Bartonella massiliensis sp. nov., a new bacterial species isolated from an Ornithodoros sonrai tick from Senegal

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Abstract

Bartonella massiliensis sp. nov., strain OS09T (= CSURB624T = DSM 23169), is the type strain of Bartonella massiliensis sp. nov., a new species within the genus Bartonella. It was isolated from a soft tick, Ornithodoros sonrai, vector of recurrent fever collected from Senegalese domestic rodent burrows. This strain is an aerobic, rod-shaped and Gram-negative bacterium. On the basis of taxonogenomic approach, we propose the creation of Bartonella massiliensis sp. nov.

Keywords: Bartonella massiliensis sp. nov., genome, Ornithodoros sonrai, senegal, soft tick

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Introduction

Bartonella is the monotypic genus of the family Bartonellaceae among Alphaproteobacteria [1]. Bartonella species are fastidious Gram-negative, slightly curved rod bacteria characterized by a small cell size (0.5–0.6 × 1.0 μm) [2]. They are facultative intracellular bacteria with a unique intraerythrocyte lifestyle. Currently the Bartonella genus includes 35 validly published species and three subspecies [3,4]. Bartonella species usually colonize the intestine of the arthropod vector or the bloodstream of the mammalian host [4,5]. In addition, our understanding of the involvement of these microorganisms in human diseases continues to grow, as does the range of clinical manifestations [6,7]. At least 13 Bartonella species are responsible for human diseases, including B. bacilliformis, B. quintana and B. henselae, which cause Carrión disease, trench fever and cat-scratch disease respectively. Bartonella species are also associated with chronic bacteraemia and/or endocarditis, bacillary angiomatosis, peliosis hepatis, prolonged fever of unknown origin, retinitis, uveitis and myocarditis in humans [6]. Other mammalian species that may host Bartonella species include dogs, coyotes, foxes, cattle, deer, elk, bats and many rodent species [8–10].

Here we present the description of Bartonella massiliensis strain OS09T (= CSURB624T = DSM 23169), a new species of the genus Bartonella isolated from a soft tick, Ornithodoros sonrai, including its complete annotated genome.

Samples and bacterial culture

Between September 2008 and May 2009, a research study on Bartonella species in Ornithodoros sonrai, as of ticks collected in Senegal (West Africa), was conducted by Mediannikov et al. [11]. Sampling was carried out in populated houses with numerous rodent burrows in room floors. Morphologically, all ticks collected in domestic rodent burrows have been identified as Ornithodoros sonrai, a nidicolous tick that inhabits small mammal burrows [11]. Globally, ticks from only two of the villages (Soulkhou Thissé and Maka Gouye) were infected with Bartonella spp., with infection in 62.5% (5/8) and 4.2% (1/24) respectively. Sequences of internal transcribed spacer (ITS) amplicons obtained from these ticks showed that the Bartonella identified in ticks collected in the two villages differed...
from each other insignificantly (0.3–3%), as well as from any other validly described species with standing in nomenclature (http://www.bacterio.net/bartonella.html). Culture of Bartonella strains was carried out as previously reported [11]. Briefly, the bacterial colonies of strains retrieved from O. sonrai were obtained after 5 to 7 days' incubation at 37°C in a 5% CO₂-enriched atmosphere on Columbia agar plates supplemented with 5% sheep’s blood (bioMérieux, Marcy l’Etoile, France).

