Alloscardovia omnicoles gen. nov., sp. nov., from human clinical samples

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The taxonomic position of 12 isolates tentatively assigned to the genus *Bifidobacterium* on the basis of a limited phenotypic characterization was examined. The isolates were collected between 1978 and 2005 in Belgium, Sweden and Norway, and originated from various human clinical samples, including urine, blood, urethra, oral cavity, tonsil, and abscesses of lung and aortic valve. On the basis of band number and clustering analysis, repetitive DNA element-based PCR fingerprinting using the BOXA1R and (GTG)₅ primers indicated that the clinical isolates represented a taxon probably not belonging to the genus *Bifidobacterium*. Analysis of 16S rRNA gene sequence similarities revealed that the isolates were most closely affiliated to *Parascardovia denticolens* LMG 18312^T (93.0–93.2%), *Scardovia inopinata* LMG 18313^T (92.9–93.1%) and other members of the *Bifidobacteriaceae*, indicating that the isolates belong to a novel genus within that family. This observation was further substantiated by the results of partial sequencing of the heat-shock protein 60 gene (*hsp60*) and determination of the DNA G+C contents (47.3–48.3 mol%). Members of the novel taxon can be phenotypically distinguished from *S. inopinata*, *P. denticolens* and *Gardnerella vaginalis* by the ability to grow on agar under aerobic conditions and on the basis of positive reactions for acid production from L-arabinose, raffinose, salicin and D-xylose. Unambiguous phenotypic differentiation from *Aeriscardovia aeriphila* and *Bifidobacterium* species may be difficult, so phenotypic analyses should be complemented by molecular methods. The values for DNA–DNA binding among four members of the novel genus were in the range of 89–100%, indicating that the strains should be considered as a single novel species of a novel genus, for which the name *Alloscardovia omnicoles* gen. nov., sp. nov. is proposed. The type strain of *Alloscardovia omnicoles* is CCUG 31649^T (=LMG 23792^T).

Upon its first description (Stackebrandt *et al.*, 1997), the family *Bifidobacteriaceae* consisted of only two genera, namely *Bifidobacterium* (a taxonomically complex genus that currently contains 29 species with validly published names; <http://www.bacterio.cict.fr/b/bifidobacterium.html>) and *Gardnerella* (containing only *Gardnerella vaginalis*). Although the members of this family are considered as essentially non-pathogenic, a number of exceptions have been reported. *G. vaginalis* has been recognized as an

opportunistic pathogen in bacterial vaginosis (Forsum *et al.*, 2005), whereas *Bifidobacterium dentium* is commonly found in human dental plaque and caries and probably contributes to caries pathology (Modesto *et al.*, 2006). In contrast, the clinical relevance of *Bifidobacterium scardovii*, which has so far been isolated exclusively from human clinical specimens, is currently unknown (Hoyle *et al.*, 2002). Recently, the family *Bifidobacteriaceae* was expanded to contain three novel genera encompassing species that were formerly considered as atypical *Bifidobacterium* species. First, Jian & Dong (2002) transferred two species isolated from human dental caries, *Bifidobacterium inopinatum* and *Bifidobacterium denticolens* (Crociani *et al.*, 1996), to two novel genera as *Scardovia inopinata* and

The GenBank/EMBL/DDBJ accession numbers for the partial 16S rRNA gene sequences and the partial *hsp60* gene sequences determined in this work are AM419458–AM419461 and AM419462–AM419467, respectively, as listed in Figs 2 and 3.

Parascardovia denticolens, respectively. A third novel genus arose from a study on the biodiversity of bifidobacteria present in a porcine caecum (Simpson *et al.*, 2003). In the latter study, a group of unknown isolates was provisionally classified as '*Bifidobacterium aerophilum*' and later named *Aeriscardovia aeriphila* (Simpson *et al.*, 2004). In the present study, we investigated the taxonomic position of 12 human clinical isolates tentatively assigned to the genus *Bifidobacterium* on the basis of limited phenotypic characterization. Taxonomic positioning based on sequencing of the 16S rRNA gene and the heat-shock protein 60 (HSP60) gene revealed that the isolates belong to a novel species and genus.

