Willems *et al.* (2001). A hybridization temperature of 40 °C was used.

REP- and (GTG)₅-PCR were used to determine the genotypic diversity of the isolates and to select strains for further analyses. Based on the combined REP and (GTG)₅ electrophoretic patterns, a dendrogram using Pearson's correlation was constructed (Fig. 1). At more than 75 % similarity, all strains except one (LMG 22283^T) could be organized into five groups. The clustering corresponds well with the origin of the strains, with groups 1–3 originating from Carmona sample 1, group 4 from sample 2, and group

5 and ungrouped strain LMG 22283^T originating from Herberstein.

Based on the rep-clustering, one representative of each group and the ungrouped strain LMG 22283^T were analysed for their nearly complete 16S rRNA gene sequence. For the larger groups (more than two strains), the partial (first ± 400 bp) or nearly complete 16S rRNA gene sequence of one additional strain was analysed. Strain pairs LMG 19502^T (1484 bp) and R-5514 (381 bp) of rep-group 1 and LMG 19501^T (1475 bp) and R-5363 (428 bp) of rep-group 2 showed 100 % partial sequence similarity. In rep-group 4, LMG 22282^T (1476 bp) and LMG 22285 (1480 bp) shared 99.9 % 16S rRNA gene sequence similarity. These results confirmed the accuracy of the rep-clustering and the delineation of the groups. In Fig. 2, the nearly complete 16S rRNA gene sequences of representatives of each rep-group and of strain LMG 22283^T are clustered together with those of closely related *Arthrobacter* species. Supplementary Fig. S1 (available in IJSEM Online) shows the phylogenetic position of the novel species within the genus *Arthrobacter*. Because no 16S rRNA gene sequence was published for *A. viscosus*, the type strain of this species was also sequenced. In a FASTA search (Pearson & Lipman, 1988), the 16S rRNA gene sequence of LMG 19502^T (rep-group 1) was most closely related to *Arthrobacter luteolus* (96.8 % similarity) and *Arthrobacter citreus* (96.3 %). Strains LMG 22281^T (rep-group 2) and LMG 19501^T (rep-group 3) both

Fig. 2. Phylogenetic positions based on neighbour-joining of the 16S rRNA gene sequences of representative mural painting isolates among related members of the genus *Arthrobacter*. Bootstrap values (expressed as percentages of 1000 replications) greater than 60% are shown at branch points.

showed highest similarity to *Arthrobacter ramosus* and *Arthrobacter pascens* (both 97.1% similarity), and LMG 22282^T (rep-group 4) was most closely related to *Arthrobacter globiformis* (97.3%) and *A. pascens* (97.1%). Ungrouped strain LMG 22283^T and one representative of rep-group 5, strain LMG 22284^T, were most similar to *Arthrobacter cummingsii* (95.6 and 95.4%, respectively) and *Arthrobacter albus* (95.4 and 95.2%, respectively). LMG 22281^T, LMG 19501^T and LMG 22282^T grouped closest to *Arthrobacter agilis* (pairwise similarity of 97.7–98.0%), although this species was not mentioned in the list of the 30 most closely related sequences obtained by using a FASTA search. The pairwise similarity between the sequenced mural painting isolates is above 97% for LMG 22281^T, LMG 19501^T and LMG 22282^T (between 98.7 and 99.5%) and for LMG 22283^T and LMG 22284^T (98.1%).

Two of the three species with uncertain position (Keddie *et al.*, 1986) have indeed been incorrectly assigned to the genus *Arthrobacter*. For *Arthrobacter duodecadis*, a 16S rRNA gene sequence is available (AB072496) that places it phylogenetically in the neighbourhood of the genus *Tetrasphaera*. From the sequence data it can be hypothesized that *A. duodecadis* is a possible novel species within *Tetrasphaera* (16S rRNA gene sequence similarity value below 97% to all recognized species of *Tetrasphaera*). However, conflicting data have been reported for *A. duodecadis* (e.g. on the cell wall peptidoglycan; Keddie *et al.*, 1986) in the literature and these data should be re-evaluated before the reclassification can be taken into consideration. *A. viscosus*, for which no 16S rRNA gene sequence was previously available, is most closely related to members of the genus *Rhizobium*, e.g. *Rhizobium gallium* (98.1% 16S rRNA gene sequence similarity), *Rhizobium mongolense* (98.0%) and *Rhizobium leguminosarum*

(97.6%). This is not unexpected, as Keddie *et al.* (1986) stated that the chemotaxonomic data for this species were in accordance with the view that it is indeed a Gram-negative rod. *A. mysorens* groups closely with *Arthrobacter nicotianae* (approx. 99.1% 16S rRNA gene sequence similarity). This supports the data of Stackebrandt *et al.* (1983), who considered *A. mysorens* as forming a distinct species within the *A. nicotianae* group. They reported a DNA–DNA relatedness level between *A. mysorens* ATCC 31021 and *A. nicotianae* DSM 20123^T of 40%. As no DNA–DNA relatedness study and chemotaxonomic analyses were performed with the type strain, it remains unclear whether *A. mysorens* deserves separate species status.

