Proposal to reclassify the three biotypes of *Bifidobacterium longum* as three subspecies: *Bifidobacterium longum* subsp. *longum* subsp. nov., *Bifidobacterium longum* subsp. *infantis* comb. nov. and *Bifidobacterium longum* subsp. *suis* comb. nov.

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In the year 2002, *Bifidobacterium longum*, *Bifidobacterium infantis* and *Bifidobacterium suis* were unified into a single species, *Bifidobacterium longum*, preserving the former species names through the creation of the three biotypes 'longum', 'infantis' and 'suis'. Consequently, the use of the species names *B. infantis* and *B. suis* was to be discontinued. The above taxonomic rearrangement of *B. longum* was based on DNA–DNA hybridizations and 16S rRNA and HSP60 gene sequence analysis. However, a variety of other genotypic techniques including ribotyping, amplified rDNA restriction analysis (ARDRA), randomly amplified polymorphic DNA (RAPD)-PCR, BOX-PCR, PCR-denaturing gradient gel electrophoresis (DGGE), comparison of the *recA*, *tuf* and *ldh* gene sequences, plasmid profiling and considerable variation in carbohydrate fermentation patterns as well as results of starch and PAGE electrophoresis experiments clearly discriminate former *B. longum*, *B. infantis* and *B. suis* strains. In the present paper we compile this published information and propose the description of *Bifidobacterium longum* subsp. *longum* subsp. nov., *Bifidobacterium longum* subsp. *infantis* comb. nov. and *Bifidobacterium longum* subsp. *suis* comb. nov. The International Committee on Systematics of Prokaryotes Subcommittee on the taxonomy of *Bifidobacterium*, *Lactobacillus* and related organisms is in favour of this proposal. The type strains of *Bifidobacterium longum* subsp. *longum* subsp. nov., subsp. *infantis* comb. nov. and subsp. *suis* comb. nov. are E194b (variant a)^T (ATCC 15707^T=DSM 20219^T), S12^T (=ATCC 15697^T=DSM 20088^T) and Su859^T (ATCC 27533^T=DSM 20211^T), respectively.

Sakata *et al.* (2002) unified *Bifidobacterium longum*, *Bifidobacterium infantis* and *Bifidobacterium suis* into the single species *Bifidobacterium longum* and, on the basis of DNA–DNA hybridization values, established the three biotypes 'longum', 'infantis' and 'suis' for strains belonging to these former species. Consequently, the species *B. infantis* and *B. suis* were published as synonyms of *B. longum*, the species name with the highest priority, as cited

first in Reuter (1963). Therefore, the use of the species names *B. infantis* and *B. suis* was to be discontinued.

The taxonomic position of *B. infantis*, *B. longum* and *B. suis* has been controversial for quite some time. *B. longum* was described by Reuter (1963) for a strain isolated from adult faeces. *B. longum* has been shown previously to be the most prevalent species in the gastrointestinal tract of human adults (Biavati *et al.*, 1984). In the same paper, Reuter (1963) proposed the name *B. infantis* for strains which generally dominate the gastrointestinal tract of infants (Biavati *et al.*, 1986; Matsuki *et al.*, 1998). *B. suis*, isolated from pig's faeces, was described by Matteuzzi *et al.* (1971).

Abbreviations: ARDRA, amplified rDNA restriction analysis; DGGE, denaturing gradient gel electrophoresis; RAPD, randomly amplified polymorphic DNA.

In the survey of DNA–DNA relatedness, *B. longum*, *B. infantis* and *B. suis* proved to have a hybridization rate around 70 % and higher (Scardovi *et al.*, 1979; Lauer & Kandler, 1983; Bahaka *et al.*, 1993; Sakata *et al.*, 2002). Moreover, *B. longum*, *B. infantis* and *B. suis* possess more than 97 % 16S rRNA gene sequence similarity (Leblond-Bourget *et al.*, 1996; Miyake *et al.*, 1998; Jian *et al.*, 2001; Sakata *et al.*, 2006). Other data obtained from 16S–23S ITS and HSP60 sequence analysis (Leblond-Bourget *et al.*, 1996; Jian *et al.*, 2001; Zhu *et al.*, 2003) revealed high similarity between *B. longum*, *B. infantis* and *B. suis*. Knowing that strains belonging to the same species should share 70 % or more DNA relatedness (at the DNA–DNA hybridization or reassociation level) and possess more than 97 % 16S rRNA gene sequence identity, the analysis of the 16S rRNA sequences and DNA–DNA hybridization rate of *B. longum*, *B. infantis* and *B. suis* do not allow the clear separation of these taxonomic entities at the species level (Wayne *et al.*, 1987; Stackebrandt *et al.*, 2002).

