NOTE

Transfer of *Bifidobacterium inopinatum* and *Bifidobacterium denticolens* to *Scardovia inopinata* gen. nov., comb. nov., and *Parascardovia denticolens* gen. nov., comb. nov., respectively

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Bifidobacterium inopinatum Crociani *et al.* 1996 and *Bifidobacterium denticolens* Crociani *et al.* 1996 have distinct phenotypic characteristics and low G+C contents compared with other bifidobacteria. In the 16S rRNA phylogenetic tree, these two species grouped in an independent subcluster. In our previous work, partial heat-shock protein 60 (HSP60) gene-sequence analysis also indicated that these two species had distinct taxonomic positions. In this work, the complete HSP60 genes of five representative bacterial strains were sequenced by using an inverse PCR method. The complete sequence similarities turned out to be at the same level as those of the partial genes, thus confirming the result based on partial sequence analysis. On the basis of all the evidence mentioned above, it is proposed that these two species should be transferred to two new genera as *Scardovia inopinata* gen. nov., comb. nov., and *Parascardovia denticolens* gen. nov., comb. nov.

Keywords: *Scardovia inopinata* gen. nov., comb. nov., *Parascardovia denticolens* gen. nov., comb. nov., 16S rRNA sequence, HSP60 gene sequence

Bifidobacterium inopinatum and *Bifidobacterium denticolens* were described by Crociani *et al.* (1996) as two *Bifidobacterium* species isolated from human dental caries. Although they meet the minimal standard criteria for the genus *Bifidobacterium*, such as producing fructose-6-phosphate phosphoketolase and producing acetic and lactic acid as end-products of glucose fermentation, these two species differ from the other *Bifidobacterium* species in their morphological characteristics, fermentation patterns and DNA base compositions (Crociani *et al.*, 1996).

The G+C contents of *Bifidobacterium inopinatum* DSM 10107^T and *Bifidobacterium denticolens* DSM 10105^T are respectively 45±1 and 55±1 mol%, the former apparently being much lower than, and the

latter at the lowest limit of, those of the other bifidobacteria, which range from 55 to 67 mol% (mostly 58–60 mol%). Such differences contradict the general opinion that organisms whose DNA base compositions differ by more than 10 mol% should not be considered as members of the same genus (Stackebrandt & Liesack, 1993).

In the phylogenetic tree constructed using 16S rRNA gene sequences (Miyake *et al.*, 1998), the bifidobacteria were divided into two subclusters. Subcluster 2 consisted of *Bifidobacterium inopinatum* DSM 10107^T and *Bifidobacterium denticolens* DSM 10105^T and subcluster 1 consisted of all the other members of the genus *Bifidobacterium* plus *Gardnerella vaginalis* ATCC 14018^T. The 16S rRNA sequence similarities to members of subcluster 1 ranged from 90.2 to 91.9% for *Bifidobacterium inopinatum* DSM 10107^T and from 90.7 to 92.8% for *Bifidobacterium denticolens* DSM 10105^T. Similarities among the members of subcluster 1 ranged from 92.3 to 99.9%. Also, the sequence similarity between *Bifidobacterium inopinatum* DSM 10107^T and *Bifidobacterium denticolens* DSM 10105^T was 95.7%. Exceptionally, even *Gardnerella vaginalis*

Abbreviation: HSP, heat-shock protein.

The GenBank accession numbers for the HSP60 gene sequences of *Bifidobacterium inopinatum* DSM 10107^T (= AS 1.2187^T), *Bifidobacterium denticolens* DSM 10105^T (= AS 1.2280^T), *Bifidobacterium adolescentis* JCM 1275^T, *Bifidobacterium lactis* DSM 10140^T and *Gardnerella vaginalis* ATCC 14018^T are respectively AY004281, AF240565, AF210319, AY004282 and AF240579.

Table 1. Restriction enzymes, inverse-PCR parameters and amplified HSP60 gene lengths of different strains

