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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**Abbreviation:** HSP, heat-shock protein.

The GenBank accession numbers for the HSP60 gene sequences of *Bifidobacterium inopinatum* DSM 10107<sup>T</sup> (Accession: AY004281), *B. denticolens* DSM 10105<sup>T</sup> (Accession: AF240565), *Bifidobacterium adolescentis* ICM 1275<sup>T</sup> (Accession: AF210319), *Gardnerella vaginalis* ATCC 14018<sup>T</sup> (Accession: AY004282) and *Parascardovia denticolens* DSM 10105<sup>T</sup> (Accession: AF240579).
Table 1. Restriction enzymes, inverse-PCR parameters and amplified HSP60 gene lengths of different strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Enzyme</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Annealing temp. (°C)</th>
<th>Extension time (min)</th>
<th>Inverse-PCR product size (kb)</th>
<th>Sequencing results of inverse-PCR products</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. denticolens DSM 10105*</td>
<td>EcoRI</td>
<td>F425 (5'-TCCAGTCGGAGGATCGTTTCA-3')</td>
<td>R79 (5'-CGAGCGATGTTACGGTCTTCA-3')</td>
<td>58</td>
<td>3</td>
<td>30</td>
<td>Complete upstream and downstream flanking HSP60 gene core region</td>
</tr>
<tr>
<td>B. adolescentis DSM 10107*</td>
<td>PstI</td>
<td>F516 (5'-GCTGCGATGTTACGGTCTTCA-3')</td>
<td>R124 (5'-CGAGCCAGGATCGTTTCA-3')</td>
<td>55</td>
<td>3</td>
<td>55</td>
<td>Complete downstream flanking HSP60 gene core region</td>
</tr>
<tr>
<td>B. lactis (B. animalis) DSM 10107</td>
<td>SfiI</td>
<td>F427 (5'-TCCAATGCTTGTACGTTTCA-3')</td>
<td>R134 (5'-GCTGCGATGTTACGGTCTTCA-3')</td>
<td>86</td>
<td>3</td>
<td>32</td>
<td>Complete upstream and downstream flanking HSP60 gene core region</td>
</tr>
<tr>
<td>Gardnerella vaginalis ATCC 14018</td>
<td>EcoRV</td>
<td>F472 (5'-GGTAAGGCCATTTGACGTTTCA-3')</td>
<td>R46 (5'-GCTGCGATGTTACGGTCTTCA-3')</td>
<td>51</td>
<td>1</td>
<td>15</td>
<td>Complete downstream flanking HSP60 gene core region</td>
</tr>
<tr>
<td>Gardnerella vaginalis ATCC 14018</td>
<td>Ndel</td>
<td>F180 (5'-CGAGAGGAGCTTGTACGTTTCA-3')</td>
<td>R123 (5'-GCTGCGATGTTACGGTCTTCA-3')</td>
<td>55</td>
<td>1</td>
<td>11</td>
<td>Complete downstream flanking HSP60 gene from position 1215</td>
</tr>
</tbody>
</table>

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

In order to confirm the accuracy of the phylogenetic tree constructed, a clone representing strains from complete HSP60 gene sequences was used to confirm the accuracy of the phylogenetic tree constructed. The complete HSP60 gene sequences of B. adolescentis DSM 10107 and B. denticolens DSM 10105 were subjected to computer analysis using the phylogenetic tree constructed. The results showed that the phylogenetic tree was consistent with the results of the phylogenetic analysis.
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 DH5α. 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 10107T, Bifidobacterium adolescentis JCM 1275T and Gardnerella vaginalis ATCC 14018T, 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 ±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 10107T and Bifidobacterium denticolens DSM 10105T 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 coccolid and of variable shape. Gram-positive. Non-acid-fast. Non-spore-forming. Non-motile. Anaerobic. Saccharo-clastic. The fermentation products from glucose are l-(+)-lactic acid and acetic acid at a molar ratio of 1:29. 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 10107T (= AS1.2187T).

Description of Parascardovia gen. nov.

Parascardovia (Par.ascard.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. Saccharo-clastic. 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’len.s. 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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**References**