Genotypic data derived from previously published information and supporting the unification of the three species are shown in Table 1.

Genotypic data supporting reclassification of *B. longum*, *B. infantis* and *B. suis* in three subspecies

A variety of other genotypic techniques, including ribotyping, amplified rDNA restriction analysis (ARDRA), randomly amplified polymorphic DNA (RAPD)-PCR, BOX-PCR, PCR-denaturing gradient gel electrophoresis (DGGE) and comparison of the *recA*, *tuf* and *ldh* gene sequences, allow the evaluation of taxonomic relationships between more closely related bacterial species. When applied to *B. longum*, *B. infantis* and *B. suis*, most techniques clearly discriminated these species. Yamamoto *et al.* (1992) showed

that it was possible to construct a species-specific probe to differentiate *B. infantis* and *B. longum*. Frothingham *et al.* (1993) compared the 16S rRNA gene sequences from *B. longum* and *B. infantis* and showed that they are distinct species differing at seven bases. Roy *et al.* (1996) indicated that *B. longum* could be differentiated by PCR from *B. infantis*, even if the respective primers differed by only three bases. Matsuki *et al.* (1998) also developed specific primers effective for the identification of *B. longum* and *B. infantis*. Roy & Sirois (2000) confirmed that, although *B. infantis* is closely related to *B. longum*, differentiation between the two species was possible using *Sau3AI* as restriction enzyme and ARDRA. Kullen *et al.* (1997), utilizing *recA* gene analysis, differentiated *B. longum* and *B. infantis*. Ventura *et al.* (2003) used the *tuf* gene as well as the *recA* gene as a phylogenetic marker: *B. longum* and *B. infantis* clearly branched separately in the *tuf*-based tree. Alignment of short regions of the *ldh* gene also confirmed that it is possible to distinguish between *B. longum* and *B. infantis* (Roy & Sirois, 2000). Fanel *et al.* (1998) revealed different electrophoretic profiles between the type strains of *B. longum*, *B. infantis* and *B. suis* using RAPD-PCR. Sakata *et al.* (2002), also using RAPD-PCR, showed that it is possible to distinguish *B. longum*, *B. infantis* and *B. suis*. Requena *et al.* (2002) reported that *B. longum*, *B. infantis* and *B. suis* are readily differentiated by transaldolase-specific PCR-DGGE. Masco *et al.* (2003) showed that *B. longum*, *B. infantis* and *B. suis* could be easily differentiated by BOX-PCR fingerprinting. In the paper of Sakata *et al.* (2006) studying the genus *Bifidobacterium* by automated ribotyping, *B. longum*, *B. infantis* and *B. suis* were divided in different clusters or subclusters (*B. longum* was allocated to cluster IX, *B. infantis* to cluster V and subclusters c and g and *B. suis* to cluster V and subclusters m and q). The clusters obtained from these species may reflect the underlying genetic diversity. Sgorbati *et al.* (1982) showed that 70 % of 186 strains genetically assigned to *B. longum* carried a large array

Table 1. Genotypic data that support unification of *B. longum*, *B. infantis* and *B. suis* in one species

The type strain of the species was always tested. Number of strains tested indicated in parentheses.

