Taxonomic Note

Reclassification of *Bifidobacterium stercoris* Kim *et al.* 2010 as a later heterotypic synonym of *Bifidobacterium adolescentis*

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The taxonomic position of *Bifidobacterium stercoris* Eg1^T (=JCM 15918^T) based on comparative 16S rRNA gene and *hsp60* sequence analyses was found to be controversial, as the strain showed high similarity to the type strain of *Bifidobacterium adolescentis*, CCUG 18363^T. Therefore, the relationship between the two species was investigated by a taxonomic study that included, in addition to re-evaluation of the 16S rRNA gene sequence, determination of DNA–DNA binding and multilocus sequence analysis (MLSA) of housekeeping genes encoding the DNA-directed RNA polymerase B subunit (*rpoC*), putative xylulose-5-phosphate/fructose-6-phosphate phosphoketolase (*xfp*), elongation factor EF-G (*fusA*), 50S ribosomal protein L2 (*rplB*) and DNA gyrase B subunit (*gyrB*). Comparative 16S rRNA gene sequence analysis showed relatively high similarity (98.9 %) between *B. stercoris* KCTC 5756^T and *B. adolescentis* ATCC 15703^T. MLSA revealed close relatedness between *B. stercoris* KCTC 5756^T and *B. adolescentis* CCUG 18363^T, with 99.3–100 % similarity between the *rpoC*, *xfp*, *fusA*, *rplB* and *gyrB* gene sequences. In addition, relatively high *dnaJ1* gene sequence similarity of 97.7 % was found between the strains. Similar phenotypes and a high DNA–DNA binding value (78.9 %) confirmed that *B. stercoris* and *B. adolescentis* are synonymous. Based on these results, it is proposed that the species *Bifidobacterium stercoris* Kim *et al.* 2010 should be reclassified as a later heterotypic synonym of *Bifidobacterium adolescentis* Reuter 1963 (Approved Lists 1980).

Bifidobacterium stercoris was described by Kim *et al.* (2010) for one strain isolated from faeces of a Korean adult mainly on the basis of phenotypic characteristics and DNA–DNA reassociation results. All results confirmed that the new bacterial isolate belonged to the genus *Bifidobacterium*. However, the authors stated, based on phylogenetic analyses, that the new isolate was closely related to *Bifidobacterium adolescentis*. Moreover, the habitats from which these bacteria were isolated suggest the necessity of clarifying their taxonomic relationship with additional genetic approaches. The affinity of *B. stercoris* KCTC 5756^T and the type strain of *B. adolescentis*, CCUG 18363^T, was therefore evaluated in this study by a polyphasic taxonomic approach.

Both strains were grown at 37 °C in TPY anaerobic broth (Scardovi, 1986). Chromosomal DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocol. The 16S rRNA gene sequence of *B. stercoris* KCTC 5756^T was resequenced by the method of Killer *et al.* (2011). A comparative analysis based on the revised 16S rRNA gene sequence of *B. stercoris* KCTC 5756^T (GenBank accession no. KF147852) and the 16S rRNA gene sequence of *B. adolescentis* ATCC 15703^T (NR_074802) revealed relatively high similarity of 98.9 % (over a total of 1520 bp). This relatively high similarity is of limited value for the resolution of bacterial species

The GenBank/EMBL/DDBJ accession numbers for the partial *rpoC*, *xfp*, *fusA*, *rplB*, *gyrB* and *dnaJ1* gene sequences of *B. stercoris* KCTC 5756^T and *B. adolescentis* CCUG 18363^T are respectively JQ363659 and JQ363660 (*rpoC*), JQ363666 and JQ363667 (*xfp*), JQ363628 and JQ363629 (*fusA*), JQ363655 and JQ363656 (*rplB*), JQ363638 and JQ363639 (*gyrB*) and JQ363622 and JQ363623 (*dnaJ1*). The GenBank/EMBL/DDBJ accession number for the revised partial 16S rRNA gene sequence of *B. stercoris* KCTC 5756^T is KF147852.

