NOTE

Phylogenetic position of *Bartonella vinsonii* subsp. *arupensis* based on 16S rDNA and *gltA* gene sequences

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The GenBank accession number for the *gltA* sequence of *B. vinsonii* subsp. *arupensis* is AF214557.

The bacterial genus *Bartonella*, which includes species formerly classified in the genera *Rochalimaea* (Brenner et al., 1993) and *Grahamella* (Birtles et al., 1995), currently contains 14 species. Seven of these species, *Bartonella bacilliformis*, *Bartonella quintana*, *Bartonella henselae*, *Bartonella elizabethae*, *Bartonella claridgeiae*, *Bartonella grahamii* and *Bartonella vinsonii*, have been associated with an increasing variety of human diseases. Thus, *Bartonella* species are currently considered as emerging human pathogens (Anderson & Neuman, 1997).

*B. vinsonii* was first isolated from voles in Grosse Isle, Quebec, Canada (Baker, 1946). The bacterium was characterized further by Weiss & Dasch (1982), who proposed the name *Rochalimaea vinsonii*, which was subsequently changed to *B. vinsonii* (Brenner et al., 1993). In 1996, a subspecies causing canine endocarditis was described and named *B. vinsonii* subsp. *berkhoffii* (Kordick et al., 1996). The Canadian vole agent was therefore renamed *B. vinsonii* subsp. *vinsonii*. However, the species *B. vinsonii* was not implicated in human disease until 1999, when a new strain was recovered from the blood of an American cattle rancher with acute febrile illness (Welch et al., 1999). The organism was recognized as a fastidious, non-motile, small, Gram-negative rod, based on its Gram-staining properties and its recovery on chocolate agar after a 7 d incubation period. Its growth on heart infusion agar plus X factor distinguished the isolate from *B. henselae* and *B. quintana* (Welch et al., 1999). DNA relatedness studies revealed rates ranging from 23 to 68% at 55 °C with *Bartonella* species other than *B. vinsonii*. With *B. vinsonii* subsp. *vinsonii* and *B. vinsonii* subsp. *berkhoffii*, the DNA was related at the species level (> 70%). These data supported the creation of a new subspecies within the species *B. vinsonii*, named *B. vinsonii* subsp. *arupensis* (Welch et al., 1999). A rodent reservoir host has been suggested for this organism by Hofmeister et al. (1998). However, no 16S rDNA or DNA sequence was available for this bacterium; such data could provide useful tools to identify other isolates of this subspecies.

In the present study, we have determined the sequences of the genes encoding 16S rRNA and citrate synthase (*gltA*) for this new subspecies for comparison with previously published sequences (obtained from databases) in order to position this subspecies within the taxonomy of the genus *Bartonella*.

**Amplification of 16S rRNA and *gltA* genes**

*B. vinsonii* subsp. *arupensis* strain ATCC 700727T was obtained from the American Type Culture Collection and cultured on blood agar at 37 °C in a 5% CO₂ atmosphere.

A 200 µl sample of a 10⁸ c.f.u. ml⁻¹ bacterial suspension was mixed with 500 µl of a 20% chelex
**Table 1.** Accession numbers of 16S rRNA and *gltA* gene sequences used for sequence alignments and construction of phylogenetic trees