Methods	Strains tested	Similarity levels (%)	Reference
16S rRNA gene sequence analysis	<i>B. longum</i> (1), <i>B. infantis</i> (1), <i>B. suis</i> (1)	99.05–99.72	Leblond-Bourget <i>et al.</i> (1996)
	<i>B. longum</i> (1), <i>B. infantis</i> (1), <i>B. suis</i> (1)	>99	Miyake <i>et al.</i> (1998)
	<i>B. longum</i> (1), <i>B. infantis</i> (4), <i>B. suis</i> (1)	99	Jian <i>et al.</i> (2001)
	<i>B. longum</i> (7), <i>B. infantis</i> (8), <i>B. suis</i> (2)	>97	Sakata <i>et al.</i> (2006)
HSP60 gene sequence analysis	<i>B. longum</i> (1), <i>B. infantis</i> (4), <i>B. suis</i> (1)	98	Jian <i>et al.</i> (2001)
	<i>B. longum</i> (5), <i>B. infantis</i> (8), <i>B. suis</i> (2)	98–100	Zhu <i>et al.</i> (2003)
16S–23S ITS sequence analysis	<i>B. longum</i> (1), <i>B. infantis</i> (1)	87.3	Leblond-Bourget <i>et al.</i> (1996)
DNA–DNA hybridization	<i>B. longum</i> (80), <i>B. infantis</i> (65)	65–80	Scardovi <i>et al.</i> (1979)
	<i>B. longum</i> (1), <i>B. infantis</i> (1)	63	Lauer & Kandler (1983)
	<i>B. longum</i> (2), <i>B. infantis</i> (2)	66–70	Bahaka <i>et al.</i> (1993)
	<i>B. longum</i> (2), <i>B. infantis</i> (3)	66–81	Sakata <i>et al.</i> (2002)
	<i>B. longum</i> (1), <i>B. suis</i> (1)	75	Lauer & Kandler (1983)
	<i>B. suis</i> (1), <i>B. longum</i> (2)	78	Sakata <i>et al.</i> (2002)
	<i>B. infantis</i> (1), <i>B. suis</i> (1)	66	Lauer & Kandler (1983)
	<i>B. infantis</i> (1), <i>B. suis</i> (1)	75	Sakata <i>et al.</i> (2002)

of plasmids, whereas none of the 63 strains of *B. infantis* did, although strains of both species were isolated from the same specimen. None of the 22 strains of *B. suis* tested possessed plasmids either. Taking into account the large diversity of extrachromosomal DNA elements in *B. longum* and awaiting the results of pending studies on the possible taxonomic and evolutionary significance of plasmids in bifidobacteria, it seems advisable to retain *B. longum* as a separate taxonomic entity.

All these data, derived from previously published information, are summarized in Table 2, supporting the reclassification in three subspecies (Staley & Krieg, 1986; Stackebrandt *et al.*, 2002).

Phenotypic data supporting reclassification of *B. longum*, *B. infantis* and *B. suis* in three subspecies

Identification of the three species using carbohydrate fermentation patterns is difficult: strains assigned to *B. longum* and *B. infantis* may show carbohydrate patterns that are different from the respective type strains (Bahaka *et al.*, 1993).

L-Arabinose is reported as not fermentable by *B. infantis* and *B. suis*, while *B. longum* characteristically ferments melezitose (Reuter, 1963; Mitsuoka, 1969; Matteuzzi *et al.*, 1971). Numerous strains isolated from infant faeces, which ferment arabinose or fail to ferment melezitose, were recognized by means of DNA–DNA hybridization as *B. infantis* and *B. longum*, respectively (Scardovi, 1986). D-Ribose is fermented only by *B. longum* and *B. infantis* (Sakata *et al.*, 2002). *B. infantis* ferments D-glucuronate and

α -fucose, which are not fermented by *B. longum* and *B. suis*. *B. longum* ferments arabinogalactan, which is not fermented by *B. infantis* and *B. suis* (Crociani *et al.*, 1994).

Scardovi *et al.* (1979) determined the isozyme patterns for transaldolase by starch electrophoresis. In 126, 65 and 22 strains allotted by DNA–DNA relatedness to *B. longum*, *B. infantis* and *B. suis*, respectively, 90 % of the *B. infantis* strains had the transaldolase more shifted to the anode (isozyme 5, migration 100) than the enzyme observed in 100 % of the *B. suis* strains (isozyme 6, migration 96), and even more shifted to the anode than the enzyme possessed by 72 % of the *B. longum* strains (isozyme 8, migration 90).