Strain	Enzyme	Forward primer	Reverse primer	Annealing temp. (°C)	Extension time (min)	Inverse-PCR product size (kb)	Sequencing results of inverse-PCR products
<i>B. denticolens</i> DSM 10105 ^T	<i>EcoRI</i>	F425 (5'-TCCAGTCAGCAGGACATCGTTCA-3')	R79 (5'-CGAGCCAGAGGTCACGTTCTTCA-3')	58.2	3	3.0	Complete upstream and downstream flanking HSP60 gene core region
<i>B. inopinatum</i> DSM 10107 ^T	(1) <i>PvuII</i> * (2) <i>EcoRV</i> *	F423 (5'-TTCCAGCCAGGATATGTTTCA-3') F423	R51 (5'-CCTCAGCCCTTCATGGACAGAGACT-3') R51	57.9	3.5	3.5	Complete upstream flanking HSP60 gene core region and downstream to position 1264
<i>B. adolescentis</i> JCM 1275 ^T	(1) <i>PstI</i> (2) <i>PvuII</i>	F510 (5'-CGAGGCTCTGCCACCTGATT-3') F955 (5'-CTGCCGACCTGATTCTGAACAACAT-3')	R124 (5'-GCTGCGACGAGTTCCTTGACGAT-3') R218 (5'-GCTTCGTCATATGCAATCATCTTG-3')	61.6	5	5.5	Complete downstream flanking HSP60 gene core region
<i>B. lactis</i> (<i>B. animalis</i>) DSM 10140 ^T	<i>Sall</i>	F529 (5'-TGCAGCAATCTGTTCTTGTTCTCA-3')	R183 (5'-ATTCTCAACAACATCGTGGCACCCTTC-3')	60.3	6	5.5	Complete upstream and downstream flanking HSP60 gene core region
<i>Gardnerella vaginalis</i> ATCC 14018 ^T	(1) <i>EcoRV</i> (2) <i>DraI</i>	F472 (5'-GGTAAGGCCATTGCTCAT-3') F1803 (5'-CGAGGAGGTTTGCTCCAGCGGTG-3')	R46 (5'-CATTCCTTAAGCCTTCGTG-3') R123 (5'-CTTTCAGCTTCTTGAGCTGCATGAC-3')	51.1	1	1.5	Complete upstream flanking HSP60 gene core region and downstream to position 1377
				55.9	1	1.1	Complete downstream region of HSP60 gene from position 1215

* The complete HSP60 gene sequence was obtained by running the inverse-PCR procedure twice (1, 2).

ATCC 14018^T, another member of the family *Bifidobacteriaceae* (Stackebrandt *et al.*, 1997), has higher sequence similarities (91.6–95.3 %) to the other *Bifidobacterium* species than do *Bifidobacterium inopinatum* DSM 10107^T and *Bifidobacterium denticolens* DSM 10105^T. This suggested that *Bifidobacterium inopinatum* and *Bifidobacterium denticolens* might be more phylogenetically distinct than *Gardnerella vaginalis* within the family *Bifidobacteriaceae*, and could be considered as two separate genera of the family *Bifidobacteriaceae*.

Heat-shock protein (HSP) 60 has been determined as another conserved molecule for use in bacterial phylogenetic analysis (Kwok *et al.*, 1999; Viale *et al.*, 1994). Recently, we analysed the sequence similarity of 538 bp fragments in the highly conserved HSP60 genes of 35 *Bifidobacterium* strains and of *Gardnerella vaginalis* ATCC 14018^T (Jian *et al.*, 2001). In the phylogenetic tree constructed, *Bifidobacterium inopinatum* DSM 10107^T, *Bifidobacterium denticolens* DSM 10105^T and the other bifidobacteria were grouped into three distinct subclusters. Both *Bifidobacterium inopinatum* DSM 10107^T and *Bifidobacterium denticolens* DSM 10105^T showed exceptionally low levels of similarity to all the other members of the genus *Bifidobacterium* (76–80 and 73–78 %, respectively), whereas all the other members exhibited sequence similarities of 81–98 %. In the phylogenetic tree, *Gardnerella vaginalis* ATCC 14018^T and *Bifidobacterium inopinatum* DSM 10107^T were grouped in one subcluster, which correlated with their DNA base compositions (42 mol % for *Gardnerella vaginalis* ATCC 14018^T and 45 mol % for *Bifidobacterium inopinatum* DSM 10107^T). However, the sequence similarity between them was just 79 %.

In order to confirm the accuracy of the phylogenetic result based on partial HSP60 gene sequences, we chose five representative strains for complete HSP60 gene sequencing in this work. These were *Bifidobacterium inopinatum* DSM 10107^T, *Bifidobacterium denticolens* DSM 10105^T, *Bifidobacterium adolescentis* JCM 1275^T, *Bifidobacterium lactis* DSM 10140^T (synonym of *Bifidobacterium animalis*; Cai *et al.*, 2000) and *Gardnerella vaginalis* ATCC 14018^T. The complete HSP60 genes of these bacteria were amplified by using an inverse PCR approach (Ochman *et al.*, 1988). First, bacterial genomic DNA was digested with different endonucleases in order to choose an appropriate enzyme(s), which was determined by examining the sequences of the HSP60 gene core region (the sequenced 538 bp fragment) and the appropriate fragment lengths (normally 3–5 kb) by Southern blotting using the core region as the probe. Once a suitable enzyme had been selected, the genomic DNA was digested with this enzyme and self-circularized by T4 ligase at a concentration of 2 ng DNA µl⁻¹ at 10–12 °C for 24–48 h. The circularized DNA was then used as the template in the inverse PCR, in which 1 pmol µl⁻¹ of each PCR primer (designed according to the core sequences) was used. Thirty thermocycles were per-

