Arthrobacter gandavensis sp. nov., for strains of veterinary origin

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Three strains of a previously undescribed, Gram-positive, coryneform bacterium, which were isolated from cattle, were subjected to polyphasic taxonomic analysis. Comparative 16S rRNA gene sequencing revealed that the unknown isolates were members of the genus *Arthrobacter* and were phylogenetically closely related to *Arthrobacter luteolus*. However, DNA–DNA hybridization indicated that the strains belonged to a new sub-lineage within the genus *Arthrobacter*. The unknown isolates can be distinguished from related species by biochemical tests. It is proposed that the *Arthrobacter*-like bacteria of veterinary origin should be classified in the genus *Arthrobacter* as *Arthrobacter gandavensis* sp. nov., with the type strain LMG 21285^T (=DSM 15046^T).

Conn (1928) described a group of bacteria that appeared as Gram-negative rods in young cultures and as Gram-positive cocci in older cultures. '*Bacterium globiforme*' was proposed for these bacteria; this species, as *Arthrobacter globiformis*, was later to become the type species of the genus *Arthrobacter* (Skerman *et al.*, 1980; Jones & Keddie, 1992). Members of the genus *Arthrobacter* are distributed widely in the environment, especially in soil, and until recently had not been isolated from clinical sources. Funke *et al.* (1996) first reported on *Arthrobacter* strains isolated from clinical specimens, leading to the description of *Arthrobacter cumminsii* and *Arthrobacter woluwensis*. In 1998, *Arthrobacter creatinolyticus* (Hou *et al.*, 1998) was described for strains isolated from human urine. Wauters *et al.* (2000) found that strains of *Arthrobacter oxydans* were present in human blood cultures. Their study also led to the description of two novel species of human origin, *Arthrobacter luteolus* and *Arthrobacter albus*. *Arthrobacter rhombi* (Osorio *et al.*, 1999), isolated from fish, and *Arthrobacter nasiphocae* (Collins *et al.*, 2002), isolated from seals, are thus far the only described *Arthrobacter* species that originated from animals.

In this article, we report the characteristics of an unknown *Arthrobacter* species that was isolated from cattle. Strains LMG 21285^T and LMG 21287 were isolated from mastitic milk of dairy cows and strain LMG 21286 was recovered from the uterus of a cow. The three strains were isolated from different animals from separate farms. Based on phenotypic and phylogenetic evidence, *Arthrobacter gandavensis* sp. nov., is proposed.

Strains were cultured on Columbia agar (BBL; Becton Dickinson) supplemented with 5 % defibrillated sheep blood at 37 °C in air. Growth in brain heart infusion (BHI) broth and in BHI broth with 6·5 % NaCl was tested; cultures were incubated at 37 °C in air. Enzymic and carbohydrate acidification tests were carried out with API Coryne and API 50 CH kits (bioMérieux) and BBL Crystal Gram-positive ID kits (Becton Dickinson).

Amino acid composition of the peptidoglycan and the major menaquinones for strain LMG 21285^T were studied by P. Schumann at DSMZ, following the methods described by Groth *et al.* (1996).

Cellular fatty acid methyl esters were prepared, separated and identified by using the Microbial Identification system (Microbial ID) as described by Vandamme *et al.* (1992).

16S rRNA genes were amplified by PCR and sequenced directly by using a BigDye Terminator Cycle Sequencing Ready Reaction kit and an automatic ABI Prism 310 Genetic Analyser (both from Applied Biosystems). The closest relatives of the new isolates were determined by performing database searches. A phylogenetic tree was constructed with the BioNumerics software package (Applied Maths), based

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains LMG 21285^T, LMG 21286 and LMG 21287 are AJ316140, AJ491107 and AJ491108, respectively.

on the neighbour-joining method (Saitou & Nei, 1987), and the stability of groupings was estimated by bootstrap analysis.

For determination of DNA base composition, DNA was degraded enzymically into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture was then separated by HPLC, using a Waters SymmetryShield C8 column that was thermostatted at 37 °C. The solvent was 0·02 M $NH_4H_2PO_4$ (pH 4·0) with 1·5 % acetonitrile. Non-methylated λ-phage DNA (Sigma) was used as the calibration reference. DNA–DNA hybridization was performed with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989) and Goris *et al.* (1998), using an HTS 7000 BioAssay Reader (Applied Biosystems) for fluorescence measurements. Hybridization temperature was 45 °C (calculated as optimal renaturation temperature in 2 × SSC and 50 % formamide). DNA was prepared by using a modification of the method of Pitcher *et al.* (1989).