Moreover, Biavati *et al.* (1982), who investigated the electrophoretic patterns of soluble proteins, could clearly distinguish three species, and Roy *et al.* (1994) showed that the β -galactosidase electrophoretic patterns are different in *B. longum* and *B. infantis*.

These data, derived from previously published information, are summarized in Table 3, supporting the reclassification in three subspecies (Wayne *et al.*, 1987; Brenner *et al.*, 2001).

On the basis of DNA–DNA relatedness values and results of 16S rRNA and HSP60 gene sequence analysis, we agree with Sakata *et al.* (2002) that *B. longum*, *B. infantis* and *B. suis* belong to a single species, *B. longum*.

However, the genotypic and phenotypic variation described above reveals considerable differences between the three former species, supporting the description of *B. longum*, *B. infantis* and *B. suis* as subspecies of *B. longum*. The International Committee on Systematics of Prokaryotes Subcommittee on the taxonomy of *Bifidobacterium*, *Lactobacillus*

Table 2. Genotypic data that support unification of *B. longum*, *B. infantis* and *B. suis* in three subspecies

The type strain of the species was always tested. Number of strains tested indicated in parentheses.

Methods	Strains tested	Results	References
RNA (or DNA) probes*	<i>B. longum</i> (1), <i>B. infantis</i> (7)	1 base difference	Yamamoto <i>et al.</i> (1992)
	<i>B. longum</i> (1), <i>B. infantis</i> (1)	7 bases difference	Frothingham <i>et al.</i> (1993)
	<i>B. longum</i> (18), <i>B. infantis</i> (6)	3 bases difference	Roy <i>et al.</i> (1996)
	<i>B. longum</i> (3), <i>B. infantis</i> (3)	>10 bases difference	Matsuki <i>et al.</i> (1999)
	<i>B. longum</i> (39), <i>B. infantis</i> (5)	Not quantified	Roy & Sirois (2000)
<i>recA</i> gene analysis	<i>B. longum</i> (1), <i>B. infantis</i> (1)	Specific sequences	Kullen <i>et al.</i> (1997)
<i>tuf</i> gene analysis	<i>B. longum</i> (2), <i>B. infantis</i> (1)	Specific sequences	Ventura <i>et al.</i> (2003)
<i>ldh</i> gene analysis	<i>B. longum</i> (1), <i>B. infantis</i> (1)	Differences in sequences (71 amino acids)	Roy & Sirois (2000)
RAPD-PCR	<i>B. longum</i> (1), <i>B. infantis</i> (1), <i>B. suis</i> (1)		Fanedl <i>et al.</i> (1998)
	<i>B. longum</i> (3), <i>B. infantis</i> (8), <i>B. suis</i> (2)		Sakata <i>et al.</i> (2002)
ARDRA-PCR	<i>B. longum</i> (39), <i>B. infantis</i> (5)	Differences in specific pattern	Roy & Sirois (2000)
PCR-DGGE	<i>B. infantis</i> (1), <i>B. suis</i> (1)		Requena <i>et al.</i> (2002)
BOX-PCR	<i>B. longum</i> (9), <i>B. infantis</i> (6), <i>B. suis</i> (2)		Masco <i>et al.</i> (2003)
Automated ribotyping	<i>B. longum</i> (7), <i>B. infantis</i> (8), <i>B. suis</i> (2)	Differences in specific pattern	Sakata <i>et al.</i> (2006)
Presence of plasmid	<i>B. longum</i> (186)	Plasmid positive: 70 %	Sgorbati <i>et al.</i> (1982)
	<i>B. infantis</i> (63), <i>B. suis</i> (22)	Plasmid negative	

*Base differences in cross-reacting species-specific primers.

Table 3. Phenotypic data that support reclassification of *B. longum*, *B. infantis* and *B. suis* in three subspecies

The type strain of the species was always tested. Number of strains tested indicated in parentheses.