Generally recommended and accepted criteria for delineating bacterial species state that strains with DNA–DNA relatedness below 70% as measured by hybridization or with 16S rRNA gene sequence dissimilarity above 3% are considered as belonging to separate species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002). For LMG 19502^T, representative of rep-group 1, 16S rRNA gene sequence similarity with all recognized *Arthrobacter* species was below 97%. Consequently, the members of rep-group 1 can be attributed to a novel genospecies and this group will be further denoted as *Arthrobacter monumenti* sp. nov. For the remaining strains, two DNA–DNA relatedness experiments were performed. The first experiment included LMG 22281^T (rep-group 2), LMG 19501^T (rep-group 3), LMG 22282^T (rep-group 4) and the type strains of *A. agilis*, *A. globiformis*, *A. pascens* and *A. ramosus* (Supplementary Table S1 available in IJSEM Online). Results showed that the three rep-groups all represent novel genospecies. Rep-group 2 will be further denoted as *Arthrobacter parietis* sp. nov., group 3 as *Arthrobacter tumbae* sp. nov. and group 4 as *Arthrobacter tecti*

sp. nov. The second DNA–DNA relatedness experiment included ungrouped strain LMG 22283^T and one representative of rep-group 5, strain LMG 22284^T. The level of DNA–DNA relatedness between these strains was 26 %, which suggests that they belong to different genospecies. LMG 22283^T will be further denoted as *Arthrobacter castelli* sp. nov. and rep-group 5 as *Arthrobacter pigmenti* sp. nov.

The G+C content of the DNA of the type strains of the novel species (Table 1) falls within the range for the genus *Arthrobacter* (59–70 mol%) reported by Keddle *et al.* (1986).

Analysis of the fatty acid contents of the cells was performed as described by Heyrman *et al.* (1999), starting from cells grown on trypticase soy agar (TSA) or marine agar (MA). Purified cell wall preparations were obtained by the method of Schleifer & Kandler (1972). The amino acids and peptides in peptidoglycan hydrolysates were analysed as described by Groth *et al.* (1999). Menaquinones were extracted as described by Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997). Polar lipids extracted by the method of Minnikin *et al.* (1979) were identified by two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Sugar analysis of purified

Table 1. Differential characteristics of the six novel *Arthrobacter* species isolated from mural paintings and related *Arthrobacter* species

Species: 1, *A. monumenti* sp. nov. (four strains); 2, *A. parietis* sp. nov. (two strains); 3, *A. tumbae* sp. nov. (five strains); 4, *A. tecti* sp. nov. (seven strains); 5, *A. castelli* sp. nov. (one strain); 6, *A. pigmenti* sp. nov. (two strains); 7, *A. agilis*; 8, *A. flavus*. +, All strains positive; (+), ≥80 % positive (6/7 or 4/5 strains); v, variable; (–), ≤20 % positive; –, all strains negative; w, all positive reactions are weak; NG, not given. Data for *A. agilis* and *A. flavus* are from Koch *et al.* (1995) and Reddy *et al.* (2000).

