International Journal of Systematic and Evolutionary Microbiology (2008), **58**, 2206–2209 **DOI** 10.1099/ijs.0.65478-0

Devriesea agamarum gen. nov., sp. nov., a novel actinobacterium associated with dermatitis and septicaemia in agamid lizards

An Martel,[1] Frank Pasmans,[1] Tom Hellebuyck,[1] Freddy Haesebrouck[1] and Peter Vandamme[2]

Correspondence
An Martel
An.Martel@ugent.be

[1]Department of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium

[2]Department of Biochemistry, Physiology and Microbiology, Faculty of Sciences, Ghent University, B-9000 Ghent, Belgium

Five bacterial isolates were recovered from dermatitis or organ lesions of five agamid lizards. Three strains were recovered from *Uromastyx* species with dermatitis or septicaemia. All five isolates were Gram-positive small rods that could not be identified using conventional phenotypic systems. They grew on sheep blood agar as small haemolytic colonies after 24 h of incubation at temperatures between 25 and 42 °C under aerobic, microaerophilic or anaerobic conditions. They were catalase-positive and non-motile. Comparative analysis of 16S rRNA gene sequences revealed that the strains represent a new taxon within the class *Actinobacteria*. Their nearest phylogenetic neighbours were determined as *Brachybacterium faecium* LMG 19847^T (95.9 % 16S rRNA gene sequence similarity) and *Dermabacter hominis* NCIMB 13131^T (95.3 % similarity). The DNA G+C content of one of the novel isolates, strain IMP2^T, was 61 mol%. On the basis of morphological, chemotaxonomic and phylogenetic differences from other species of coryneform bacteria, it is proposed that this novel taxon be classified as *Devriesea agamarum* gen. nov., sp. nov. The type strain is IMP2^T (=LMG 24257^T=CCUG 55056^T).

Desert-dwelling lizards belonging to the genera *Agama* and *Uromastyx* and inhabiting North African steppe and desert areas are nowadays kept and bred in captivity in Europe. Not infrequently, these animals suffer from skin infections, which may develop into septicaemia. Coryneform bacteria are frequently associated with these lesions either as causative agents or as complicating factors (Pasmans *et al.*, 2008). In the present communication, a description is given of five bacterial isolates from saurian skin lesions that have been shown to belong to a new genus.

Strain IMP1 was recovered from a male *Agama impalearis* with proliferative dermatitis lesions. Strain IMP2^T was isolated from the liver of a female *Agama impalearis* that died from proliferative dermatitis lesions. Three strains were recovered from *Uromastyx* species: UGE1 and UAC1 were isolated from cheylitis lesions from *Uromastyx geyri* and *Uromastyx acanthinura*, respectively; and UGE2 was isolated from bone marrow of another *Uromastyx geyri*, which died after showing dermatitis lesions.

Abbreviation: *m*-Dpm, *meso*-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMP2^T is EU009865.

Strains were isolated by culturing swabs on Columbia agar with 5 % sheep blood (Oxoid). This medium was also used to determine the effect of temperature on growth at 25, 30 and 37 °C and to examine growth under aerobic, microaerophilic (5 % CO_2 atmosphere) and anaerobic conditions. Gram staining was performed with reagents produced by bioMérieux. Acid fastness was determined by Ziehl–Neelsen staining (Lanyi, 1987). Catalase and oxidase activities and NaCl tolerance were examined by standard methods as described by Lanyi (1987). The API 20 Strep, API 50 CH test and API Coryne identification systems (bioMérieux) were used for determination of additional biochemical characteristics. All five isolates grew well on Columbia agar with 5 % sheep blood at 37 °C under aerobic, microaerophilic and anaerobic conditions. The strains were also able to grow at 25 and 30 °C and, to a lesser extent, at 42 °C. They formed smooth, mucoid, whitish, small colonies surrounded by a narrow zone of haemolysis. They were positive when tested with 3 % H_2O_2 and consisted of non-sporulating Gram-positive short rods. The five isolates showed an identical biochemical profile, which is given in the species description. All five isolates were categorized as 'unidentified' by all three commercially available identification systems used.