Method/strains tested	Results	References
Carbohydrate fermentation		
<i>B. longum</i> (>20), <i>B. infantis</i> (>20) and <i>B. suis</i> (>20)	Arabinose +: <i>B. longum</i> and <i>B. suis</i> Arabinose -: <i>B. infantis</i>	Reuter (1963), Matteuzzi <i>et al.</i> (1971), Sakata <i>et al.</i> (2002)
<i>B. longum</i> (>20), <i>B. infantis</i> (>20) and <i>B. suis</i> (>20)	Melezitose +: <i>B. longum</i> Melezitose -: <i>B. infantis</i> and <i>B. suis</i>	Reuter (1963), Matteuzzi <i>et al.</i> (1971), Scardovi (1986)
<i>B. longum</i> (11), <i>B. infantis</i> (12) and <i>B. suis</i> (10)	Ribose +: <i>B. longum</i> and <i>B. infantis</i> Ribose -: <i>B. suis</i>	Sakata <i>et al.</i> (2002)
<i>B. longum</i> (10), <i>B. infantis</i> (10) and <i>B. suis</i> (10)	D-Glucuronate +: <i>B. infantis</i> D-Glucuronate -: <i>B. longum</i> and <i>B. suis</i>	Crociani <i>et al.</i> (1994)
Transaldolase electrophoresis		
<i>B. longum</i> (>80), <i>B. infantis</i> (>40) and <i>B. suis</i> (22)		Scardovi <i>et al.</i> (1979)
Soluble protein electrophoresis	Differences in specific patterns	
<i>B. longum</i> (275), <i>B. infantis</i> (22) and <i>B. suis</i> (7)		Biavati <i>et al.</i> (1982)
β -Galactosidase electrophoresis		
<i>B. longum</i> (6) and <i>B. infantis</i> (5)		Roy <i>et al.</i> (1994)

and related organisms is in favour of this proposal (Klein, 2006).

Based on Sakata *et al.* (2002), the species name retained is *Bifidobacterium longum*, with the type strain E194b (variant a)^T (=ATCC 15707^T), isolated from faeces of a human adult (Reuter, 1963). The three subspecies withheld are: *Bifidobacterium longum* subsp. *longum* subsp. nov. with the type strain E194b (variant a)^T Reuter 1963 (=ATCC 15707^T), isolated from faeces of a human adult, *Bifidobacterium longum* subsp. *infantis* comb. nov. with the type strain S12^T Reuter 1963 (=ATCC 15697^T), isolated from faeces of a human infant, and *Bifidobacterium longum* subsp. *suis* comb. nov. with the type strain Su 859^T (=ATCC 27533^T), isolated from faeces of a piglet (Matteuzzi *et al.*, 1971).

Description of *Bifidobacterium longum* subsp. *longum* subsp. nov.

Bifidobacterium longum subsp. *longum* (long'um. L. neut. adj. *longum* long) (Reuter 1963).

On TPY agar, strains of this species show mostly very elongated and relatively thin cellular elements with slightly irregular contours and rare branchings. In TPY broth, most strains develop with uniform turbidity, clearing is slow and the sediment is viscous. *B. longum* is apparently the only species among those usually found in human faeces which possesses a large variety of plasmids (Sgorbati *et al.*, 1982). Concerning DNA–DNA relatedness (63–81%), this subspecies is most closely related to *B. longum* subsp. *infantis* and *B. longum* subsp. *suis* (Scardovi *et al.* 1979; Lauer & Kandler, 1983; Bahaka *et al.*, 1993; Sakata *et al.*, 2002). The genome of *B. longum* NCC 2705 (GenBank accession no. AE014295) was the first to be completely sequenced among the genus *Bifidobacterium*. Sequencing the DNA of this

strain has revealed much about its metabolic diversity and generated insights into other members of the genus (Schell *et al.*, 2002). Bioinformatic analysis revealed several physiological traits that could partially explain the successful adaptation of this bacterium to the colon. An unexpectedly large number of the predicted proteins appeared to be specialized for catabolism of a variety of oligosaccharides, some possibly released by rare or novel glycosyl hydrolases acting on 'non-digestible' plant polymers or host-derived glycoproteins and glycoconjugates. *B. longum* DJ010A is presently available as a partly assembled genome (GenBank accession no. AABM000000000) (Leahy *et al.*, 2005). Strains have been isolated from infant and adult human faeces, from faeces of suckling calf, from human vagina and from sewage. For the original description, see Reuter (1963). The genomic G + C content is 61 mol% (T_m).