With the exception of isolate LMG 23791, which originated in Belgium from a patient with a lung abscess, all isolates were obtained from the Culture Collection, University of Göteborg (CCUG), Sweden (<http://www.ccug.se>). The CCUG isolates were collected through clinical laboratory services in Sweden and Norway from 1978 to 2005 and originated from various human clinical samples, including urine, blood, urethra, oral cavity, tonsil and abscesses of the lung and aortic valve (for details, see Fig. 1). Bacterial reference strains for the genus *Bifidobacterium* were obtained from the BCCM/LMG Bacteria Collection, Ghent University, Belgium (<http://bccm.belspo.be/db/>). Isolates were routinely grown on modified Columbia agar [MCA, containing (l⁻¹ distilled water) 23 g special peptone (L37; Oxoid), 1 g soluble starch, 5 g NaCl, 0.3 g L-cysteine hydrochloride, 5 g glucose and 15 g agar] at 37 °C under anaerobic conditions (N₂/H₂/CO₂, 84:8:8) unless otherwise stated.

Because the 12 human clinical isolates were received as *Bifidobacterium*-like organisms, they were first examined using repetitive DNA element PCR (rep-PCR) fingerprinting using the primer BOXA1R (BOX-PCR). Previously, a taxonomic framework based on BOX-PCR profiles was constructed, allowing the identification of a wide range of bifidobacteria at species, subspecies and, potentially, strain level (Masco *et al.*, 2003). DNA extraction, BOX-PCR and data processing were performed essentially as described previously (Masco *et al.*, 2003). BOX-PCR analysis of the 12 clinical isolates yielded atypical fingerprints showing 5–7 bands (data not shown); bifidobacterial BOX-PCR fingerprints typically contain >20 bands (Masco *et al.*, 2003).

Subsequently, a second rep-PCR fingerprinting technique based on the use of the (GTG)₅ primer [(GTG)₅-PCR] was employed as described previously (Masco *et al.*, 2003). (GTG)₅-PCR fingerprinting yielded more bands than BOX-PCR, and clustering analysis reconfirmed that the clinical isolates represented a group of isolates probably not belonging to the genus *Bifidobacterium* (Fig. 1). In addition, each of the isolates displayed a unique (GTG)₅-PCR fingerprint, indicating that the collection under study consisted of 12 different strains.

The complete 16S rRNA gene sequences of strains CCUG 34444 (GenBank accession no. AJ278695) and CCUG 26938 (accession no. AJ278694) were previously deposited in GenBank/EMBL/DDBJ under the provisional unpublished species name '*Bifidobacterium urinalis*'. In the present study, the 16S rRNA gene sequences of four additional strains, CCUG 18650, CCUG 31649^T, CCUG 44766 and LMG 23791, were determined. In addition, partial sequences (~590 bp) of the gene encoding HSP60 were determined for the same six strains. The *hsp60* gene has been proposed as an alternative phylogenetic marker for the genus *Bifidobacterium* (Jian *et al.*, 2001) and has since been included in the description of several novel members of the *Bifidobacteriaceae* (Jian & Dong, 2002; Simpson *et al.*, 2004). Genomic DNA was extracted as described previously (Masco *et al.*, 2003). Amplification, purification and sequencing of 16S rRNA and *hsp60* genes were performed according to Vancanneyt *et al.* (2006) and Jian *et al.* (2001), respectively. The sequences obtained for both genes were used for BLAST searches in EMBL; the highest levels of similarities were found with respect to *Scardovia*, *Parascardovia* and other members of the *Bifidobacteriaceae*. Phylogenetic trees that included reference sequences of members of the family *Bifidobacteriaceae* were constructed for the 16S rRNA and *hsp60* gene sequences by using the neighbour-joining method and the BioNumerics (version 4.0) software package (Applied Maths). Unknown bases were excluded from the analysis. The statistical reliability of the trees was evaluated by bootstrap analysis of 500 replicates, and the tree topology was also confirmed using maximum-parsimony and maximum-likelihood cluster analysis (data not shown). Strains CCUG 18650, CCUG 26938, CCUG 31649^T, CCUG 34444, CCUG 44766 and LMG 23791 showed 16S rRNA gene sequence similarity in the range 99.5–100 %, indicating

Fig. 1. Dendrogram showing clustering of digitally inverted (GTG)₅-PCR fingerprints of the 12 *Bifidobacterium*-like human clinical isolates in comparison with the type strains of *Bifidobacterium adolescentis* and *Bifidobacterium longum*. The dendrogram was obtained with the UPGMA, using Pearson's product-moment similarity coefficient. The clinical source, geographical origin (B, Belgium; N, Norway; S, Sweden) and year of isolation of the 12 unknown isolates are indicated.

