NOTE

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

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The nucleotide sequences of the genes encoding 16S rRNA and citrate synthase (*gltA*) from a recently described member of the genus *Bartonella*, *Bartonella vinsonii* subsp. *arupensis*, isolated from mice and from the blood of a patient suffering from bacteraemia, were determined and compared with sequences of the 16S rDNA and *gltA* genes of other members of the genus *Bartonella* in order to determine its relative phylogenetic position. *B. vinsonii* subsp. *arupensis* appeared to be closely related to *B. vinsonii* subsp. *vinsonii*, another rodent-associated taxon, and to *B. vinsonii* subsp. *berkhoffii*, which was described recently in dogs.

Keywords: *Bartonella vinsonii* subsp. *arupensis*, 16S rRNA, citrate synthase, *gltA*

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 clarridgeiae*, *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 700727^T 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

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

Table 1. Accession numbers of 16S rRNA and *gltA* gene sequences used for sequence alignments and construction of phylogenetic trees

Taxon	Strain	Accession numbers	
		16S rRNA	<i>gltA</i>
<i>B. koehlerae</i>	C-29	AF076237	AJ005494
<i>B. henselae</i>	FR96/BK38	AJ223779	L38987
<i>B. alsatica</i>	IBS382	AJ002139	NA
<i>B. tribocorum</i>	IBS506	AJ003070	AJ005494
<i>B. quintana</i>	Fuller	U28268	Z70014
<i>B. vinsonii</i> subsp. <i>vinsonii</i>	Baker	Z31352	Z70015
<i>B. vinsonii</i> subsp. <i>berkhoffii</i>	93-C01	U26258	(this study)
<i>B. vinsonii</i> subsp. <i>arupensis</i>	OK94-513	AF214558	AF214557 (this study)
<i>B. taylorii</i>	M6	Z31350	Z70013
<i>B. grahamii</i>	V2	Z31349	Z70016
<i>B. clarridgeiae</i>	Houston-2	U64691	U84386
<i>B. doshiae</i>	R18	Z31351	Z70017
<i>B. bacilliformis</i>	LA6.3	Z70003	Z70021
<i>B. elizabethae</i>	F9251	L01260	Z70009

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

primers forward CS140F and reverse BhCS1137n (Birtles & Raoult, 1996). Seven microlitres of the DNA preparation was amplified in a 17.5 µl reaction mixture containing 12.5 pM of each primer, 5 nM of each dNTP, 15 nM dUTP (Life Technologies), 0.75 U EuroblueTaq DNA polymerase (Eurobio), 0.8 µl of a 25 mM solution of MgCl₂ (Perkin-Elmer) and 2.5 µl 10× reaction buffer. The primer annealing temperatures were 55 °C for the pair fD1/rp2 and 50 °C for the pair CS140F/BhCS1137n. The PCR product was visualized by ethidium bromide staining after electrophoresis through 1% agarose gel.

Sequencing and phylogenetic analysis

For sequencing, the samples were purified with a QIAquick PCR purification kit (Qiagen) and then incorporated into the D-rhodamine Terminator Cycle Sequencing Ready Reaction buffer (DNA Sequencing kit, Perkin-Elmer). Nucleotide sequences were determined with an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer). Sequencing of the 16S rRNA gene and the *gltA* gene was performed using primers described previously (Weisburg *et al.*, 1991; Birtles & Raoult, 1996).

The newly determined 16S rDNA and *gltA* sequences were aligned with sequences of recognized *Bartonella* species (obtained from GenBank; accession numbers given in Table 1) by using the CLUSTAL multisequence alignment program, which is part of the BISCANCE software package (Dessen *et al.*, 1990). Inserts and 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, 1987). In addition, the data were examined using the parsimony program DNAPARS in PHYLIP. Bootstrapping was carried out in order to investigate the stability of the trees obtained with the different methods. Bootstrap values were obtained from a consensus tree based 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 97.3–99.2% for the 16S rDNA gene and 86.0–98.8% 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* and *B. vinsonii* subsp. *berkhoffii*. 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. *berkhoffii*. Our study also confirmed the fact that the phylogenies based on different genes showed slight discrepancies.

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