Type strain: E194b (variant a)^T (=ATCC 15707^T=DSM 20219^T), from faeces of human adult (Reuter, 1963).

Description of *Bifidobacterium longum* subsp. *infantis* (Reuter 1963) comb. nov.

Bifidobacterium longum subsp. *infantis* (in.fan'tis. L. gen. n. *infantis* of an infant).

Cellular morphology does not present specific traits, so that it is similar to that of many other species of the genus. These bifidobacteria do not ferment pentoses. They are found as predominant forms in breast-fed infant faeces.

Strains have been isolated from infant faeces, from faeces of suckling calf and from human vagina. Regarding DNA–DNA relatedness (63–81%), this subspecies is most closely related to *B. longum* subsp. *longum* and to *B. longum* subsp. *suis*

(Scardovi *et al.* 1979; Lauer & Kandler, 1983; Bahaka *et al.*, 1993; Sakata *et al.*, 2002). For the original description see Reuter (1963). The genomic G + C content is 60.5 mol% (T_m).

Type strain: S12^T (=ATCC 15697^T=DSM 20088^T), from faeces of human infant (Reuter, 1963).

Description of *Bifidobacterium longum* subsp. *suis* (Matteuzzi, Crociani, Zani and Trovatelli 1971) comb. nov.

Bifidobacterium longum subsp. *suis* (su'is. L. n. *sus* pig; L. sing. gen. n. *suis* of a pig).

Slender cells, elongated (2–6 µm long), with rare terminal bifurcations in the shape of clubs. Non-motile. Gram-positive. Colonies are circular, soft, smooth, white with entire margins. Liquid cultures are at first turbid, after 24–36 h become clear with sediment dispersible by agitation. Anaerobic. CO₂ without effect on O₂ sensitivity or anaerobic growth. Catalase-negative. Optimum growth at 38–39 °C, minimum 19–20 °C, maximum 44.5–45 °C. Optimum growth at pH 7–8; no growth at pH 5.0 or 9.5. Sugars fermented: arabinose, xylose, glucose, fructose, mannose, galactose, maltose, sucrose, lactose, melibiose, raffinose. Rhamnose, melezitose, cellobiose, trehalose, dextrin, starch, inulin, sorbitol, mannitol, glycerol, salicin, gluconate and lactate are never fermented. No growth in carbohydrate-free media. The end products of glucose fermentation are lactic (LA) and acetic acids (AA), in the ratio 1.0(LA) : 1.7(AA) to 1.0(LA) : 2.0(AA). Glucose is fermented by fructose-6-phosphate shunt. Cell-free extracts contain fructose-6-phosphate phosphoketolase, aldolase and HMP dehydrogenase. Nitrites not produced from nitrates. Indole and acetyl-methyl-carbinol not produced. Most strains possess a constitutive urease activity, which is not influenced by urea or organic nitrogen sources. Skimmed milk: acidification followed by coagulation in 1–2 days. Riboflavin is the only growth factor required for growth. The above description is based on data obtained from Matteuzzi *et al.* (1971).

Based on DNA relatedness (66–81%), this subspecies is most closely related to *B. longum* subsp. *infantis* and *B. longum* subsp. *longum* (Lauer & Kandler, 1983; Bahaka *et al.*, 1993; Sakata *et al.*, 2002). So far, found only in the faeces of piglets. The genomic G + C content is 62 mol% (T_m).

Type strain: Su859^T (ATCC 27533^T=DSM 20211^T), from faeces of piglet (Matteuzzi *et al.*, 1971).