that they represent a phylogenetically homogeneous taxon (Fig. 2). The highest levels of similarity with respect to members of the *Bifidobacteriaceae* were found with *P. denticolens* LMG 18312^T (93.0–93.2%) and *S. inopinata* LMG 18313^T (92.9–93.1%). Sequence similarities for the six strains with respect to the other family members, namely *Aeriscardovia aeriphila* LMG 21773^T, *G. vaginalis* LMG 7832^T and *Bifidobacterium* species, ranged from 89.8 to 91.8%. Analysis of a partial *hsp60* gene sequence confirmed the high levels of similarity among the six strains of the *Bifidobacterium*-like group (97.1–99.6%) and clearly separated this group from its closest phylogenetic neighbours, *G. vaginalis* LMG 7832^T (80.3–81.5% sequence similarity), *S. inopinata* LMG 18313^T (75.0–77.7%) and *P. denticolens* LMG 18312^T (74.6–77.3%) (Fig. 3). Collectively, the 16S rRNA and *hsp60* gene sequence analyses indicate that the group of 12 clinical strains is positioned on a distinct branch within the family *Bifidobacteriaceae* and is sufficiently divergent from the other members of this family to require placement in a novel genus.

The G + C contents (mol%) of four strains were determined using the enzymic degradation method described by Mesbah *et al.* (1989). The nucleoside mixture was separated by HPLC using a Waters SymmetryShield C8 column maintained at 37 °C. The solvent was 0.02 M (NH₄)H₂PO₄ (pH 4.0) with 1.5% acetonitrile. Non-methylated λ -phage DNA (Sigma) was used as the calibration reference. The DNA G + C contents of strains CCUG 26938, CCUG 31649^T, CCUG 34444 and LMG 23791 were 48.3, 48.0, 47.3 and 48.0 mol%, respectively. These values are consistent with the phylogenetic positioning of these strains within the family *Bifidobacteriaceae*, with *S. inopinata* (45 mol%),

Fig. 2. Neighbour-joining phylogenetic tree, derived from analysis of nearly-complete 16S rRNA gene sequences (stretches of 1404 bp), showing the position of six of the novel strains in the family *Bifidobacteriaceae*. *Lactobacillus brevis* ATCC 14869^T was used as an outgroup. Bootstrap percentages (based on 500 replications) at or above 50% are indicated at branch points. Bar, 1 nucleotide substitution per 100 nucleotides.

Fig. 3. Neighbour-joining phylogenetic tree, derived from analysis of partial *hsp60* gene sequences (stretches of 458 bp), showing the position of six of the novel strains in the family *Bifidobacteriaceae*. *Lactobacillus delbrueckii* subsp. *bulgaricus* NCC641^T was used as an outgroup. Bootstrap percentages (based on 500 replications) at or above 50% are indicated at branch points. Bar, 10 nucleotide substitutions per 100 nucleotides.

P. denticolens (55 mol%) and *G. vaginalis* (41.8–43.0 mol%) as the closest relatives (Table 1).

To determine the DNA relatedness among members of the novel taxon at species level, DNA–DNA hybridizations were performed between strains CCUG 26938, CCUG 31649^T, CCUG 34444 and LMG 23791. Total genomic DNA was prepared using a combination of the protocols of Marmur (1961) and Pitcher *et al.* (1989), as described previously (Goris *et al.*, 1998) and using mutanolysin for cell lysis. DNA–DNA hybridizations were performed with biotin-labelled probes in microplate wells according to Ezaki *et al.* (1989), with the modifications of Goris *et al.* (1998), using an HTS7000 Bio Assay Reader (Perkin Elmer) for the fluorescence measurements. Hybridization was performed at 37 °C in the presence of 50% formamide. Reciprocal experiments were performed for every pair of strains: the standard deviations ranged from 3 to 12%. The values for DNA–DNA binding among strains CCUG 26938, CCUG 31649^T, CCUG 34444 and LMG 23791 were in the range 89–100%. As the recognized DNA–DNA binding threshold for allocating bacterial isolates to the same species is $\geq 70\%$ (Wayne *et al.*, 1987), it can be concluded that these four strains [along with the other eight CCUG strains included in (GTG)₅-PCR fingerprinting and phylogenetic analyses] should be considered as representing a single novel species.

Growth characteristics and primary phenotypic characteristics of the 12 clinical strains were determined on MCA or on modified MRS (mMRS) agar containing MRS agar (CM361; Oxoid) supplemented with 0.05% (w/v) cysteine hydrochloride. Aerobic and anaerobic growth was tested at 28, 37, 40, 45 and 50 °C. The carbohydrate-fermentation

Table 1. Differential characteristics of the novel strains (*Alloscardovia omnicoles* gen. nov., sp. nov.) and other members of the *Bifidobacteriaceae*

Taxa: 1, *Alloscardovia omnicoles*; 2, *Scardovia inopinata*; 3, *Parascardovia denticolens*; 4, *Aeriscardovia aeriphila*; 5, *Gardnerella vaginalis*; 6, *Bifidobacterium*. Data are from this study and previous studies (Crociani *et al.*, 1996; Gavini *et al.*, 1991; Jian & Dong, 2002; Piot *et al.*, 1980; Simpson *et al.*, 2004). +, Positive; –, negative; v, variable reaction.