Classification and features

The ITS, ftsZ, rpoB and gltA genes as well as the 16S ribosomal RNA (rRNA) gene were amplified and sequenced to identify isolated Bartonella strains. After the sequences analysis, two strains, OS09T and OS23T, showed almost the same genetic similarity: they had 100% identity for the 16S rRNA and rpoB genes, 99.8% for the ftsZ gene and 99.9% for the gltA gene. No mutation was detected for the ITS gene, and only a 5 bp deletion was found for the OS23T strain. The similarities of the sequences of the OS09T and OS23T strains with respect to the different species closest to the genus Bartonella for the 16S rRNA, ITS, ftsZ, rpoB and gltA genes were 99.5%, 79.5–81.3%, 96.8–96.9%, 93.2% and 94.5–94.6% respectively. According to La Scola et al. [8], these data support the notion that strains OS09T and OS23T may be identified as a new and same species. We focus here on the analysis of Bartonella massiliensis strain OS09T (Table 1). Similarities in sequences of the OS09T strain with the different closest members of the Bartonella species for the 16S rRNA gene, ITS, ftsZ, rpoB and gltA were 99.5%, 79.5%, 96.6%, 93.2% and 94.5%, with Bartonella queenslandensis (EU111758), Bartonella elizabethae (JF766264), Bartonella grahamii (CP001562), Bartonella tribocorum (JF766251) and Bartonella grahamii (CP001562) respectively. All 16S rRNA sequences of Bartonella species are used in Fig. 1 to highlight the phylogenetic position of this bacterium relative to other species.

MALDI-TOF MS was performed on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [9]. The obtained spectra (Fig. 2) were imported against the main spectra of bacteria included in two databases (Bruker as well as Microbes Evolution Phylogeny and Infections (MEPHI), which is constantly updated). No identification was obtained because the strain displayed scores below 1.7, supporting the suggestion that our isolate was not a member of a known species. The spectrum of strain OS09T has been added to the local MEPHl database. A dendrogram made with Biotyper 3.0 software comparing the spectrum of the OS09 strain to those of the other Bartonella species is shown in Fig. 3.

**TABLE 1. Classification and general features of Bartonella massiliensis sp. nov., strain OS09T**

| MIGS ID | Property | Term | Evidence code |
|---------|----------|------|---------------|
| Current classification | Domain: Bacteria | TAS [12] |
| | Phylum: Proteobacteria | TAS [13,14] |
| | Class: Alphaproteobacteria | TAS [15] |
| | Order: Rhizobiales | TAS [16,17] |
| | Family: Bartonellaceae | TAS [18,19] |
| | Genus: Bartonella | TAS [18.20–22] |
| | Species: Bartonella massiliensis | IDA |
| Type strain: OS09T | IDA |
| Gram stain | Negative | IDA |
| Cell shape | Rod | IDA |
| Motility | Nonmotile | IDA |
| Sporulation | Nonsporulating | IDA |
| Temperature range | Mesophilic | IDA |
| Optimum temperature | 32°C | IDA |
| Oxygen requirement | Aerobic | IDA |
| Carbon source | Unknown | IDA |
| Energy source | Unknown | IDA |
| Habitat | Tick gut | IDA |
| Biotic relationship | Facultative intracellulard | IDA |
| Pathogenicity | Unknown | IDA |
| Biosafety level | 3 | IDA |
| Isolation | Ornithodoros sonrai | IDA |
| Geographic location | Senegal | IDA |
| Sample collection | May 2009 | IDA |
| Latitude | 14°03’N | IDA |
| Longitude | 15°31’E | IDA |
| Depth | <0.5 m under surface | IDA |
| Altitude | 5 m above sea level | IDA |

**Biochemical characterization**

Different growth temperatures (32, 37 and 42°C) were tested. Optimal colony growth was observed at 32°C on Columbia agar supplemented with 5% sheep’s blood in an atmosphere enriched with 5% CO₂. Colonies appeared grey and opaque, with a diameter of 0.3 to 1 mm on Columbia blood-enriched agar. The bacterial cells were Gram negative and had a mean length of 1.34 ± 0.26 μm and a width of 0.49 ± 0.13 μm. Neither flagella nor pili were observed by electron microscopy (Fig. 4). Strain OS09T exhibited no catalase or oxidase activity. Biochemical characteristics were assessed by API strips ZYM, 50 CH and Coryne (bioMérieux). None of the available biochemical tests was positive. Similar patterns have been previously observed for Bartonella senegalensis and Bartonella mastomysidis [10,24].

**Genome sequencing information**

**Genome project history.** The OS09 strain was selected for sequencing on the basis of its phylogenetic position and phenotypic differences with other members of the Bartonellaceae family. This strain was isolated in a study on the role of the soft tick, O. sonrai, as a host of Bartonella [11]. Currently 29
Genomes are available in GenBank database for the genus Bartonella. The genome of strain OS09\textsuperscript{T} is the first genome of Bartonella massiliensis sp. nov., and is assembled and deposited under GenBank accession numbers CABFVS010000001 to CABFVS010000091. A summary of the project information is presented in Table 2.

