Bifidobacterium gallicum sp. nov. Isolated from Human Feces

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A bifidobacterial isolate from human feces was found to have very low genetic relatedness to any previously described species of the genus. This strain, which also contained a unique type of peptidoglycan, L-lysine-L-alanine-L-serine (A3c), is considered to represent a new species, which is designated Bifidobacterium gallicum. Its description is presented. The type strain is strain DSM 20093.

On the basis of biochemical and DNA-DNA hybridization studies (4), the existence of at least 20 well-defined species within the genus Bifidobacterium Orla-Jensen 1924 has been clearly substantiated. The principal differentiating characteristics have been shown to be the type of peptidoglycan and the relative electrophoretic mobilities of some enzymes, including NAD-dependent L- (+)-lactate dehydrogenase (EC 1.1.1.27) and transaldolase (EC 2.2.1.2).

Based on the application of the same methods for phenotypic and genotypic characterization, an additional genus species is now presented, which has unique characteristics. The description of the new species, which is designated Bifidobacterium gallicum, has not been published for more than 10 years, because of the unfortunate fact that additional strains could not be isolated.

The origins of strains (Table 1) and the methods used for phenotypic characterization and in vitro DNA-DNA hybridization have been described previously (3, 4). By using the filter technique of Denhardt (1) in the hybridization experiments, specific DNA binding was obtained at 55°C in 3× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate) containing 30% formamide, which according to McComnaughy et al. (6) is equivalent to the reaction conditions at 25°C below the melting point of the test DNA, which has a G+C content of 61 mol%. The G+C content was determined in 0.1× SSC by using the usual thermal denaturation method (5) and DNA of Escherichia coli ATCC 8739 as the reference (G+C content, 50 mol%).

The type strain of B. gallicum sp. nov., which was originally designated strain P6, was obtained from H. Beerens (Institut Pasteur de Lille, Lille, France) in 1967 as a human fecal isolate. However, the true habitat of the new species remains uncertain for the time being, because additional strains have not been isolated in the 20 years during which the strain has survived as a “dormant” collection strain (strain DSM 20093T [T = type strain]) that was not known to researchers in intestinal ecology. Its morphological appearance is shown in Fig. 1.

The assignment of strain P6T to the genus Bifidobacterium is evident from the usual bacteriological characteristics (see below), which were obtained by testing as described by Mitsuoka et al. (7), as well as from the kind of metabolism, which is in accordance with the typical bifidoshunt (3). This is evident from the strictly anaerobic growth of the strain on carbohydrate-containing complex media and the formation of acetic and lactic acids at concentrations ranging from 50 to 70 and 2 to 4 μmol/ml, respectively. In addition, relatively high concentrations of ethanol (about 8 to 10 μmol/ml) were found, indicating an extensive phosphorolytic split of catabolically intermediary pyruvate. Key enzymes of the bifidoshunt could also be demonstrated (see below).

The phenotypic differentiation of B. gallicum sp. nov. at the species level is based mainly on the type of peptidoglycan, the most important feature in the taxonomy of bifidobacteria (4). As Table 1 shows, the peptidoglycan of strain P6T is a unique type, L-lysine-L-alanine-L-serine (A3c). The same type has been found previously in Lactobacillus viridescens, Lactobacillus halotolerans (2), and some variants of Leuconostoc oenos (unpublished data).

Identification of B. gallicum sp. nov. is possible from its carbohydrate fermentation pattern; pentoses and starch are fermented, whereas lactose, melibiose, and raffinose are not. This unusual pattern shares some similarities only with Bifidobacterium cuniculi, which was isolated from rabbits (8). However, this species is also distinct, because it is melibiose (and celllobiose) positive (4, 8), provided that the species-specific characteristics of strain P6T are confirmed with further isolates.

As Table 1 also shows, the levels of genetic relatedness of strain P6T to other representative strains are low (only 8 to 25% DNA-DNA relatedness). Unfortunately, the species Bifidobacterium gallinarum was not available at the time that the hybridization experiments with strain P6T were done. However, according to its description (9), B. gallinarum is clearly different based on its DNA G+C content (64 to 67 mol%), whereas a value of 61 mol% has been found for strain P6T.

A definition of the new genospecies based on the thermal denaturation method, as recommended by Wayne et al. (10), is not stressed here, because only one strain was available, and the levels of relatedness to other species are low.

Description of Bifidobacterium gallicum sp. nov. Bifidobacterium gallicum (gal′li.cum. N.L. adj. gallicus, of or belonging to the Gauls, although ethnic relationships to the French remain speculatative). Gram positive. Short rods with rounded ends, generally 0.7 to 0.9 by 1.5 to 3.0 μm, mostly arranged in pairs or short chains when grown in liquid culture. Irregular elongations and swellings occur in cells obtained from colony growth. Spores are not formed. Nonmotile. Not flagellated. Slimy, capsulike material is excreted in complex media containing meat extract, bacteriological peptone, salts, and fermentable carbohydrates.

Colonies are whitish, opaque, round, and entire and have a soft consistency. A weak odor of acetic acid is produced in fresh cultures.

The main fermentation product is acetic acid, and its concentration is about 15 times that of L- (+)-lactic acid; in addition, variable amounts of ethanol (and certainly formic acid) are formed. No D-(-)-lactic acid or gas is produced. Metabolism is strictly anaerobic. Obligatory saccharolytic via the characteristic bifidoshunt.

