Reclassification of *Actinobacillus muris* as *Muribacter muris* gen. nov., comb. nov.

Werner Nicklas,¹ Magne Bisgaard,² Bent Aalbæk,³ Peter Kuhnert⁴ and Henrik Christensen⁵

¹Microbiological Diagnostics, German Cancer Research Centre, D-69120 Heidelberg, Germany  ⁠
²Professor emeritus, Horsevænget 40, DK-4130 Viby Sjælland, Denmark  ⁠
³Department of Veterinary Disease Biology, VetSchool, University of Copenhagen, 4 Stigbejen, DK-1870 Frederiksberg C, Denmark  ⁠
⁴Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Laenggass-Strasse 122, CH-3001 Bern, Switzerland

To reinvestigate the taxonomy of *Actinobacillus muris*, 474 strains, mainly from mice and rats, were characterized by phenotype and 130 strains selected for genotypic characterization by 16S rRNA and partial *rpoB* gene sequencing. The type strain was further investigated by whole-genome sequencing. Phylogenetic analysis of the DNA sequences showed one monophyletic group with intragroup similarities of 96.7 and 97.2 % for the 16S rRNA and *rpoB* genes, respectively. The highest 16S rRNA gene sequence similarity to a taxon with a validly published name outside the group was 95.9 %, to the type strain of *Pasteurella pneumotropica*. The closest related taxon based on *rpoB* sequence comparison was *Haemophilus influenzae-murium*, with 88.4 % similarity. A new genus and a new combination, *Muribacter muris* gen. nov., comb. nov., are proposed based on a distinct phylogenetic position based on 16S rRNA and *rpoB* gene sequence comparisons, with major divergence from the existing genera of the family *Pasteurellaceae*. The new genus has the characteristics of *A. muris* with the emendation that acid formation from (-)-d-mannitol and hydrolysis of aesculin are variable, while the *α*-glucosidase test is positive. There is no requirement for exogenously supplied NAD (V factor) for the majority of strains investigated; however, one strain was found to require NAD. The major fatty acids of the type strain of *Muribacter muris* were C₁₄ : 0, C₁₄ : 0 3-OH/iso-C₁₆ : ₁ I, C₁₆ : ₁ 9/₇c and C₁₆ : ₀, which is in line with most genera of the *Pasteurellaceae*. The type strain of *Muribacter muris* is CCUG 16938T (=NCTC 12432T=ATCC 49577T).

*Actinobacillus muris* was originally described based on 19 strains isolated from the oral cavity of healthy mice, with the provisional designation Bisgaard taxon 12 and the selection of a previously described reference strain (Ackerman80-443D⁴⁵=NCTC 12432T) as the type strain (Bisgaard, 1986) (Table S1, available in the online Supplementary Material). DNA reassociation studies showed that *A. muris* as a species was unrelated to other members of *Actinobacillus*, and further that *Pasteurella pneumotropica* and *A. muris* were also unrelated to each other at the species level (Piechulla et al., 1985; Ryll et al., 1991). Phylogenetic analysis based on 16S rRNA gene sequence comparison documented a ‘rodent group’ within the *Pasteurellaceae* that included *P. pneumotropica* and *A. muris* (Dewhirst et al., 1993), both taxa being unrelated to *Actinobacillus sensu stricto* and *Pasteurella sensu stricto*. Further investigations have confirmed that *A. muris* is unrelated to *Actinobacillus sensu stricto* (Christensen & Bisgaard, 2004). Recently, a new genus, *Necropsobacter*, unrelated to *P. pneumotropica* and *A. muris*, has been described that mainly included organisms from rodents (Christensen et al., 2011). In the 16S rRNA gene multiple alignment, strains of *Necropsobacter* had two characteristic deletions through positions 203–206 and 213–216 (GenBank accession no. AY078999 for *Pasteurella multocida* NCTC 10322T) compared with other members of *Pasteurellaceae* (Christensen et al., 2011). *Mesocricetibacter intestinalis* and *Cricetibacter*
osteomyelitidis were recently described based on the characterization of strains isolated from hamsters (Christensen et al., 2014). In the present study, a collection of strains belonging to the Pasteurellaceae obtained from rodents was subjected to extended phenotypic characterization. Partial rpoB sequences were used to evaluate characters used for phenotypic identification and separation of taxa, and comparison of 16S rRNA gene sequences was used to evaluate genotypic diversity mainly at the genus level. The study aimed to reclassify [Actinobacillus] muris away from Actinobacillus sensu stricto as a new combination in a separate new monotypic genus, Muribacter muris gen. nov., comb. nov., and further to investigate the diversity of this taxon, which may lead to improved identification and consequently better understanding of its epidemiology and clinical implications. Phenotypic diversity of [A.] muris has been reported with respect to acid production from cellobiose, mannitol and salicin, hydrolysis of ascelulin, production of indole and urease activity (Nicklas, 2007); however, most biochemical profiles of A. muris have never been mentioned in the literature, and some members of this taxon may have been misidentified (e.g. as P. multocida) or not identified at all. The current investigation shows that all members of the taxon [A.] muris are frequently found in colonies of laboratory mice.