The biochemical analyses listed above were also performed on the type strains of *Brachybacterium faecium* and *Dermabacter hominis*. Phenotypic characteristics that differentiate the representative strain IMP2^T from the type strains of *B. faecium* and *Dermabacter hominis* are listed in Table 1.

Cellular fatty acid analysis of strain IMP2^T and the *B. faecium* and *Dermabacter hominis* type strains was carried out with a loopful of well-grown cells after an incubation period of 48 h under standard conditions as recommended by the manufacturer (i.e. 28 °C, aerobic conditions, BBL trypticase soy agar; Microbial ID). Fatty acid methyl esters were prepared, separated and identified using the Microbial Identification System (Microbial ID) as described previously (Vandamme *et al.*, 1992). Strain IMP2^T and the type strains of *B. faecium* and *Dermabacter hominis* contained large amounts of branched-chain fatty acids. Although all three strains had similar fatty acid profiles, the proportions of the individual fatty acids differed (Table 2).

Table 1. Differential phenotypic characteristics of the type strains of *Devriesea agamarum* gen. nov., sp. nov., *Dermabacter hominis* and *Brachybacterium faecium*

Strains: 1, IMP2^T; 2, *Dermabacter hominis* NCIMB 13131^T; 3, *B. faecium* LMG 19847^T.

Characteristic	1	2	3
Growth rate*	R	R	S
Haemolysis	+	−	−
Oxidase activity	−	−	+
Gelatin hydrolysis	+	+	−
Starch hydrolysis	−	+	+
Urease production	+	−	−
Nitrate reduction	+	−	+
Voges–Proskauer test	+	−	−
Alkaline phosphatase activity	−	+	−
Pyrazinamidase activity	+	−	−
Methyl α-D-glucopyranosidase activity	−	+	−
Acid production from:			
N-Acetylglucosamine	+	+	−
Amygdalin	−	+	−
Arbutin	+	−	−
D-Arabinose	−	−	+
L-Arabinose	−	−	+
D-Fructose	+	+	−
D-Galactose	+	+	−
Gentiobiose	−	+	−
Glycerol	+	−	−
Lactose	−	+	−
D-Mannose	+	+	−
Melezitose	−	+	+
Melibiose	+	+	−
Raffinose	+	+	−
D-Ribose	−	+	+
Trehalose	+	+	−

*Growth rate determined on Columbia agar with 5 % sheep blood at 37 °C under microaerophilic conditions. R, Rapid; S, slow.

Table 2. Fatty acid composition (%) of the type strains of *Devriesea agamarum* gen. nov., sp. nov., *Dermabacter hominis* and *Brachybacterium faecium*

Strains: 1, strain IMP2^T; 2, *Dermabacter hominis* NCIMB 13131^T; 3, *B. faecium* LMG 19847^T. Those fatty acids for which the mean amount for all taxa was less than 1 % are not given; therefore, the percentages for each group do not total 100 %. Mean percentages are given. ND, Not detected.

Fatty acid	1*	2	3†
14 : 0 iso	ND	2.32	1.24
14 : 0	2.11	2.36	2.16
15 : 0 iso	4.70	13.92	9.83
15 : 0 anteiso	40.13	26.35	55.49
16 : 0 iso	5.65	18.89	7.46
16 : 0	9.59	3.62	4.04
17 : 0 iso	1.14	1.71	2.27
17 : 0 anteiso	33.31	30.82	13.42
18 : 0	ND	ND	1.76

*Strain IMP2^T also contained 3.35 % 16 : 0 N alcohol.
†Strain LMG 19847^T also contained 1.35 % of an unidentified fatty acid with equivalent chain-length value of 18.814.