Characteristic	1*	2	3	4	5*	6*	7	8
Colony colour	Light yellow	Yellow–orange	Yellow–orange	Yellow	Light yellow	Light yellow	Rose-red‡	Yellow‡
Alkaliphilic	–	–	+	–	–	+	NG	–
Growth at 5 % NaCl	+	+	+	+	+	+	–	+
Oxidase	–	–	–	–	–	–	+	–
Nitrate reduction	+	+	v	–	–	v	–	–
β-Glucuronidase	+	–	+	–	+	+	NG	NG
N-Acetyl-β-glucosaminidase	–	–	–	+	–	–	NG	NG
Urease	v	v	v	–	+	–	–	–
Gelatin hydrolysis	+	+	(+)	(+)	–	+	+	+
Alkaline phosphatase	+	–	(–)w	+w	+	+	NG	NG
Esterase lipase (C ₈)	(+)	–	(+)w	(+)	+	+w	NG	NG
Lipase (C ₁₄)	–	–	–	–	+w	–	–§	+§
α-Galactosidase	–	+	(–)	–	–	–	NG	NG
Peptidoglycan type†	Lys–Ala ₄	Lys–Thr–Ala ₂	Lys–Thr–Ala ₃	Lys–Thr–Ala ₃	Lys–Ala–Ser–Ala ₃	Lys–Ala ₄	Lys–Thr–Ala ₃	Lys–Thr–Ala ₃
Cell wall sugars†	Gal (Xyl, Rha)	Gal	Gal	Gal (Man)	Gal (Xyl, Rha)	Gal, Rha	GlcN	Gal, Glc, Rib
Percentage of menaquinones†								
MK-7(H ₂)	4	–	6	–	–	14		
MK-8(H ₂)	4	2	4	–	–	–	Minor	
MK-9(H ₂)	87	69	54	41	75	66	Major	Major
MK-10(H ₂)	4	20	25	28	6	13		
MK-11(H ₂)	–	4	7	11	–	–		
MK-12(H ₂)	–	–	–	2	–	–		
MK-9	–	–	–	7	11	3		
MK-10	–	–	–	4	–	–		
MK-11	–	–	–	1	–	–		
G+C content (mol%)†	62.2	63.8	64.7	63.7	68.1	61.6	67–70	62–66

**A. monumenti*, *A. castelli* and *A. pigmenti* differ from the phylogenetically closest related species listed in Fig. 2 based on their peptidoglycan type and cell wall sugars (the latter trait has not been determined for all species).

†Data for the novel species were determined for the type strain only. Abbreviations of cell wall sugars: Gal, galactose; Glc, glucose; GlcN, glucosamine; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose. Minor components are given in parentheses. For *A. agilis* and *A. flavus* the menaquinone content given in the literature is only presented as major or minor amounts.

‡Colony colour was not tested on NA.

§Data were not recorded using the API ZYM system.

cell walls was performed with the chemical method as described by Stanek & Roberts (1974). Cell morphology and Gram staining were examined by phase-contrast and normal light microscopy, respectively. Catalase and oxidase tests were performed according to the methods described by Smibert & Krieg (1994). Optimal growth temperature was tested on NA (incubation at 4, 10, 15, 20, 28, 37, 45 and 50 °C); growth at various pH and salt range was tested in nutrient broth (NB) (with 0, 5, 10 and 15 % salt added and pH adjusted to 4.0–10.0 at 0.5 intervals). Anaerobic growth was recorded in an anaerobic chamber on NA. API ZYM and API CORYNE strips (bioMérieux) were used according to the manufacturer's instructions.

The novel species described here have a type A3 α peptidoglycan, have dihydrogenated menaquinones with nine isoprene units [MK-9(H₂)] as the major menaquinone, and contain the polar lipids diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. They share these features with most of the recognized *Arthrobacter* species (Keddie *et al.*, 1986). The novel *Arthrobacter* species can be distinguished on the basis of peptidoglycan type, relative amounts of menaquinones and cell wall sugars (Table 1).

The fatty acid compositions of the novel species (Supplementary Table S2 available in IJSEM Online) are in good agreement with the genus description (Keddie *et al.*, 1986): they contain major amounts of iso- and anteiso-methyl-branched acids (iso-C_{15:0} and anteiso-C_{15:0}) together with small amounts of straight-chain saturated acids (C_{16:0}); monounsaturated acids are absent or present only in trace amounts.

Results of the physiological characterization are given in the species descriptions below. Characters that distinguish the novel species are listed in Table 1. The phenotypic analyses seem to support the division suggested by the genomic data. Together, these data support the delineation of six novel *Arthrobacter* species.

Description of *Arthrobacter castelli* sp. nov.

Arthrobacter castelli (cas.tel'li. L. gen. n. *castelli* of the castle).