Fig. 1. Phylogenetic tree based on 1597 bp fragments of HSP60 DNA sequences. The tree was rooted with *Bacillus subtilis* W168 and constructed using the Fitch method with bootstrap values calculated from 1000 trees. The numbers at each branch-point represent the percentage bootstrap support. The GenBank accession numbers of the HSP60 gene sequences are given in parentheses. Bar, 5% sequence divergence.

formed, each cycle consisting of denaturation at 94 °C for 30 s, annealing at different temperatures (shown in Table 1) for 30 s and elongation at 72 °C for 1–6 min. The PCR products were ligated with pUCm-T and the recombinant plasmids were transformed into *Escherichia coli* DH5 α . The inserts containing the HSP60 gene were sequenced and the sequenced fragments were joined into complete gene sequences. Table 1 lists the experimental conditions for sequencing of the five bacterial HSP60 genes, including the endonucleases, inverse PCR primers and thermocycling parameters used and the lengths of the amplified products. For complete HSP60 sequencing of *Bifidobacterium inopinatum* DSM 10107^T, *Bifidobacterium adolescentis* JCM 1275^T and *Gardnerella vaginalis* ATCC 14018^T, two enzymes were used and a second run of inverse PCRs was conducted.

The complete HSP60 sequences determined in this study were compared by using CLUSTAL W software (version 1.5). Continuous fragments of 1597 bp, ranging from positions 1 to 1597 (accounting for 99%) of the reading frame of the HSP60 genes, were used for similarity analysis and a phylogenetic tree rooted with *Bacillus subtilis* W168 was constructed using the Fitch method with the PHYLIP software package (Fig. 1). Analysis showed that the similarities of the whole genes among the five bacteria were at the same level as the similarities for their partial genes, the differences being approximately $\pm 2.4\%$. The results demonstrate that the 538 bp segments were highly representative of the whole HSP60 gene in phylogenetic studies.

On the basis of the results obtained from the phylogenetic analysis of both 16S rRNA and HSP60 sequences, the DNA base compositions and some phenotypic characteristics, we propose that *Bifidobacterium inopinatum* DSM 10107^T and *Bifidobacterium denticolens* DSM 10105^T should be transferred to the new genera *Scardovia* gen. nov. and *Parascardovia* gen. nov. as *Scardovia inopinata* comb. nov. and *Parascardovia denticolens* comb. nov., respectively.

Description of *Scardovia* gen. nov.

Scardovia (Scar.do'vi.a. N.L. fem. n. *Scardovia* named after Vittorio Scardovi, an Italian microbiologist who has made many contributions to our knowledge of bifidobacteria).

Rod-shaped cells that are small and coccoid and of variable shape. Gram-positive. Non-acid-fast. Non-spore-forming. Non-motile. Anaerobic. Saccharoclastic. The fermentation products from glucose are L-(+)-lactic acid and acetic acid at a molar ratio of 1:2.9. Dextran is fermented. The G + C content of the DNA of the type species of this genus is 45 ± 1 mol%. Found in human dental caries. According to 16S rRNA analysis and HSP60 gene-sequence comparisons, *Scardovia* represents a new genus belonging to the family *Bifidobacteriaceae*. Only one species, the type species *Scardovia inopinata* comb. nov., has been described.

Description of *Scardovia inopinata* (Crociani et al. 1996) comb. nov.

Scardovia inopinata (in.o.pin.a'ta. L. fem. adj. *inopinata* unexpected, referring to the very unusual morphology).

The description of the species was provided by Crociani et al. (1996). The type strain is DSM 10107^T (= AS1.2187^T).

Description of *Parascardovia* gen. nov.

Parascardovia (Pa.ra.scar.do'vi.a. N.L. fem. n. *Parascardovia* resembling *Scardovia*).

Small, slender rods of variable shape. Gram-positive. Non-acid-fast. Non-spore-forming. Non-motile. Anaerobic. Saccharoclastic. The fermentation products from glucose are L-(+)-lactic acid and acetic acid at a molar ratio of 1:2. Dextran is fermented. The G + C content of the DNA of the type species of this genus is 55 ± 1 mol%. Found in human dental caries. According to 16S rRNA analysis and HSP60 gene-sequence analysis, *Parascardovia* represents a new genus belonging to the family *Bifidobacteriaceae*. Only one species, the type species *Parascardovia denticolens* comb. nov., has been described.

Description of *Parascardovia denticolens* (Crociani et al. 1996) comb. nov.

Parascardovia denticolens (den.ti.co'lens. L. masc. n. *dens*, dentis tooth; L. v. *colere* to dwell; L. pres. part. *colens* dwelling; N.L. adj. *denticolens* tooth-dwelling).

The description of the species was provided by Crociani *et al.* (1996). The type strain is DSM 10105^T (= AS 1.2280^T).

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