Two supplementary figures and a supplementary table are available with the online version of this paper.

(Tindall *et al.*, 2010). Protein-coding housekeeping gene sequences have recently been recommended for the determination of genomic relatedness at the bacterial species level because of their ability to provide higher taxonomic resolution compared with 16S rRNA gene sequence analysis (Ventura *et al.*, 2006). Therefore, comparative sequence analyses were done between *B. stercoris* KCTC 5756^T and *B. adolescentis* CCUG 18363^T based on *rpoC*, *xfp*, *fusA*, *rplB*, *gyrB* and *dnaJ1* gene sequences. Multilocus sequence analysis (MLSA) has been proposed as an alternative to DNA hybridization, enabling inter- and intra-specific genomic relatedness to be established. In addition, the authors who described the species *B. stercoris* reported high *hsp60* gene sequence similarity (99.4 %) between *B. stercoris* Eg1^T and *B. adolescentis* JCM 1275^T (Kim *et al.*, 2010). Primers and PCR conditions for amplification of partial *fusA*, *rplB* and *gyrB* gene sequences were obtained from Delétoile *et al.* (2010). Partial sequences of *rpoC* and *dnaJ1* genes were amplified under conditions described by Ventura *et al.* (2006). The gene encoding the xylulose-5-phosphate/fructose-6-phosphate phosphoketolase (*xfp*) was proposed as a suitable phylogenetic marker for bifidobacteria by Berthoud *et al.* (2005). Amplified DNA fragments were subsequently checked by electrophoresis on 1.5 % PCR agarose gel (Top-Bio), purified using a PCR purification kit (Qiagen) and sequenced by using an automatic ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems). The values of sequence similarity based on *rpoC*, *xfp*, *fusA*, *rplB*, *gyrB* and *dnaJ1* gene sequences using the jPHYDIT program (Jeon *et al.*, 2005) were 99.3, 99.6, 99.6, 99.8, 100.0 and 97.7 %, respectively. Multilocus sequence typing based on partial *fusA*, *rplB* and *gyrB* gene sequences revealed $\geqslant$99 % similarity between different strains within bifidobacterial species (Delétoile *et al.*, 2010). Ventura *et al.* (2006) determined means of 88.25 and 65.09 % sequence similarity for the *rpoC* and *dnaJ1* genes between 31 bifidobacterial strains.

The concatenation of protein-encoding housekeeping genes has been shown to be extremely useful in order to infer bacterial phylogeny (Teichmann & Mitchison, 1999). Therefore, phylogenetic analysis to reveal the relationship between *B. stercoris* KCTC 5756^T and *B. adolescentis* CCUG 18363^T was based on concatenated sequences of the *hsp60*, *xfp* and *dnaJ1* genes. These housekeeping genes were chosen because their sequences are available for a wider range of bifidobacterial species. Phylogenetic trees were reconstructed by MEGA version 5.05 and the Gblocks program using the maximum-likelihood algorithm as described previously (Killer *et al.*, 2013). The topology of the phylogenetic tree reconstructed on the basis of concatenated *hsp60* and *xfp* gene sequences revealed a very close relationship between the two tested strains. The phylogenetic branch length between the two strains was shorter than the length of the phylogenetic branches between distinctive subspecies of bifidobacteria such as *Bifidobacterium animalis* subsp. *animalis* and *B. animalis* subsp. *lactis* and *Bifidobacterium pseudolongum* subsp. *pseudolongum* and *B. pseudolongum* subsp. *globosum* (Fig. 1). Very similar results were obtained using concatenated *hsp60*, *xfp* and *dnaJ1* gene sequences (Fig. S1, available in IJSEM Online). The GenBank accession numbers for the partial *hsp60*, *xfp* and *dnaJ1* gene sequences of type strains of bifidobacterial species used and generated in this study are listed in Table S1. A close relationship of the two bacterial strains was also confirmed by the phylogenetic tree of the family *Bifidobacteriaceae* reconstructed on the basis of sequences of the 16S rRNA gene (Fig. S2).