Cells are Gram-positive, short rods and cocci (diameter 0.8–1 μ m) occurring in pairs and chains. They are non-motile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), light yellow, round with entire margins, of low convexity, opaque and smooth. No growth in an anaerobic chamber on NA. Optimal temperature for growth is 22–37 °C. Weak growth at 15 °C and no growth at 10 or 45 °C. Poor growth on media without NaCl. Catalase-positive and oxidase-negative. By using the API CORYNE system, positive reactions are observed for pyrazinamidase, alkaline phosphatase, β -glucuronidase, β -galactosidase, α -glucosidase and urease. Negative reactions are obtained for nitrate reduction, pyrrolidonyl arylamidase, *N*-acetyl- β -glucosaminidase, aesculin (β -glucosidase), gelatinase and fermentation of glucose, ribose, xylose, mannitol, maltose,

lactose, sucrose and glycogen. By using the API ZYM system, activity is detected for alkaline phosphatase, acid phosphatase (weak), esterase C₄ (weak), esterase lipase C₈, lipase C₁₄ (weak), leucine arylamidase, trypsin (weak), phosphoamidase (weak) and α -glucosidase. No activity is detected for valine arylamidase, cystine arylamidase, chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase. Predominant fatty acids are anteiso-C_{15:0} and iso-C_{15:0} (approximately 51 and 23 %, respectively). Peptidoglycan type, menaquinone content and cell wall sugars are given in Table 1. Polar lipids are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid and one unknown glycolipid.

The G+C content of the DNA, determined for the type strain LMG 22283^T (=DSM 16402^T), is 68.1 mol%. Isolated from a rosy biofilm overgrowing a mural painting in the Saint-Catherine chapel (castle of Herberstein, Austria).

Description of *Arthrobacter monumenti* sp. nov.

Arthrobacter monumenti (mo.nu.men'ti. L. gen. n. *monumenti* of the monument).

Cells are Gram-positive, short rods and cocci (diameter 0.8–1 μ m) occurring in pairs or clusters. They are non-motile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), light yellow, round with entire margins, of low convexity, opaque and smooth. No growth in an anaerobic chamber on NA. Optimal temperature for growth is 22–30 °C. Weak growth at 4 and 37 °C, and no growth at 45 °C. Growth on medium with 15 % NaCl. Optimum pH for growth is 7–8. Catalase-positive and oxidase-negative. Using the API CORYNE system, positive reactions are observed for nitrate reduction, pyrazinamidase, β -glucuronidase, β -galactosidase, α -glucosidase, aesculin (β -glucosidase) and gelatinase. Negative reactions are obtained for *N*-acetyl- β -glucosaminidase and fermentation of ribose, xylose, mannitol and glycogen. Variable reactions are obtained for pyrrolidonyl arylamidase, urease and fermentation of glucose, lactose, maltose and sucrose. Using the API ZYM system, activity is detected for alkaline phosphatase, esterase C₄, esterase lipase C₈, leucine arylamidase, trypsin, acid phosphatase (weak) and phosphoamidase (weak). No activity is detected for lipase C₁₄, valine arylamidase, cystine arylamidase, chymotrypsin, α -galactosidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase or α -fucosidase. Variable reactions are obtained for β -galactosidase, α -glucosidase and α -mannosidase. Predominant fatty acids are anteiso-C_{15:0} and iso-C_{15:0} (approximately 57 and 25 %, respectively). Peptidoglycan type, menaquinone content and cell wall sugars are given in Table 1. Polar lipids of the type strain are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid and one unknown glycolipid.

The G+C content of the DNA, determined for the type

strain LMG 19502^T (= DSM 16405^T), is 62.2 mol%. In the variable characters listed above for the API CORYNE tests, the type strain is positive for pyrrolidonyl arylamidase but negative for urease and fermentation of glucose, lactose, maltose and sucrose. In API ZYM the type strain is positive for β -galactosidase and α -glucosidase (weak) but negative for α -mannosidase. Isolated from a biofilm overgrowing a mural painting in the Servilia tomb (Roman necropolis of Carmona, Spain).

Description of *Arthrobacter parietis* sp. nov.

Arthrobacter parietis (pa.ri.et'is. L. gen. n. *parietis* of a wall).