Respiratory quinone and polar lipid analyses were carried out by the Identification Service and Dr Brian Tindall, DSMZ, Braunschweig, Germany. The major quinone component corresponded to unsaturated menaquinones with eight isoprene units (MK-8) (Table 3). TLC analysis of whole-organism methanolysates revealed the presence of single spots attributable to non-hydroxylated long-chain fatty acid methyl esters.

The peptidoglycan was isolated and purified using the methods described by Schleifer & Kandler (1972), Schleifer (1985), MacKenzie (1987), Rhuland *et al.* (1955) and Groth *et al.* (1996). The total hydrolysate (4 M HCl, 100 °C, 15 h) contained the amino acids *meso*-diaminopimelic acid

Table 3. Menaquinone and polar lipid compositions (%)

Strains: 1, IMP2^T (*Devriesea agamarum* gen. nov., sp. nov.); 2, *Dermabacter hominis* NCFB 2769^T; 3, *B. faecium* NCIB 9860^T. ND, Not detected.

Compound	1	2	3
MK-7	12	9	88
MK-8	83	21	11.5
MK-9	5	70	ND
Polar lipids*	PG, GL, PL	DPG, PG, PL, GL	DPG, PG, GL

*DPG, Diphosphatidylglycerol; GL, glycolipid; PG, phosphatidylglycerol; PL, phospholipid.

(*m*-Dpm), alanine, glycine, aspartic acid and glutamic acid in an approximate molar ratio of 1.0 : 2.0 : 2.1 : 0.9 : 2.4, respectively. The Dpm isomer was analysed by TLC according to Rhuland *et al.* (1955). The molar ratio of the amino acids was determined by GC (GC 14A; Shimadzu) and GC-MS (320-MS Quadrupol; Varian) according to MacKenzie (1987) and Groth *et al.* (1996). The partial hydrolysate (4 M HCl, 100 °C, 45 min) contained the dipeptides L-Ala–D-Glu, D-Ala–D-Glu, Gly–D-Glu and *m*-Dpm–D-Ala. From these data, it was concluded that strain IMP2^T (ID 07-1122) displays the peptidoglycan type A4γ *m*-Dpm–D-Asp–D-Glu, with the α-carboxyl group of D-Glu at position 2 substituted by Gly (type A31.3 according to http://www.dsmz.de/species/murein.htm). This peptidoglycan structure has been detected so far in *Dermabacter hominis* DSM 7083^T, *Brachybacterium alimentarium* DSM 10672^T, *Brachybacterium tyrofermentans* DSM 10673^T (see www.dsmz.de) and *Brachybacterium muris* DSM 15460^T (Buczolits *et al.*, 2003).

DNA of strain IMP2^T was isolated as described by Pitcher *et al.* (1989) and its G+C content was determined as described by Mesbah *et al.* (1989). DNA was degraded enzymically into nucleotides. The obtained nucleotide mixture was then separated by HPLC using a Waters Symmetry Shield C8 column thermostatted at 37 °C. The solvent was 0.02 M $(NH_4)H_2PO_4$ (pH 4.0) with 1.5 % acetonitrile. Non-methylated phage lambda (Sigma) was used as the calibration reference. The DNA G+C content was 61 mol%.

For phylogenetic analysis, DNA of all five isolates was extracted from a loopful of cells using the alkaline lysis method (Baele *et al.*, 2000). PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were performed as described previously (Baele *et al.*, 2001). The sequence reactions were electrophoresed on an ABI Prism 310 automatic sequencer (Applied Biosystems). Sequences were compared with those maintained in the GenBank database through BLAST. Sequences were compared and aligned with those retrieved from GenBank using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analyses were subsequently performed using the BioNumerics 4.5 software package (Applied Maths). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987); bases that could not be unequivocally identified were discarded from the analyses. The near-complete sequences of the 16S rRNA gene (1470 nt) of the five isolates were almost identical [strain UGE1 differed in 1 nt (257G) from all the others, which were identical]. When compared with sequences in public databases, the highest similarity values (about 95 %) were obtained with the type strains of *Brachybacterium* species and *Dermabacter hominis*. Numerical analysis of the sequences revealed that the five isolates occupied a distinct phylogenetic position within the class *Actinobacteria* (Fig. 1). The overall tree topology (Fig. 1) was supported by maximum-parsimony and maximum-likelihood cluster analyses.