**Growth conditions and DNA isolation.** The OS09\textsuperscript{T} strain of Bartonella massiliensis (= CSUR B624T = DSM 23169) was cultured on Columbia agar enriched with sheep’s blood (bioMérieux) with 5% CO\textsubscript{2} at 32°C. Bacteria growing on two petri dishes were harvested and resuspended in 6 × 100 \(\mu\)L of G2 buffer. A first mechanical lysis was performed with glass powder using the Fastprep-24 device (MP Biomedicals, Graffenstaden, France) during 2 × 20 seconds. Then after 30 minutes’ lysozyme incubation at 37°C, DNA was extracted on the EZ1 biorobot (Qiagen, Hilden, Germany) with the EZ1 DNA tissue kit. DNA was quantified by Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA) to 68.6 ng/\(\mu\)L.

**Genome sequencing and assembly.** Five micrograms of DNA was fragmented mechanically on the Hydroshear device (Digilab, Holliston, MA, USA) with an enrichment size of 3 to 4 kb. The DNA fragments were visualized through an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) on a DNA lab chip 7500 with an optimal size of 3.75 kb. The library was constructed according to the 454 Titanium paired end rapid library protocol and the manufacturer. Circularization and nebulization were performed and generated a pattern optimal at 591 bp. After PCR amplification through 20 cycles, the double-stranded paired end library was then quantified on the Quant-it Ribogreen kit (Invitrogen) on the Genios_Tecan fluorometer at 7360 pg/\(\mu\)L. The library concentration

![Phylogenetic tree showing position of Bartonella massiliensis sp. nov., strain OS09\textsuperscript{T}. Relative to other phylogenetically close neighbours. Sequences were aligned by ClustalW parameters within MEGA7 software. Evolutionary history was inferred using minimum evolution method. Respective GenBank accession numbers for 16S rRNA genes are indicated in parentheses. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 1000 times to generate majority consensus tree. Scale bar indicates 5% nucleotide sequence divergence.](image-url)
FIG. 2. MALDI-TOF MS reference mass spectrum of *Bartonella massiliensis* sp. nov. Spectra from 12 individual colonies were compared and reference spectrum generated.

FIG. 3. Dendrogram comparing MALDI-TOF MS spectra of *Bartonella massiliensis* sp. nov., strain OS09T, with those of other members of *Bartonella* genus.
equivalence was calculated as 1.14E + 10 mol/μL. The library was stored at −20°C until use. The library was clonal amplified with 0.40 cpb in three emulsion PCR (emPCR) reactions with the GS Titanium SV emPCR Kit (Lib-L) v2 (Roche, Basel, Switzerland). The yield of the emPCR was 13.47%, within the range of 5% to 20% from the Roche procedure. A total of 790 000 beads were loaded on a quarter region of the GS Titanium PicoTiterPlate PTP Kit 70x75 and sequenced with the GS Titanium Sequencing Kit XLR70. The run was performed overnight, then analysed on the cluster through gsRunBrowser and gsAssembler (Roche). Overall, 119 842 passed filter wells were obtained and generated 38.01 Mb with an average length of 317 bp. The passed filter sequences were assembled on the gsAssembler with 90% identity and 40 bp as overlap. It led to 25 scaffolds and 234 large contigs (>1500 bp) and generated a genome size of 2.05 Mb, which corresponds to a coverage of 17.27× genome equivalent.

Genome annotation
Open reading frames (ORFs) were predicted using Prodigal [25] with default parameters, but predicted ORFs were excluded if they spanned a sequencing gap region. The predicted bacterial protein sequences were searched against the GenBank database using BLASTP and the Clusters of Orthologous Groups (COGs) database using Cognitor [26]. The prediction of RNA genes, including rRNAs, transfer RNAs and other RNAs, was carried out using the RNAmer[27] and ARAGORN [28] algorithms. The transmembrane helices and signal peptides were identified by TMHMM [29] and SignalP [30] respectively.