Optimum growth occurs at 37 to 39°C; no growth occurs
B. pseudocatenulatum

**TABLE 1. DNA-DNA hybridization values for *B. gallicum* sp. nov. and peptidoglycan types in the genus *Bifidobacterium***

<table>
<thead>
<tr>
<th>Strain</th>
<th>Peptidoglycan type</th>
<th>% Relatedness to <em>B. gallicum</em> P6&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. gallicum</em> P6&lt;sup&gt;+&lt;/sup&gt; (= DSM 20093&lt;sup&gt;T&lt;/sup&gt;)</td>
<td>Lys-Ala-Ser</td>
<td>100</td>
</tr>
<tr>
<td><em>B. magnus</em> DSM 20222&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Ala-Ala-Ala</td>
<td>23</td>
</tr>
<tr>
<td><em>B. catenulatum</em> DSM 20103&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Ala-Ser</td>
<td>15</td>
</tr>
<tr>
<td><em>B. catenulatum</em> DSM 20244</td>
<td>Lys-Ala-Ser(Ser)</td>
<td>17</td>
</tr>
<tr>
<td><em>B. catenulatum</em> DSM 20437</td>
<td>Lys-Ala-Ser</td>
<td>18</td>
</tr>
<tr>
<td><em>B. pseudocatenulatum</em> DSM 20438&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Ala-Ser(Ser)</td>
<td>21</td>
</tr>
<tr>
<td><em>B. pseudolongum</em> DSM 20099&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Ala-Ala-Ala</td>
<td>22</td>
</tr>
<tr>
<td><em>B. pseudolongum</em> DSM 20092</td>
<td>Lys-Ala-Ala-Ala</td>
<td>25</td>
</tr>
<tr>
<td><em>B. minimum</em> DSM 20102&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Ser</td>
<td>16</td>
</tr>
<tr>
<td><em>B. cheenterium</em> DSM 20434&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Ser-Ala-Ala</td>
<td>18</td>
</tr>
<tr>
<td><em>B. cuniculii</em> DSM 20435&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Ser-Ala-Ala</td>
<td>17</td>
</tr>
<tr>
<td><em>B. animis DSM</em> 20104&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Ser-Ala-Ala</td>
<td>20</td>
</tr>
<tr>
<td><em>B. longum</em> DSM 20219&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Ser-Ala-Thr-Ala</td>
<td>18</td>
</tr>
<tr>
<td><em>B. suis</em> DSM 20211&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Ser-Ala-Thr-Ala</td>
<td>16</td>
</tr>
<tr>
<td><em>B. infantis</em> DSM 20088&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Ser-Ala-Thr-Ala</td>
<td>18</td>
</tr>
<tr>
<td><em>B. breve</em> DSM 20213&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Glu</td>
<td>11</td>
</tr>
<tr>
<td><em>B. asteroides</em> DSM 20089&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Glu</td>
<td>8</td>
</tr>
<tr>
<td><em>B. asteroides</em> DSM 20431</td>
<td>Lys-Glu</td>
<td>9</td>
</tr>
<tr>
<td><em>B. bifidum</em> DSM 20456&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Ser-Asp</td>
<td>16</td>
</tr>
<tr>
<td><em>B. bifidum</em> DSM 20082</td>
<td>Orn-Ser-Ala-Ala</td>
<td>15</td>
</tr>
<tr>
<td><em>B. bauri</em> DSM 20432&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Glu-Ala-Ala</td>
<td>16</td>
</tr>
<tr>
<td><em>B. thermophilum</em> DSM 20210&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Glu</td>
<td>20</td>
</tr>
<tr>
<td><em>B. thermophilum</em> DSM 20209</td>
<td>Orn-Glu</td>
<td>18</td>
</tr>
<tr>
<td><em>B. thermophilum</em> DSM 20440</td>
<td>Orn-Glu</td>
<td>18</td>
</tr>
<tr>
<td><em>B. subtile</em> DSM 20096&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Asp</td>
<td>13</td>
</tr>
<tr>
<td><em>B. adolescentis</em> DSM 20086</td>
<td>Lys-Asp</td>
<td>24</td>
</tr>
<tr>
<td><em>B. dentium</em> DSM 20436&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Orn-Asp</td>
<td>19</td>
</tr>
<tr>
<td><em>B. dentium</em> DSM 20221</td>
<td>Lys-Asp</td>
<td>19</td>
</tr>
<tr>
<td><em>B. angulatum</em> DSM 20099&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Asp</td>
<td>18</td>
</tr>
<tr>
<td><em>B. pullorum</em> DSM 20433&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Asp</td>
<td>22</td>
</tr>
<tr>
<td><em>B. indicum</em> DSM 20214&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Lys-Asp</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> As determined by DNA-DNA hybridization.

The cell wall polysaccharide comprises a galactan polymer of unknown structure; minor amounts of rhamnose may be found in addition to galactose after growth on less complex media.

The G+C content of the DNA is 61 mol% (thermal denaturation method).

The levels of DNA-DNA relatedness to other bifidobacteria range from 8 to 25%.

Habitat: not known. Only one strain has been isolated from human feces.

The type strain is strain DSM 20093 (= P6).

**LITERATURE CITED**