Cells are Gram-positive, short rods and cocci (diameter 0.8–1 μ m) occurring in pairs or clusters. They are non-motile and do not form endospores. Colonies on NA after 48 h are 1–2 mm in diameter, yellow–orange, round with entire margins, of low convexity, opaque and smooth. No growth in an anaerobic chamber on NA. Optimum temperature for growth is 22–30 °C. No or only weak growth at 37 °C and no growth at 45 °C. Good growth after 1 week at 4 °C. Growth on medium with 15 % NaCl. Growth occurs at pH 6–9, with an optimum of 7–8. Catalase-positive and oxidase-negative. Using the API CORYNE system, positive reactions are observed for nitrate reduction, pyrazinamidase, β -galactosidase, α -glucosidase, aesculin (β -glucosidase), gelatinase and fermentation of glucose. Negative reactions are obtained for alkaline phosphatase, β -glucuronidase, *N*-acetyl- β -glucosaminidase and fermentation of ribose, xylose, mannitol and glycogen. Variable reactions are obtained for pyrrolidonyl arylamidase, urease, and fermentation of maltose, lactose and sucrose. Using the API ZYM system, activity is detected for acid phosphatase (weak), esterase C₄, leucine arylamidase, trypsin, phosphoamidase (weak), α -galactosidase, β -galactosidase and α -glucosidase. No activity is detected for alkaline phosphatase, esterase lipase C₈, lipase C₁₄, valine arylamidase, chymotrypsin, β -glucuronidase, *N*-acetyl- β -glucosaminidase and α -fucosidase. Variable results (if positive, weak) were obtained for cystine arylamidase, β -glucosidase and α -mannosidase. Predominant fatty acids are anteiso-C_{15:0} and iso-C_{15:0} (approximately 51 and 29 %, respectively). Peptidoglycan type, menaquinone content and cell wall sugars are given in Table 1. Polar lipids of the type strain are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid and two unknown glycolipids.

The G+C content of the DNA, determined for the type strain LMG 22281^T (= DSM 16404^T), is 63.8 mol%. The type strain is negative for the variable characters listed above. Isolated from a biofilm overgrowing a mural painting in the Servilia tomb (Roman necropolis of Carmona, Spain).

Description of *Arthrobacter pigmenti* sp. nov.

Arthrobacter pigmenti (pig.men'ti. L. gen. n. *pigmenti* of pigment or paint).

Cells are Gram-positive, short rods and cocci (diameter 0.8–1 μ m) occurring in pairs, chains or clusters. They are non-motile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), light yellow, round with entire margins, of low convexity, opaque and smooth. No growth in an anaerobic chamber on NA. Optimum temperature for growth is 22–30 °C. No or only weak growth at 37 or 10 °C. No growth at 45 or 4 °C. Growth on medium with 15 % NaCl; optimal growth at 10 % NaCl. Growth at pH 7–10; optimal at pH 8–9. Catalase-positive and oxidase-negative. Using the API CORYNE system, positive reactions are observed for pyrazinamidase, β -glucuronidase, β -galactosidase, α -glucosidase and gelatinase. Negative reactions are obtained for pyrrolidonyl arylamidase, *N*-acetyl- β -glucosaminidase, urease, and fermentation of ribose, xylose, mannitol, lactose and glycogen. Variable reactions are obtained for nitrate reduction, aesculin (β -glucosidase) and fermentation of glucose, maltose and sucrose. Using the API ZYM system, activity is detected for alkaline phosphatase, esterase lipase C₈ (weak), leucine arylamidase, trypsin, phosphoamidase (weak), α -galactosidase (weak) and β -glucuronidase. No activity is detected for lipase C₁₄, valine arylamidase, cystine arylamidase, chymotrypsin, α -galactosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase or α -fucosidase. Variable results (if positive, weak) were obtained for esterase C₄, acid phosphatase and α -mannosidase. Predominant fatty acids are iso-C_{15:0} and anteiso-C_{15:0} (approximately 48 and 40 %, respectively). Peptidoglycan type, menaquinone content and cell wall sugars are given in Table 1. Polar lipids of the type strain are phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol, one unknown phospholipid and one unknown glycolipid.

The G+C content of the DNA, determined for the type strain LMG 22284^T (= DSM 16403^T), is 61.6 mol%. In the variable characters listed above, the type strain is positive. Isolated from a rosy biofilm overgrowing a mural painting in the Saint-Catherine chapel (castle of Herberstein, Austria).

Description of *Arthrobacter tecti* sp. nov.

Arthrobacter tecti (tec'ti. L. gen. n. *tekti* from the ceiling).