Overall, the results of the present study demonstrate that the five strains represent a novel taxon in the class *Actinobacteria*. Given the homogeneity in terms of

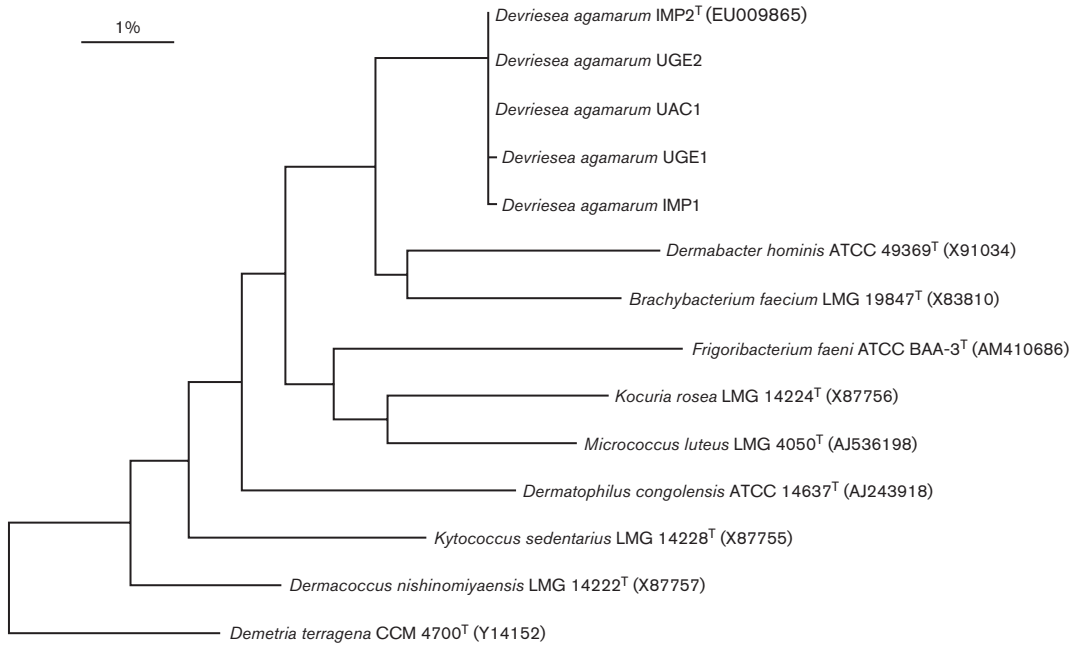

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of *Devriesea agamarum* gen. nov., sp. nov. and its nearest neighbours. Bar, 1 % sequence dissimilarity.

phenotype and 16S rRNA gene sequences of the five strains examined and the phylogenetic and phenotypic differences between the novel taxon and its nearest phylogenetic neighbours, it is proposed that the five isolates should be allocated to a single novel species within a novel genus, *Devriesea agamarum* gen. nov., sp. nov.

Description of *Devriesea* gen. nov.

Devriesea (De′vrie.se.a. N.L. fem. n. *Devriesea* referring to the veterinary microbiologist L. A. Devriese).