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Strain</th>
<th>Accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>16S rRNA</td>
</tr>
<tr>
<td><em>B. koehlerae</em></td>
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<td>AF076237</td>
</tr>
<tr>
<td><em>B. henselae</em></td>
<td>FR96/BK38</td>
<td>AJ223779</td>
</tr>
<tr>
<td><em>B. alsatica</em></td>
<td>IBS382</td>
<td>A1002139</td>
</tr>
<tr>
<td><em>B. tribocorum</em></td>
<td>IBS506</td>
<td>A1003070</td>
</tr>
<tr>
<td><em>B. quintana</em></td>
<td>Fuller</td>
<td>U28268</td>
</tr>
<tr>
<td><em>B. vinsonii</em> subsp. <em>vinsonii</em></td>
<td>Baker</td>
<td>Z31352</td>
</tr>
<tr>
<td><em>B. vinsonii</em> subsp. <em>berkhoffii</em></td>
<td>93-C01</td>
<td>U26258 (this study)</td>
</tr>
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<td><em>B. vinsonii</em> subsp. <em>arupensis</em></td>
<td>OK94-513</td>
<td>AF214558</td>
</tr>
<tr>
<td><em>B. taylorii</em></td>
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<td>Z31350</td>
</tr>
<tr>
<td><em>B. grahamii</em></td>
<td>V2</td>
<td>Z31349</td>
</tr>
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<td>Houston-2</td>
<td>U64691</td>
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<td><em>B. doshiae</em></td>
<td>R18</td>
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<td>LA6.3</td>
<td>Z70003</td>
</tr>
<tr>
<td><em>B. elizabethae</em></td>
<td>F9251</td>
<td>L01260</td>
</tr>
</tbody>
</table>

NA, Not available.

**Fig. 1.** Phylogenetic trees derived from the 16S rRNA gene (a) and the *gltA* gene (b) for *Bartonella* species, using the parsimony method. The support of each branch, as determined from 100 bootstrap samples, is indicated at the nodes. The lengths of vertical and horizontal lines are not significant. *Brucella melitensis* and *Rickettsia conorii* served as outgroups to establish the roots of the 16S rRNA tree and the *gltA* tree, respectively.

Suspension (Bio-Rad) and the mixture was boiled for 30 min and centrifuged (14000 g for 10 min). The supernatant was kept at 4 °C until amplification.

The 16S rRNA gene was amplified using the primers forward fD1 and reverse rp2 described by Weisburg *et al.* (1991) and the *gltA* gene was amplified using the
Analysis of the phylogenetic trees derived from 16S–B other subsp. dNTP, 15 nM dUTP (Life Technologies), 0–containing 12

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incorporated into the QIAquick PCR purification kit (Qiagen) and then

For sequencing, the samples were purified with a

(Birtles & Raoult, 1996). Seven microlitres of the DNA

et al alignment program, which is part of the

species (obtained from GenBank; accession numbers

were aligned with sequences of recognized

Bartonella species.

regions of ambiguity were removed from sequences

prior to phylogenetic inference. Phylogenetic relationships were inferred with PHYLIP, version 3.4 (Felsenstein, 1989). The evolutionary distance values, generated by DNADIST, were determined by the method of Jukes & Cantor (1969). These values were used to construct dendrograms by the neighbour-joining and maximum-likelihood methods (Saitou & Nei, 1990). Inserts and regions of ambiguity were removed from sequences obtained with the different methods. Bootstrap values were calculated on 100 randomly generated trees using SEQBOOT and CONSENSE in the same package.

A 1472 bp fragment of the 16S rDNA gene and a 951 bp fragment of the gltA gene were sequenced. A pair-wise comparison of DNA sequences between B. vinsonii subsp. arupensis and other Bartonella species revealed similarity values of 97–99.2% for the 16S rDNA gene and 86–98.8% for the gltA gene. Analysis of the phylogenetic trees derived from 16S rDNA and gltA sequences (Fig. 1) confirmed that B. vinsonii subsp. arupensis was closely related to the other B. vinsonii subspecies, B. vinsonii subsp. vinsonii arupensis and B. vinsonii subsp. berkthoffii. For the two genes tested, the branching orders generated with the three different analysis methods were consistent. Consensus trees, however, showed low bootstrap values and many of the observed clusters should therefore be confirmed. The branching of our trees had only moderate support, since no branch of the genus had support of more than 90% of the bootstrap samples, except for Bartonella tribocorum with B. elizabethae on the 16S rDNA tree and B. bacilliformis on the same tree. The instability of the proposed architectures was confirmed, as different branching orders were produced with each gene.

Conclusion

Based on the 16S rDNA and gltA sequences, we have positioned B. vinsonii subsp. arupensis close to the other B. vinsonii subspecies, B. vinsonii subsp. vinsonii and B. vinsonii subsp. berkthoffii. Our study also confirmed the fact that the phylogenies based on different genes showed slight discrepancies.

References