Characteristic	1	2	3	4	5	6
Aerobic growth on agar	+	–	–	+	+	(+)*
Temperature range for growth (°C)	28–45	27–44	27–44	30–46	30–37	25–46
Acid from:						
L-Arabinose	+	–	v	+	v	v
Raffinose	+	+	+	+	–	v
Salicin	+	+	+	+	–	v
D-Xylose	+	+	–	v	v	v
DNA G+C content (mol%)	47.3–48.3	45	55	54.7	41.8–43.0	55–67

**Bifidobacterium minimum* grows at the initial inoculation point on mMRS agar.

patterns of the strains were determined using API Rapid ID 32A and API 20A test strips (bioMérieux), according to the manufacturer's instructions, at 37 °C under anaerobic conditions. Tests in the API Rapid ID 32A and API 20A systems were read after 4 and 48 h, respectively. Haemolysis activity was checked on MCA supplemented with 5 % horse blood at 37 °C under anaerobic conditions. The novel taxon can be distinguished phenotypically from *S. inopinata*, *P. denticolens* and *G. vaginalis* by its ability to grow on agar under aerobic conditions and on the basis of its positive reactions for acid production from L-arabinose, raffinose, salicin and D-xylose (Table 1). Unambiguous phenotypic differentiation from *Aeriscardovia aeriphila* and *Bifidobacterium* species may be difficult, so phenotypic tests should be complemented by molecular methods, as described here. A detailed phenotypic description of the novel taxon is given below.

Taken together, the evidence provided by (GTG)₅-PCR fingerprinting, 16S rRNA and *hsp60* gene sequencing, G+C content analysis, DNA–DNA hybridizations and phenotypic characterization indicate that the 12 strains listed in Fig. 1 represent a novel taxon within the family *Bifidobacteriaceae*, for which the name *Alloscardovia denticolens* gen. nov., sp. nov. is proposed. The genus name *Alloscardovia* is in line with the nomenclature of the genera that are, on the basis of 16S rRNA gene sequence similarity, most closely related phylogenetically (i.e. *Scardovia*, *Parascardovia* and *Aeriscardovia*).

Description of *Alloscardovia* gen. nov.

Alloscardovia (Al.lo.scar.do'vi.a. Gr. adj. *allos* different; N.L. fem. n. *Scardovia* a bacterial generic name; N.L. fem. n. *Alloscardovia* organism related to, but different from, *Scardovia* and related genera).

Gram-positive, catalase- and oxidase-negative, non-motile, non-spore-forming, short irregularly shaped rods. Optimal growth occurs under anaerobic conditions on MCA or mMRS after 24 h at 37 °C, but slow aerobic growth (72 h), producing pinpoint-sized colonies, is also observed on MCA. The DNA G+C content of the type species is 47.3–48.3 mol%. Isolated from various human clinical samples, but no data are currently available on potential pathogenic relevance or virulence factors. On the basis of 16S rRNA and HSP60 gene sequence analyses, *Alloscardovia* is a member of the family *Bifidobacteriaceae*. Only one species has been described for this genus, i.e. the type species, *Alloscardovia omnicoles*.

Description of *Alloscardovia omnicoles* sp. nov.

Alloscardovia omnicoles (om.ni.co'lens. L. masc. adj. *omnis* every; L. v. *colere* to dwell; L. pres. part. *colens* dwelling; N.L. part. adj. *omnicoles* dwelling everywhere in the human body).