We included the type strain of [A.] muris and 473 additional isolates in the characterization (Tables S1 and S2). The strains were isolated during the period 1980–2014 and represented mainly isolates from mice and a few isolates from rats. Mice and rats sampling positive were received from other research institutions and universities or from commercial breeders of laboratory rodents; other bacterial isolates were received from other diagnostic laboratories. In addition to laboratory mice and rats, wild rodents were trapped. In addition, mice and rats were bought from 10 different pet shops. Isolates were subjected to phenotypic characterization using 40 biochemical criteria examined by conventional tests as reported previously (Christensen et al., 2014). In these tests, acid formation from carbohydrates was tested in phenol red broth base (Difco Laboratories) supplemented with 1 % of the respective carbohydrate and read after 2–3 days of incubation at 37 °C. All other reactions were read after 18–24 h of incubation or as recommended by the author cited below. Hydrolysis of aseculin was tested in aseculioin broth (Merck). Urease, indole and amino acid decarboxylase tests were performed as recommended by Kilian (1976). The requirement for growth factors was tested with filter paper discs containing 12.5 μg NAD (Roche Diagnostics) or 25 μg haem (Sigma) on Mueller–Hinton agar (Heipha). The ability to synthesize porphyrins from δ-aminolaevulinc acid was demonstrated under UV light in a dark room and by addition of Kovac’s indole reagent (Merck) as described by Kilian (1976). Phenotypic characters shared by all strains investigated (some included for genus-level separation; Table 1) were in accordance with the description of [A.] muris (Bisgaard, 1986) except for variable reactions in acid formation from (−)-D-mannitol as well as variable reactions for indole production, urease and hydrolysis of aseculin. The α-glucosidase test (PNPG; 4-nitrophenyl α-D-glucopyranoside) was found to be positive.

On the basis of phenotypic diversity, 16 biovars were identified (Table S1). We then selected isolates for further characterization by DNA sequencing from each biovar, representing animals coming from different sources and different years (Table S1).

16S rRNA gene sequencing of 125 strains was performed as reported previously (Angen et al., 2003; Christensen et al., 2002). In addition, the 16S rRNA gene of the reference strain of ‘Haemophilus influenzae-murium’ was sequenced, since numerous ambiguous positions were present in the published sequence (GenBank accession no. AF024530). Partial sequencing of the rpoB gene of 127 strains was performed according to previously described protocols (Korczak et al., 2004; Korczak & Kuhnert, 2008; Kuhnert et al., 2004). All GenBank accession numbers are listed in Table S1.

Searches for sequences in public databases were performed by BLAST (Altschul et al., 1997). Pairwise similarity was determined by the water program of EMBOS (Rice et al., 2000). In addition to the 16S rRNA gene sequences determined in the current investigation, published sequences were included of strains of [A.] muris, ‘H. influenzae-murium’ and taxon 17 of Bisgaard as well as type strains of type species of genera of the Pasteurellaceae, in addition to the type strain of [P.] pneumotropica (biovar Jawetz) and the reference strain of biovar Heyl of this species (Fig. 1).

Genome sequencing of the type strain of [A.] muris was done by Illumina Hiseq 2000 and reads were assembled by CLC Genomic Workbench version 7.5. Automatic annotation was performed by RAST (http://rast.nmpdr.org/; Overbeek et al., 2014). The DNA G + C content was 43.7 %, as determined by whole-genome sequencing. The DNA G + C content was previously reported as 46.9 mol%, as determined by the DNA renaturation method (Pielchulla et al., 1985), and the difference may be related to the different methodologies applied.