Cells are Gram-positive, short rods and cocci (diameter 0.8–1 μ m) occurring in pairs or clusters. They are non-motile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), yellow, round with entire margins, of low convexity, opaque and smooth. No growth in an anaerobic chamber on NA. Optimum temperature for growth is 22–30 °C. Growth on medium with 15 % NaCl added. Growth at pH 6–9; optimal growth at pH 8. Catalase-positive and oxidase-negative. Using the API CORYNE system, a positive reaction is observed for *N*-acetyl- β -glucosaminidase. Most strains, including the type strain, test positive for gelatinase but negative for pyrrolidonyl arylamidase and fermentation of ribose and mannitol. Negative reactions are obtained for nitrate reduction,

β -glucuronidase, urease, and fermentation of ribose, xylose, maltose, lactose and glycogen. Variable reactions are obtained for pyrazinamidase, alkaline phosphatase, β -galactosidase, α -glucosidase, aesculin (β -glucosidase), and fermentation of glucose and sucrose. Using the API ZYM system, activity is detected for alkaline phosphatase (weak), acid phosphatase (weak), leucine arylamidase and phosphoamidase (weak). Most strains test positive for esterase C_4 , esterase lipase C_8 , trypsin (negative for the type strain), *N*-acetyl- β -glucosaminidase (negative for the type strain) and α -mannosidase (negative for the type strain). No activity is detected for lipase C_{14} , valine arylamidase, cystine arylamidase, α -galactosidase, β -glucuronidase or α -fucosidase. Variable reactions are obtained for chymotrypsin, β -galactosidase, α -glucosidase and β -glucosidase. Predominant fatty acids are anteiso- $C_{15:0}$ and iso- $C_{15:0}$ (approximately 44 and 37 %, respectively). Peptidoglycan type, menaquinone content and cell wall sugars are given in Table 1. Polar lipids of the type strain are phosphatidyl-inositol, phosphatidylglycerol, diphosphatidylglycerol and one unknown phospholipid.

The G + C content of the DNA, determined for the type strain LMG 22282^T (=DSM 16407^T), is 63.7 mol%. The type strain is negative for all variable reactions in the API CORYNE system listed above. The type strain is positive for chymotrypsin and α -glucosidase, and negative for β -galactosidase and β -glucosidase in the API ZYM system. Isolated from a biofilm overgrowing the ceiling of the main room of the Servilia tomb (Roman necropolis of Carmona, Spain).

Description of *Arthrobacter tumbae* sp. nov.

Arthrobacter tumbae (tum'bae. L. gen. n. *tumbae* of the tomb).

Cells are Gram-positive, short rods and cocci (diameter 0.8–1 μ m) occurring singly, in pairs or in clusters. They are non-motile and do not form endospores. Colonies on NA after 48 h are small (<1 mm), yellow–orange, round with entire margins, of low convexity, opaque and smooth. No growth in an anaerobic chamber on NA. Optimum temperature for growth is 22–30 °C. Weak growth at 37 °C, and no growth at 52 °C. Growth at 4 °C after 1 week of incubation. Growth on medium with 10 % NaCl, but not with 15 % NaCl. Catalase-positive and oxidase-negative. Alkaliphilic; pH range for growth of 7–10 with an optimum of 8–9. Using the API CORYNE system, positive reactions are observed for pyrazinamidase, β -glucuronidase, β -galactosidase and α -glucosidase. Most strains, including the type strain, test positive for gelatinase. Negative reactions are obtained for *N*-acetyl- β -glucosaminidase and fermentation of ribose, xylose, mannitol and glycogen. Variable reactions are obtained for nitrate reduction, pyrrolidonyl arylamidase, alkaline phosphatase, aesculin (β -glucosidase), urease, and fermentation of glucose, maltose, lactose and sucrose. Using the API ZYM system, activity is detected for esterase C_4 , leucine arylamidase

and phosphoamidase (weak). Most strains, including the type strain, test positive for esterase lipase C_8 and negative for alkaline phosphatase, valine arylamidase, α -galactosidase and β -galactosidase. No activity is detected for lipase C_{14} , cystine arylamidase, β -glucuronidase, β -glucosidase, *N*-acetyl- β -glucosaminidase or α -fucosidase. Variable reactions are obtained for trypsin, chymotrypsin, acid phosphatase, α -glucosidase and α -mannosidase. Predominant fatty acids are anteiso- $C_{15:0}$ and iso- $C_{15:0}$ (approximately 57 and 17 %, respectively). Peptidoglycan type, menaquinone content and cell wall sugars are given in Table 1. Polar lipids of the type strain are phosphatidyl-inositol, phosphatidylglycerol, diphosphatidylglycerol and one unknown phospholipid.

The G + C content of the DNA, determined for the type strain LMG 19501^T (=DSM 16406^T), is 64.7 mol%. The type strain is negative for all variable characters listed above, except for trypsin in the API ZYM system. Isolated from a biofilm overgrowing a mural painting in the Servilia tomb (Roman necropolis of Carmona, Spain).

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