Displays the following properties in addition to those given in the genus description. Cells are arranged singly or in pairs. Anaerobic growth on MCA occurs at 28 °C (after 72 h), 37–40 °C (after 24 h) and 45 °C (after 96 h), but not at 50 °C (after 96 h). Little or no β -haemolysis is produced on blood-supplemented MCA. All strains ferment D-glucose, sucrose, D-maltose, salicin, D-xylose, L-arabinose and raffinose. None ferments rhamnose (except strain CCUG 31736), sorbitol (except strains CCUG 27412 and CCUG 31736), mannitol (except strains CCUG 27412 and CCUG 31736) or glycerol (except strain CCUG 31736). All strains exhibit α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, α -arabinosidase (except strain CCUG 44766), arginine arylamidase, proline arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase.

activities. None of the strains exhibits arginine dihydrolase, β -galactosidase 6-phosphate, β -glucuronidase, *N*-acetyl- β -glucosaminidase, glutamic acid decarboxylase, α -fucosidase, alkaline phosphatase, leucyl glycine arylamidase, pyroglutamic acid arylamidase, alanine arylamidase, glutamyl glutamic acid arylamidase or urease activities or indole production. Aesculin is hydrolysed. Gelatin is not liquefied and nitrate is not reduced to nitrite. Isolated from various human clinical samples, including urine, blood, urethra, oral cavity, tonsil and abscesses of lung and aortic valve.

The type strain, CCUG 31649^T (=LMG 23792^T), was isolated from the tonsil of a 25-year-old woman from Kristianstad, Sweden, in 1993. The DNA G+C content of this strain is 48.0 mol%. Strains CCUG 7132, CCUG 50589 and CCUG 26938, which also belong to the species, have been deposited in the BCCM/LMG Bacteria Collection as LMG 23793–LMG 23795, respectively.

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References

- Crociani, F., Biavati, B., Alessandrini, A., Chiarini, C. & Scardovi, V. (1996). *Bifidobacterium inopinatum* sp. nov. and *Bifidobacterium denticolens* sp. nov., two new species isolated from human dental caries. *Int J Syst Bacteriol* **46**, 564–571.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane-filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Forsum, U., Holst, E., Larsson, P. G., Vasquez, A., Jakobsson, T. & Mattsby-Baltzer, I. (2005). Bacterial vaginosis – a microbiological and immunological enigma. *APMIS* **113**, 81–90.
- Gavini, F., Pourcher, A.-M., Neut, C., Monget, D., Romond, C., Oger, C. & Izard, D. (1991). Phenotypic differentiation of bifidobacteria of human and animal origin. *Int J Syst Bacteriol* **41**, 548–557.
- Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1–7.
- Hoyles, L., Inganäs, E., Falsen, E., Drancourt, M., Weiss, N., McCartney, A. L. & Collins, M. D. (2002). *Bifidobacterium scardovii* sp. nov., from human sources. *Int J Syst Evol Microbiol* **52**, 995–999.
- Jian, W. & Dong, X. (2002). Transfer of *Bifidobacterium inopinatum* and *Bifidobacterium denticolens* to *Scardovia inopinata* gen. nov., comb. nov., and *Parascardovia denticolens* gen. nov., comb. nov., respectively. *Int J Syst Evol Microbiol* **52**, 809–812.
- Jian, W., Zhu, L. & Dong, X. (2001). New approach to phylogenetic analysis of the genus *Bifidobacterium* based on partial HSP60 gene sequences. *Int J Syst Evol Microbiol* **51**, 1633–1638.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Masco, L., Huys, G., Gevers, D., Verbruggen, L. & Swings, J. (2003). Identification of *Bifidobacterium* species using rep-PCR fingerprinting. *Syst Appl Microbiol* **26**, 557–563.
- 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.
- Modesto, M., Biavati, B. & Mattarelli, P. (2006). Occurrence of the family *Bifidobacteriaceae* in human dental caries and plaque. *Caries Res* **40**, 271–276.
- Piot, P., Van Dyck, E., Goodfellow, M. & Falkow, S. (1980). A taxonomic study of *Gardnerella vaginalis* (*Haemophilus vaginalis*) Gardner and Dukes 1955. *J Gen Microbiol* **119**, 373–396.
- 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.
- Simpson, P. J., Stanton, C., Fitzgerald, G. F. & Ross, R. P. (2003). Genomic diversity and relatedness of bifidobacteria isolated from a porcine cecum. *J Bacteriol* **185**, 2571–2581.
- Simpson, P. J., Ross, R. P., Fitzgerald, G. F. & Stanton, C. (2004). *Bifidobacterium psychraerophilum* sp. nov. and *Aeriscardovia aeriphila* gen. nov., sp. nov., isolated from a porcine caecum. *Int J Syst Evol Microbiol* **54**, 401–406.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Vancanneyt, M., Naser, S. M., Engelbeen, K., De Wachter, M., Van der Meulen, R., Cleenwerck, I., Hoste, B., De Vuyst, L. & Swings, J. (2006). Reclassification of *Lactobacillus brevis* strains LMG 11494 and LMG 11984 as *Lactobacillus parabrevis* sp. nov. *Int J Syst Evol Microbiol* **56**, 1553–1557.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.