The determination of DNA–DNA relatedness is the mandatory reference method for determining whether micro-organisms belong to the same species (Tindall *et al.*, 2010). DNA–DNA relatedness between *B. stercoris* KCTC 5756^T and *B. adolescentis* CCUG 18363^T was re-evaluated as follows. Wet biomass (3 g) from the tested strains suspended in isopropanol/water (1 : 1, v/v) was disrupted using a Constant Systems TS 0.75 kW disruptor (IUL Instruments) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) under consideration of the modifications described by Huss *et al.* (1983) using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 $\times$ 6 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). The results showed that the tested strains had a binding level of 78.9 % (mean of three experiments, SD = 0.2 %), higher than the 70 % species boundary limit.

The DNA G + C contents of *B. stercoris* KCTC 5756^T and *B. adolescentis* CCUG 18363^T were re-evaluated as follows. DNA was degraded enzymically into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture was then separated by HPLC as described previously (Killer *et al.*, 2011). The determined values were not significantly different between the studied strains (60.6 and 61.2 mol%, respectively).

API 50 CHL and Rapid ID 32A commercial kits (bioMérieux) were used for comparison of biochemical characteristics of *B. stercoris* KCTC 5756^T and *B. adolescentis* CCUG 18363^T to determine intra- or inter-species divergence. For this purpose, the strains were cultivated in anaerobic TPY broth. Tests were performed according to the manufacturer's instructions, except that the API 50 CHL test strips were incubated under anaerobic conditions (anaerobic jars; Oxoid) at 37 °C for 48 h. Minor differences in biochemical characteristics between *B. adolescentis* CCUG 18363^T and *B. stercoris* KCTC 5756^T are shown in Table 1. These results proved the close biochemical similarity of the tested strains.

On the basis of the phenotypic and important genotypic results presented in this study, it is proposed that *B. stercoris* and *B. adolescentis* represent the same species and should be united under the same name. It is concluded that