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References

Bahaka, D., Neut, C., Khattabi, A., Monget, D. & Gavini, F. (1993). Phenotypic and genomic analyses of human strains belonging or

related to *Bifidobacterium longum*, *Bifidobacterium infantis*, and *Bifidobacterium breve*. *Int J Syst Bacteriol* **43**, 565–573.

Biavati, B., Scardovi, V. & Moore, W. E. C. (1982). Electrophoretic patterns of proteins in the genus *Bifidobacterium* and proposal of four new species. *Int J Syst Bacteriol* **32**, 358–373.

Biavati, B., Castagnoli, P., Crociani, F. & Trovatelli, L. D. (1984). Species of the *Bifidobacterium* in the feces of infants. *Microbiologica* **7**, 341–345.

Biavati, B., Castagnoli, P. & Trovatelli, L. D. (1986). Species of the genus *Bifidobacterium* in the feces of human adults. *Microbiologica* **9**, 39–45.

Brenner, D., Staley, J. & Krieg, N. (2001). Classification of procaryotic organisms and the concept of bacterial speciation. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 27–31. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.

Crociani, F., Alessandrini, A., Mucci, M. M. & Biavati, B. (1994). Degradation of complex carbohydrates by *Bifidobacterium* spp. *Int J Food Microbiol* **24**, 199–210.

Fanedi, L., Nekrep, F. V. & Avgustin, G. (1998). Random amplified polymorphic DNA analysis and demonstration of genetic variability among bifidobacteria isolated from rats fed with raw kidney beans. *Can J Microbiol* **44**, 1094–1101.

Frothingham, R., Duncan, A. J. & Wilson, K. H. (1993). Ribosomal DNA sequences of bifidobacteria: implications for sequence-based identification of the human colonic flora. *Microb Ecol Health Dis* **6**, 23–27.

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.

Klein, G. (2006). International Committee on Systematics of Prokaryotes Subcommittee on the taxonomy of *Bifidobacterium*, *Lactobacillus* and related organisms. Minutes of the meetings, 1 and 2 April 2005, Stuttgart-Hohenheim, Germany. *Int J Syst Evol Microbiol* **56**, 2501–2503.

Kullen, M. J., Brady, L. J. & O'Sullivan, D. J. (1997). Evaluation of using a short region of the *recA* gene for rapid and sensitive speciation of dominant bifidobacteria in the human large intestine. *FEMS Microbiol Lett* **154**, 377–383.

Lauer, E. & Kandler, O. (1983). DNA-DNA homology, murein types and enzyme patterns in the type strains of the genus *Bifidobacterium*. *Syst Appl Microbiol* **4**, 42–64.

Leahy, S. C., Higgins, D. G., Fitzgerald, G. F. & van Sinderen, D. (2005). Getting better with bifidobacteria. *J Appl Microbiol* **98**, 1303–1315.

Leblond-Bourget, N., Philippe, H., Mangin, I. & Decaris, B. (1996). 16S rRNA and 16S to 23S internal transcribed spacer sequence analyses reveal inter- and intraspecific *Bifidobacterium* phylogeny. *Int J Syst Bacteriol* **46**, 102–111.

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.

Matsuki, T., Watanabe, K., Tanaka, R. & Oyaizu, H. (1998). Rapid identification of human intestinal bifidobacteria by 16S rRNA-targeted species- and group-specific primers. *FEMS Microbiol Lett* **167**, 113–121.

Matsuki, T., Watanabe, K., Tanaka, R., Fukuda, M. & Oyaizu, H. (1999). Distribution of bifidobacterial species in human intestinal microflora examined with 16S rRNA-gene-targeted species-specific primers. *Appl Environ Microbiol* **65**, 4506–4512.

Matteuzzi, D., Crociani, F., Zani, G. & Trovatelli, L. D. (1971). *Bifidobacterium suis* n. sp.: a new species of the genus *Bifidobacterium* isolated from pig feces. *Z Allg Mikrobiol* **11**, 387–395 (in German).