The three isolates were relatively small (0·5–1 μm) coccobacilli, often with one pointed end, which resembled cells of members of the genus *Arcanobacterium*. They grew equally well at 25, 30 and 37 °C, but grew less well at 42 °C. No growth was seen when cultured anaerobically and growth was not enhanced by 5 % CO_2. The isolates produced yellow-pigmented, smooth, glistening, circular colonies up to 1 mm in diameter. They were motile, sedimented partially in broth and did not grow in broth that contained 6·5 % NaCl. Strains were catalase-positive and non-fermentative, but produced acid weakly from fructose and ribose when grown aerobically. Strain LMG 21287 also showed a weak reaction with ribose and aesculin. Strain LMG 21285^T was D-glucose-negative and was weakly positive for mannose and rhamnose. The three strains reacted in tests for nitrate reduction, pyrazinamidase, β-galactosidase, α-glucosidase, urease (one strain only weakly) and enzymic hydrolysis of L-phenylalanine-AMC, 4MU-β-D-glucoside, arginine, *p*-nitrophenyl β-D-glucoside, leucine- and proline-*p*-nitroanilide, *p*-nitrophenyl phosphate and 4MU-phosphate. Strains LMG 21287 and LMG 21285^T liquefied gelatin. Growth at 37 °C distinguishes *A. citreus* from *A. luteolus* and *A. gandavensis*.

Cellular fatty acid analysis revealed that a predominant amount of anteiso-$C_{15:0}$ and significant amounts of iso-$C_{15:0}$ and anteiso-$C_{17:0}$ were present, as is the case for *A. luteolus* (Wauters *et al.*, 2000).

Strain LMG 21285^T possesses peptidoglycan of the type A3α (L-Lys–L-Thr–L-Ala–L-Ala), which supports its assignment to the *A. globiformis*/*A. citreus* group (Jones & Keddie, 1992). The major menaquinone is MK-9(H_2).

Table 1 contains phenotypic characteristics that are useful for differentiation from the related species *A. luteolus* and *A. citreus*, to which the novel species was otherwise very similar phenotypically, notably in its production of bright yellow pigment (Wauters *et al.*, 2000).

Table 1. Characteristics that differentiate *A. gandavensis* from its nearest phylogenetic relatives

Taxa: 1, *A. gandavensis*; 2, *A. luteolus* DSM 13067^T; 3, *A. citreus* LMG 16338^T.

Characteristic	1	2	3
β-Galactosidase	+	−	+
Urease	+	−	−
Hydrolysis of:			
Aesculin	+	−	−
L-Isoleucine-AMC	−	+	+
p-Nitrophenyl phosphate	+	−	−
p-Nitrophenyl β-D-glucoside	+	−	−
Proline- and leucine-*p*-nitroanilide	+	−	−
p-Nitrophenyl phosphate	+	−	−
ONPG and *p*-nitrophenyl α-D-galactoside	+	−	−

Almost-complete 16S rRNA gene sequences of the three isolates were determined and revealed 100 % similarity between LMG 21285^T and LMG 21287 and 99·9 % similarity between these strains and LMG 21286 (corresponding to a single base difference), showing their high phylogenetic relatedness. A tree showing the phylogenetic affinity of the new isolates to other members of the genus *Arthrobacter* is shown in Fig. 1 and reveals that the unknown bacterial species was most closely related to *A. luteolus* and *A. citreus* (98·6 and 97·2 % similarity by pairwise comparison, respectively). These clustered together with a bootstrap resampling value of 99 % (1000 tree replications).

DNA–DNA hybridizations were performed between the three strains and also with their closest relatives, *A. citreus* and *A. luteolus*. Hybridization values are given in Table 2; they show clearly that the three unknown strains belong to the same novel species within the genus *Arthrobacter*. The DNA G+C content of these three isolates is 65 mol%.

Description of *Arthrobacter gandavensis* sp. nov.

Arthrobacter gandavensis (gan.da.ven′sis. M.L. masc. adj. *gandavensis* of *Gandavum*, the Latin name for Ghent, referring to the place where these strains were first isolated).

Cells are Gram-positive, relatively small coccobacilli with one pointed end. They are catalase-positive. Bright yellow pigment is produced. Growth at 25 and 37 °C is about equal; growth is less abundant at 42 °C. Obligately aerobic. Cells precipitate partially in BHI broth; unable to grow in 6·5 % NaCl. Acid is produced weakly from aesculin, D-fructose and ribose. No acid is produced from adonitol, amygdalin, D- or L-arabinose, D- or L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- or L-fucose, galactose, β-gentiobiose, gluconate, glycogen, glycerol, inositol, inulin, 2- or 5-ketogluconate, lactose, D-lyxose, maltose, maltotriose, mannitol, melezitose, melibiose, methyl β-glycoside, methyl α-D-mannoside, methyl α-D-glucoside, D-raffinose, sucrose,