Gram-positive, short (1–2 μm) rods that occur singly, in pairs or in short chains. Non-motile. On Columbia agar with 5 % sheep blood, colonies are small, smooth and mucoid, surrounded by a narrow zone of haemolysis. Colour is whitish. Positive for β-galactosidase and catalase activity, but no oxidase activity is observed. Acid is produced from glycerol and arbutin. Starch is not hydrolysed. Urease- and pyrazinamidase-positive. Voges–Proskauer-positive. Additional biochemical characteristics are given above. Cell wall peptidoglycan type is A4γ *m*-Dpm–D-Asp–D-Glu, with the α-carboxyl group of D-Glu at position 2 substituted by Gly. The principal menaquinone is MK-8. Major polar lipids consist of phosphatidylglycerol and some unidentified phospholipids and glycoplipids. The following fatty acid components are present: 14 : 0, 15 : 0 iso, 15 : 0 anteiso, 16 : 0, 16 : 0 iso, 17 : 0 iso, 17 : 0 anteiso. The type species is *Devriesea agamarum*.

Description of *Devriesea agamarum* sp. nov.

Devriesea agamarum (ag.a.mar′um. N.L. n. *Agama* an Old World reptile genus of *Sauria*; N.L. gen. pl. n. *agamarum* of lizards of the genus *Agama*, of agamid lizards).

Exhibits the following properties in addition to those given in the genus description. Cells are non-spore-forming, non-acid-fast, short rods. Acid is produced from *N*-acetylglucosamine, D-fructose, D-galactose, D-glucose, maltose, D-mannose, melibiose, raffinose, sucrose, trehalose and turanose. Acid is not produced from adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, cellobiose, dulcitol, erythritol, D-fucose, gentiobiose, glycogen, inositol, inulin, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, lactose, D-lyxose, D-mannitol, melezitose, L-rhamnose, D-ribose, salicin, D-sorbitol, D-tagatose, xylitol, D-xylose or L-xylose. Aesculin, arginine and gelatin are hydrolysed. Hippurate is not hydrolysed. Positive for α-galactosidase, *N*-acetyl-β-glucosaminidase, α-glucosidase and leucine aminopeptidase. Negative for β-glucuronidase, alkaline phosphatase, methyl β-D-xylopyranosidase, methyl α-D-mannopyranosidase and methyl α-D-glucopyranosidase. Nitrate is reduced.

The type strain is IMP2^T (=LMG 24257^T=CCUG 55056^T). The DNA G+C content of the type strain is 61 mol%. Strains have been isolated from skin and organ lesions of different lizard species, but the habitat and pathogenic activity are unknown.

Acknowledgements

We are grateful to Luc A. Devriese for his help with the phenotypic identification, and to Jean Euzéby for support with the nomenclature.

References

Baele, M., Baele, P., Vaneechoutte, M., Storms, V., Butaye, P., Devriese, L., Verschraegen, G., Gillis, M. & Haesebrouck, F. (2000). Application of tRNA intergenic spacer PCR for identification of *Enterococcus* species. *J Clin Microbiol* **38**, 4201–4207.

Baele, M., Chiers, K., Devriese, L. A., Smith, H. E., Wisselink, H. J., Vaneechoutte, M. & Haesebrouck, F. (2001). The gram-positive tonsillar and nasal flora of piglets before and after weaning. *J Appl Microbiol* **91**, 997–1003.

Buczolits, S., Schumann, P., Weidler, G., Radax, C. & Busse, H.-J. (2003). *Brachybacterium muris* sp. nov., isolated from the liver of a laboratory mouse strain. *Int J Syst Evol Microbiol* **53**, 1955–1960.

Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.

Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyryl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.

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

Pasmans, F., Blahak, S., Martel, A. & Pantchev, N. (2008). Introducing reptiles into a captive collection: the role of the veterinarian. *Vet J* **175**, 53–68.

Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.

Rhuland, L. E., Work, E., Denman, R. F. & Hoare, D. S. (1955). The behavior of the isomers of α,ε-diaminopimelic acid on paper chromatograms. *J Am Chem Soc* **77**, 4844–4846.

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

Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

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

Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., Dewettinck, D., Vlaes, L., Van Den Borre, C., Higgins, R. & other authors (1992). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int J Syst Bacteriol* **42**, 344–356.