The genome could be assembled to 2 684 001 nt on 148 contigs, and 2810 coding sequences were identified by RAST, 1376 of which could be associated with a known function based on the database search included with RAST.

Multiple alignments of DNA sequences were constructed by Clustal X2 (Larkin et al., 2007). Columns with gaps were removed from the multiple alignment by the use of BioEdit (Hall, 1999). Phylogenetic analysis of the 16S rRNA and rpoB gene sequences was carried out by neighbour-joining using the Jukes–Cantor correction and included calculation of bootstrap support. MEGA6 (Tamura et al., 2011) was used for graphical representation of trees. Two sequences were excluded from the 16S rRNA
Table 1. Phenotypic separation of *Muribacter* gen. nov. from existing genera of the Pasteurellaceae

Genera: 1, *Muribacter* gen. nov.; 2, *Mesocricetibacter* (data from Christensen et al., 2014); 3, *Cricetibacter* (Christensen et al., 2014); 4, *Haemophilus sensu stricto* (Kilian, 2005; Nørskov-Lauritsen et al., 2005; Winslow et al., 1917; Zinnemann & Biberstein, 1974); 5, *Actinobacillus sensu stricto* (Brumpt, 1910; Christensen & Bisgaard, 2004); 6, *Lonepinella* (Osawa et al., 1995); 7, *Mannheimia* (Angen et al., 1999); 8, *Pasteella sensu stricto* (Trevisan, 1887; Mutters et al., 1985; Christensen & Bisgaard, 2006); 9, *Phocoenobacter* (Foster et al., 2000); 10, *Galibacterium* (Bisgaard et al., 2009); 11, *Volucribacter* (Christensen et al., 2004a); 12, *Histophilus* (Angen et al., 2003); 13, *Avibacterium* (Blackall et al., 2005); 14, *Gallibacterium* (Kuhnert et al., 2004); 15, *Bibersteinia* (Blackall et al., 2007); 16, *Aggregatibacter* (Nørskov-Lauritsen & Kilian, 2006); 17, *Bafsa* (Kuhnert et al., 2010); 18, *Chelonobacter* (Gregersen et al., 2009); 19, *Necropsobacter* (Christensen et al., 2011); 20, *Bisgaardia* (Foster et al., 2011); 21, *Oriatribacter* (Hansen et al., 2012); 22, *Frederiksenia* (Bisgaard & Mutters, 1986; Korczak et al., 2014); 23, *Vespertilibacter* (Mühlendorf et al., 2014). Characters are scored as: +, 90 % or more of strains positive within 1–2 days; −, less than 10 % of strains positive within 14 days; ( +), 90 % or more of the strains positive within 3–14 days; d, 11–89 % of strains positive; w, weakly positive; ND, no data available. All tests were performed at 37 °C.

<table>
<thead>
<tr>
<th>Character</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>d</td>
<td>d</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td><em>d</em></td>
<td>−</td>
<td>d</td>
<td>+</td>
<td>−</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>X-factor requirement†</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>V-factor requirement‡</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td><em>d</em></td>
<td>−</td>
<td>d</td>
<td>+</td>
<td>−</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>−</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Methyl red test</td>
<td>−</td>
<td>W</td>
<td>W</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Voges–Proskauser reaction</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Hydrolysis of Tween 80</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+)-l-Arabinose</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>d</td>
<td>+</td>
<td>d</td>
<td>+</td>
<td>d</td>
<td>ND</td>
<td>d</td>
<td>d</td>
<td>ND</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>−</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>d</td>
<td>+</td>
<td>d</td>
<td>d</td>
<td>ND</td>
<td>d</td>
<td>ND</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>(−)-d-Ribose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>d</td>
<td>+</td>
<td>d</td>
<td>+</td>
<td>ND</td>
<td>d</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>d</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>(−)-d-Mannose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>d</td>
<td>+</td>
<td>d</td>
<td>+</td>
<td>ND</td>
<td>d</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>d</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>d</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dextrin</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>d</td>
<td>−</td>
<td>d</td>
<td>−</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>d</td>
<td>−</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Arbutin</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Growth on MacConkey agar</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>W</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>α-Glucosidase (PNPG)</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>d</td>
<td>ND</td>
<td>ND</td>
<td>d</td>
<td>ND</td>
<td>d</td>
<td>−</td>
<td>d</td>
<td>ND</td>
<td>d</td>
<td>ND</td>
<td>d</td>
<td>ND</td>
<td>d</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>43.7%</td>
<td>47.5%</td>
<td>41.9%</td>
<td>39%</td>
<td>35.5%</td>
<td>37.5%</td>
<td>39.2%</td>
<td>37.7%</td>
<td>41.5%</td>
<td>39.9%</td>
<td>40.8%</td>
<td>44.2%</td>
<td>ND</td>
<td>44.2%</td>
<td>44.2%</td>
<td>42.6%</td>
<td>42.4%</td>
<td>42.5%</td>
<td>47.2%</td>
<td>52.5%</td>
<td>39.5%</td>
<td>36.2%</td>
<td>43.5%</td>
</tr>
</tbody>
</table>