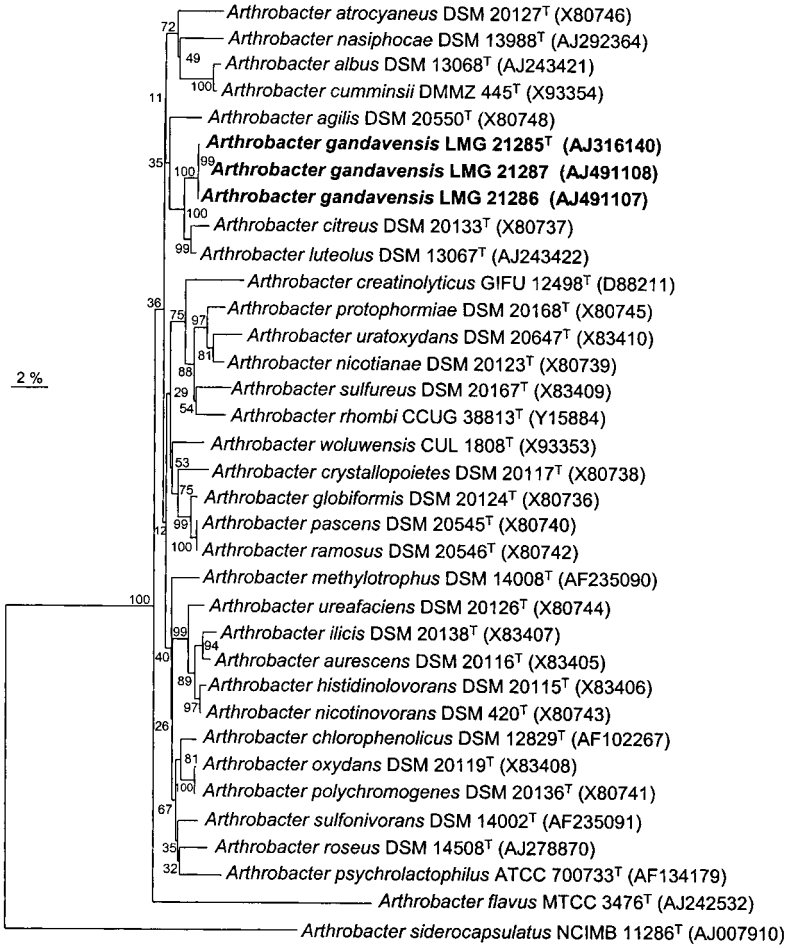

Fig. 1. Phylogenetic tree derived from analysis of 16S rRNA gene sequences of three *A. gandavensis* strains and type strains of other species within the genus *Arthrobacter*. The tree was constructed by using the neighbour-joining method, based on approximately 1350 nt, and the UPGMA grouping method. Bootstrap values are given at branching points (1000 replications). *Arthrobacter siderocapsulatus*, used as outgroup, is a later subjective synonym of *Pseudomonas putida* (Chun *et al.*, 2001). Bar, 2 % sequence divergence.

Table 2. DNA–DNA hybridization values and DNA G+C content (mol%)

Species	G+C content	DNA–DNA hybridization (%) with:				
		1	2	3	4	5
1. *A. gandavensis* LMG 21285^T	65	100				
2. *A. gandavensis* LMG 21286	65	98	100			
3. *A. gandavensis* LMG 21287	65	97	95	100		
4. *A. citreus* LMG 16338^T	65	36	30	36	100	
5. *A. luteolus* DSM 13067^T	66	49	41	50	36	100

salicin, sorbitol, L-sorbose, starch, D-tagatose, trehalose, D-turanose, xylitol or D- or L-xylose. Variable in tests with D-glucose, mannose and rhamnose. In addition to the reactions described in the text, strains are negative for pyrrolidonyl arylamidase (hydrolysis of L-pyroglutamic acid-AMC), β-glucuronidase, *N*-acetyl β-glucosaminidase, enzymic hydrolysis of L-valine-AMC, 4MU-α-D-glucoside, 4MU-β-D-glucuronide, L-isoleucine-AMC, *p*-nitrophenyl β-D-cellobioside and *p*-nitrophenyl α-D-maltoside. Variable in tests for L-arginine-AMC, L-pyroglutamic acid-AMC, L-tryptophan-AMC, 4MU-*N*-acetyl β-D-glucosaminide, 4MU-phosphate, 4MU-β-D-glucuronide and gelatin liquefaction. Cell-wall peptidoglycan is based on the A3α type (L-Lys–L-Thr–L-Ala–L-Ala); major menaquinone is MK-9(H_2). Predominant cellular fatty acid is anteiso-$C_{15:0}$; significant amounts of iso-$C_{15:0}$ and anteiso-$C_{17:0}$ are also present. DNA G+C content is 65 mol%.

The type strain is LMG 21285^T (=DSM 15046^T). Habitat is unknown. Isolated from mammary and uterine infections in cattle; its pathogenic role in these processes is uncertain.

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