- Mitsuoka, T. (1969). Comparative studies on bifidobacteria isolated from the alimentary tract of man and animals. *Zentralbl Bakteriol [Orig]* **210**, 52–64.
- Miyake, T., Watanabe, K., Watanabe, T. & Oyaizu, H. (1998). Phylogenetic analysis of the genus *Bifidobacterium* and related genera based on 16S rDNA sequences. *Microbiol Immunol* **42**, 661–667.
- Requena, T., Burton, J., Matsuki, T., Munro, K., Simon, M. A., Tanaka, R., Watanabe, K. & Tannock, G. W. (2002). Identification, detection, and enumeration of human *Bifidobacterium* species by PCR targeting the transaldolase gene. *Appl Environ Microbiol* **68**, 2420–2427.
- Reuter, G. (1963). Vergleichende Untersuchung über die Bifidus-Flora im Säuglings- und Erwachsenenstuhl. *Zentralbl Bakteriol Orig* **191**, 486–507 (in German).
- Roy, D. & Sirois, S. (2000). Molecular differentiation of *Bifidobacterium* species with amplified ribosomal DNA restriction analysis and alignment of short regions of the *ldh* gene. *FEMS Microbiol Lett* **191**, 17–24.
- Roy, D., Berger, J. L. & Reuter, G. (1994). Characterization of dairy-related *Bifidobacterium* spp. based on their beta-galactosidase electrophoretic patterns. *Int J Food Microbiol* **23**, 55–70.
- Roy, D., Ward, P. & Champagne, G. (1996). Differentiation of bifidobacteria by use of pulsed-field gel electrophoresis and polymerase chain reaction. *Int J Food Microbiol* **29**, 11–29.
- Sakata, S., Kitahara, M., Sakamoto, M., Hayashi, H., Fukuyama, M. & Benno, Y. (2002). Unification of *Bifidobacterium infantis* and *Bifidobacterium suis* as *Bifidobacterium longum*. *Int J Syst Evol Microbiol* **52**, 1945–1951.
- Sakata, S., Ryu, C. S., Kitahara, M., Sakamoto, M., Hayashi, H., Fukuyama, M. & Benno, Y. (2006). Characterization of the genus *Bifidobacterium* by automated ribotyping and 16S rRNA gene sequences. *Microbiol Immunol* **50**, 1–10.
- Scardovi, V. (1986). Genus *Bifidobacterium* Orla-Jensen 1924, 472^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1418–1434. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Scardovi, V., Casalicchio, F. & Vincenzi, N. (1979). Multiple electrophoretic forms of transaldolase and 6-phosphogluconic dehydrogenase and their relationships to the taxonomy and ecology of the bifidobacteria. *Int J Syst Bacteriol* **29**, 312–327.
- Schell, M. A., Karmirantzou, M., Snel, B., Vilanova, D., Berger, B., Pessi, G., Zwahlen, M.-C., Desiere, F., Bork, P. & other authors (2002). The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proc Natl Acad Sci U S A* **99**, 14422–14427.
- Sgorbati, B., Scardovi, V. & LeBlanc, D. J. (1982). Plasmids in the genus *Bifidobacterium*. *J Gen Microbiol* **128**, 2121–2131.
- Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C., Nesme, X., Rosselló-Mora, R., Swings, J. & other authors (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* **52**, 1043–1047.
- Staley, J. & Krieg, N. R. (1986). Bacterial classification I. Classification of procaryotic organisms: an overview. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 965–968. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Ventura, M., Canchaya, C., Meylan, V., Klaenhammer, T. R. & Zink, R. (2003). Analysis, characterization, and loci of the *tuf* genes in *Lactobacillus* and *Bifidobacterium* species and their direct application for species identification. *Appl Environ Microbiol* **69**, 6908–6922.
- 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.
- Yamamoto, T., Morotomi, M. & Tanaka, R. (1992). Species-specific oligonucleotide probes for five *Bifidobacterium* species detected in human intestinal microflora. *Appl Environ Microbiol* **58**, 4076–4079.
- Zhu, L., Li, W. & Dong, X. (2003). Species identification of genus *Bifidobacterium* based on partial HSP60 gene sequences and proposal of *Bifidobacterium thermacidophilum* subsp. *porcinum* subsp. nov. *Int J Syst Evol Microbiol* **53**, 1619–1623.