*Not part of the formal genus description.
†Dependence on haeminy for growth in vitro.
‡Dependence on NAD (or related substances) for growth in vitro.
§Percentage value determined from the whole-genome sequence of the type strain.
gene sequence-based multiple alignments, since they were too short (Table S1). The multiple alignment included at least 1173 nt of the remaining strains.

The 16S rRNA gene sequence-based phylogenetic comparisons documented a monophyletic group of strains isolated from rodents including the type strain of *A. muris* (Figs. 1 and S1). This group is referred to as *Muribacter* in the following discussion. The lowest similarity within the group was 96.7 %. This is slightly below the normal lower limit of 16S rRNA gene sequence similarity within a species (Stackebrandt & Goebel, 1994); however, all members of the taxon were related in a continuum, and neither genotypic nor phenotypic differences justified separation into more species. The highest similarity outside the group was found to the reference strain (HIM565-1) of *H. influenzae-murium*, with 96.4 % similarity to

![Phylogenetic tree](image-url)

**Fig. 1.** Phylogenetic relationships between the type strain of *Muribacter muris* gen. nov., comb. nov. and members of other genera of the *Pasteurellaceae* as well as some reference taxa, based on neighbour-joining analysis of 16S rRNA gene sequences. Support for monophyletic groups by bootstrap analysis is indicated as numbers out of 100. Bar, 0.01 substitutions per nucleotide position, considering the model for nucleotide substitution (Jukes & Cantor) and tree shape used in the neighbour-joining analysis.
strain 1999906011, which is below the recognized 16S rRNA gene sequence similarity threshold of 97 % for species separation (Stackebrandt & Goebel, 1994). Characterization of ‘H. influenzae-murium’ was not the aim of this paper and, since this taxon is unrelated at the species level to M. muris, it is left out of the current taxonomic treatment. The 16S rRNA gene sequence similarity between the type strains of [A.] muris and [P.] pneumotropica (biovar Jawatz) was only 94.8 %, which is below the similarity between most genera within the Pasteurellaceae, which is around 95 % (Christensen et al., 2007).

In the 16S rRNA gene sequence multiple alignment, all strains of Muribacter had a characteristic deletion of five nucleotides in the region 211–217 (numbering according to the sequence of P. multocida NCTC 10322) that may be utilized for identification, since the signature is at a slightly different location compared with those found in Necropsobacter rosrorum (positions 203–206 and 213–216) (Christensen et al., 2011).

The type strain of [P.] pneumotropica (biovar Jawatz) was used as an outgroup for the rpoB phylogeny (Fig. S2). The rpoB sequences were identical for Ackerman80-443D and 24 other strains (group I). Another large group included 25 strains with identical rpoB sequences (group VIII) (Table S1). Eight other groups and a singleton could be identified. Strain 2005150026 (group XI) diverged from the other groups; however, analysis of rpoB still showed high similarity within M. muris of 97.2–100 %. The closest related taxon outside the M. muris group based on rpoB sequence comparison was ‘H. influenzae-murium’, with 88.4 % similarity, which is at the lower range of similarity between species of the Pasteurellaceae, which is within the range 91–99 % (Bisgaard et al., 2012; Korczak et al., 2014). The 16S rRNA gene sequence similarity between ‘H. influenzae-murium’ and M. muris was also below the species level, and further taxonomic investigation will eventually show whether ‘H. influenzae-murium’ belongs to Muribacter as a genospecies.

16S rRNA gene sequence-based phylogenetic analysis confirmed four of the groups (I, III, V, VI) identified by rpoB gene sequence analysis, whereas six groups were further subdivided in the 16S rRNA gene sequence analysis into two or three (II, VIII) subgroups. The singleton (XI) was also recognized by 16S rRNA gene sequence-based phylogenetic analysis (Figs S1 and S2, Table S1).