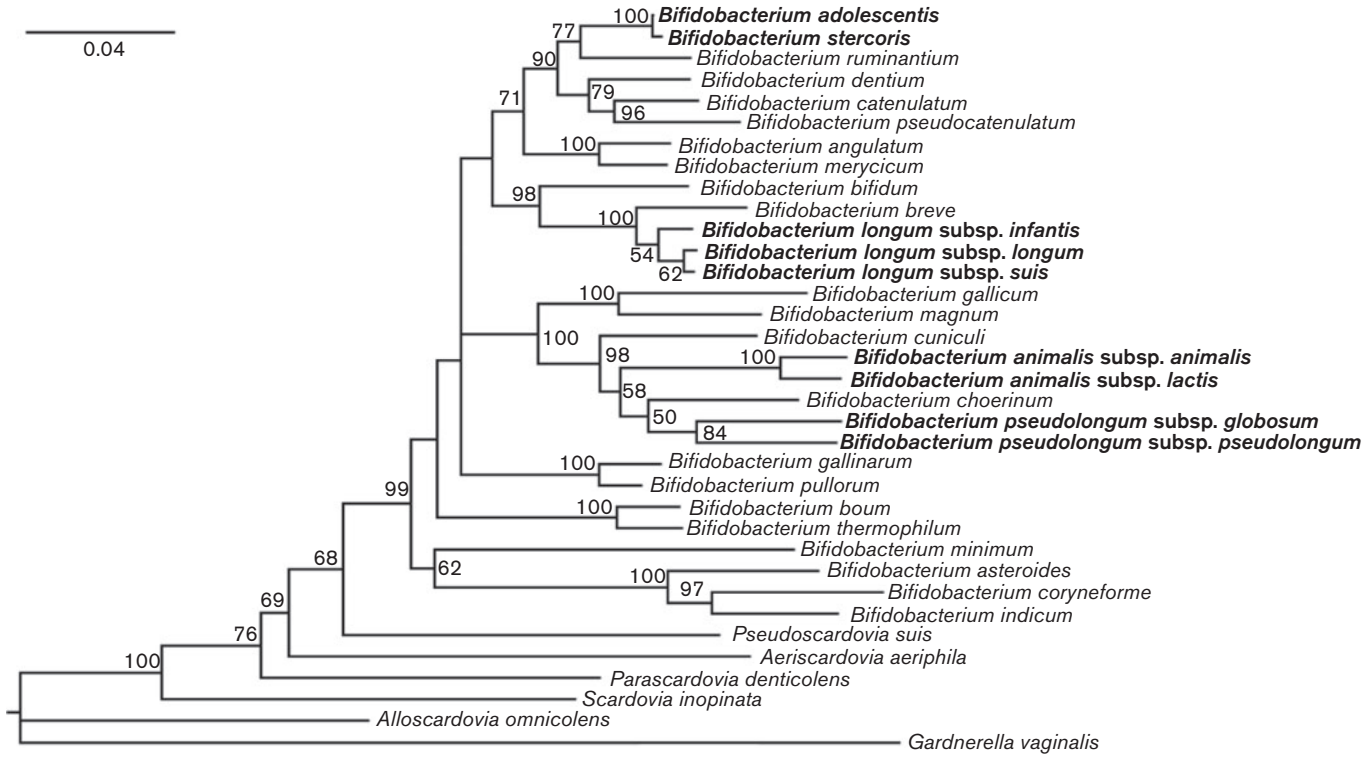

Fig. 1. Phylogenetic tree of the family *Bifidobacteriaceae* showing the very close relationship of *B. adolescentis* CCUG 18363^T and *B. stercoris* KCTC 5756^T, reconstructed by the maximum-likelihood method based on concatenated partial sequences of the *hsp60* (539 nt) and *xfp* (418 nt) genes using MEGA version 5.05 software after removing hypervariable positions by using the program Gblocks. Sequence data were aligned using the CLUSTAL W algorithm. The Tamura–Nei model was used for reconstruction of the phylogenetic tree. Bootstrap values, expressed as percentages of 1000 datasets, are given at nodes. GenBank accession numbers of partial gene sequences derived from type strains are presented in Table S1. The tree was rooted by *Gardnerella vaginalis* ATCC 14018^T. Bar, 0.04 substitutions per nucleotide position.

Table 1. Minor differences between *B. stercoris* KCTC 5756^T and *B. adolescentis* CCUG 18363^T

Both strains produced acids from D-ribose, D-galactose, D-glucose, amygdalin, arbutin, aesculin (hydrolysis), maltose, lactose, melibiose, sucrose, raffinose, gentiobiose and turanose. Neither strain produced acids from glycerol, erythritol, D- or L-arabinose, D- or L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, cellobiose, trehalose, inulin, melezitose, starch, glycogen, xylitol, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate or 2- or 5-ketogluconate. Both strains were positive for α-galactosidase, α-glucosidase, β-galactosidase, β-glucosidase, α-arabinosidase, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. Both strains were negative for urease, arginine dihydrolase, β-galactosidase-6-phosphate, β-glucuronidase, *N*-acetyl-β-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, nitrate reduction, indole production, alkaline phosphatase, pyroglutamic acid arylamidase, glutamyl glutamic acid arylamidase, esterase (C4), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-mannosidase, gelatin hydrolysis, catalase and oxidase. +, Positive reaction; W, weakly positive reaction; −, negative reaction.

Characteristic	*B. stercoris* KCTC 5756^T	*B. adolescentis* CCUG 18363^T
DNA G+C content (mol%)	60.6	61.2
Fermentation of:		
D-Fructose	−	+
D-Sorbitol	−	W
Salicin	−	W
Production of:		
Esterase lipase (C8)	−	W
Naphthol-AS-BI-phosphohydrolase	W	−

Bifidobacterium stercoris Kim *et al.* 2010 is a later heterotypic synonym of *Bifidobacterium adolescentis* Reuter 1963 (Approved Lists 1980), which has priority.

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