Sequence-based comparison indicated that strain R002094 belongs to M. muris. This strain was classified as a variant of biovar 6, phenotypically related to taxon 17 of Bisgaard and to Pasteurella dagmatis; however, the 16S rRNA gene sequence of the reference strain of Bisgaard taxon 17 (CCUG 17206; accession no. AY362902) showed only 94.0 % similarity to that of the type strain of M. muris. Strain 33696Asv8, classified as biovar 1, which also represents Bisgaard taxon 26 biovar 4 (non-haemolytic), was recently excluded from the novel species Actinobacillus anseriformium, which was based on the classification of taxon 26 of Bisgaard (Bisgaard & Christensen, 2012).

Partial infB gene (translation initiation factor 2) sequencing was performed for a few strains as described by Christensen et al. (2004b). The analysis documented 98.1 % similarity between the type strain (accession no. EU350935) and strain 3996-85 of biovar 15 (Bisgaard taxon 27) in M. muris genotypic group VIII (KNN44115). Comparison of partial infB gene sequences between the type strain of M. muris, ‘H. influenzae-murium’ HIM565-1 (accession no. KP664114) and the type strain of [P.] pneumotropica (biovar Jawatz) (accession no. AJ438124) demonstrated only 84.5 and 80.2 % similarity, respectively. Comparable levels of partial infB gene sequence similarity are found for genera of the Pasteurellaceae range from 83 to 85 % (Frederiksenia and Actinobacillus) (Korczak & Kuhnert, 2008; Korczak et al., 2014), thus confirming the genus-level classification of M. muris.

Fatty acids were investigated by the Culture Collection, University of Göteborg (CCUG). The major fatty acids of the type strain of M. muris were C14 : 0, C16 : 0, 3-OH/iso-C16 : 1 I, C16 : 0, C17 : 0, C17 : 1, C18 : 0 and C18 : 1 (Table S4). No obvious differences were found from the fatty acid profiles of type strains of
12 other genera of the Pasteurellaceae that were available for comparison.

Phenotypic characters shared by all strains investigated and included for genus-level separation are listed in Table 1. Muribacter can be separated phenotypically from the existing genera of the Pasteurellaceae in at least two characters.

**Description of Muribacter gen. nov.**

Muribacter [Mu.ri.bac.*ter. L. n. mus, muris the mouse; N.L. masc. n. bact.*er (derived from bact*rum) rod; N.L. masc. n. Muribacter rod from mice].

The description is based on that of Actinobacillus muris (Bisgaard, 1986) with the following emendations. Reactions for urease and indole production are variable. Acid formation from (−)-d-mannitol and hydrolysis of ascelin are variable. The α-glucosidase test (PNPG; 4-nitrophenyl α-d-glucopyranoside) is positive. There is no requirement for X factor (haemin). Major fatty acids of the type strain of the type species are C_{14:0}, C_{14:0} 3-0H/iso-C_{16:0} I, C_{16:1} 07c and C_{16:0}. The DNA G+C content of the type strain of the type species is 43.7%, as determined by whole-genome sequencing. The type species is *Muribacter muris*.

**Description of Muribacter muris comb. nov.**


The description is based on that of Actinobacillus muris (Bisgaard, 1986), with the following additions. Acid formation from (−)-d-xylose, myo-inositol, (+)-d-ribose, (+)-melibiose, cellobiose and salicin is variable. Acid is formed from trehalose, but one negative strain has been observed. Acid is usually not formed from sorbitol, but four strains have been observed to be positive. Some phenotypes show a weak haemolysis or CAMP reaction (e.g. genotypic group VIII). The ONPG (α-nitrophenyl d-galactopyranoside) β-galactosidase test is variable, as are tests for β-glucosidase, α-fucosidase and β-glucuronidase, whereas the β-xylosidase test is negative. The bacteria have been isolated mainly from mice, but also from rats.

The type strain is Ackerman80-443D^T (=NCTC 12432^T =CCUG 16938^T =ATCC 49577^T), isolated from a mouse uterus, with the phenotypic properties originally reported by Bisgaard (1986).

**Acknowledgements**

Hans G. Trüper is thanked for helping with the Latin name.

**References**


Christensen, H., Kuhnert, P., Olsen, J. E. & Bisgaard, M. (2004b). Comparative phylogenies of the housekeeping genes atpD, infB and...