Genome properties
The genome is 2 277 694 bp long with 37.76 mol% GC content (Table 3, Fig. 5). It is composed of 91 contigs. Of the 1967 predicted genes, 1925 were protein-coding genes and 42 were RNAs (including one 16S rRNA, one 23S rRNA, one 5S rRNA and 39 transfer RNA genes). A total of 1309 genes (68%) were assigned a putative function (by COGs or NR BLAST). A total of 111 genes were identified as ORFans (5.77%). The remaining genes (n = 386) were annotated as hypothetical proteins (20.05%). The distribution of genes into COGs functional categories is presented in Table 4. The properties and statistical information of the genome are summarized in Tables 3 and 4. The degree of genomic similarity of OS09T closely related species was estimated by OrthoANI software [31]. Values among closely related species ranged from 81.45% between Bartonella massiliensis strain OS09T and Bartonella rataustraliani AUST NH4 to 91.49% between Bartonella queenslandensis strain AUST NH15 and Bartonella tribocorum strain CIP 105476 (Fig. 6). When the isolate was compared to these closely species, values ranged from 81.45% with Bartonella rattaustraliani AUST NH4 to 89.09% with Bartonella mastonydis strain 008.

FIG. 4. Transmission electron micrograph of Bartonella massiliensis strain OS09T using Morgagni 268D (Philips, Amsterdam, The Netherlands) transmission electron microscope at operating voltage of 60 kV. Scale bar represents 500 nm.
**FIG. 5.** Graphical circular map of chromosome. From outside to centre: genes on forward strand coloured by Clusters of Orthologous Groups database (COGs) categories (only genes assigned to COGs), genes on reverse strand coloured by COGs categories (only gene assigned to COGs), RNA genes (transfer RNAs green, rRNAs red), GC content and GC skew (three circles), GC content.

**TABLE 4.** Number of genes associated with 25 general COGs functional categories

| Code | Value | % of total | Description                                              |
|------|-------|------------|----------------------------------------------------------|
| J    | 146   | 7.58       | Translation                                              |
| A    | 0     | 0          | RNA processing and modification                          |
| K    | 86    | 4.47       | Transcription                                            |
| L    | 128   | 6.65       | Replication, recombination and repair                    |
| B    | 0     | 0          | Chromatin structure and dynamic                          |
| D    | 24    | 1.25       | Cell cycle control, mitosis and meiosis                 |
| Y    | 0     | 0          | Nuclear structure                                        |
| V    | 12    | 0.62       | Defense mechanisms                                       |
| T    | 44    | 2.29       | Signal transduction mechanisms                           |
| M    | 100   | 5.19       | Cell wall/membrane biogenesis                            |
| N    | 8     | 0.42       | Cell motility                                            |
| Z    | 3     | 0.05       | Cytoskeleton                                             |
| W    | 12    | 0.62       | Extracellular structures                                 |
| U    | 80    | 4.16       | Intracellular trafficking and secretion                  |
| Q    | 73    | 3.79       | Posttranslational modification, protein turnover, chaperones |
| C    | 79    | 4.10       | Energy production and conversion                         |
| G    | 73    | 3.79       | Carbohydrate transport and metabolism                    |
| E    | 130   | 6.75       | Amino acid transport and metabolism                      |
| F    | 47    | 2.44       | Nucleotide transport and metabolism                      |
| H    | 58    | 3.01       | Coenzyme transport and metabolism                        |
| I    | 41    | 2.13       | Lipid transport and metabolism                           |
| P    | 84    | 4.36       | Inorganic ion transport and metabolism                   |
| Q    | 15    | 0.78       | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 209   | 10.86      | General function prediction only                         |
| S    | 119   | 6.18       | Function unknown                                         |
| —    | 563   | 29.25      | Not in COGs                                             |

COGs, Clusters of Orthologous Groups database.

*Total is based on total number of protein-coding genes in annotated genome.*
Conclusion

On the basis of unique phenotypic and genotypic characteristics, including MALDI-TOF MS spectrum, sequencing of the 16S rRNA, ITS, ftsZ, rpoB and gltA genes (sequence divergences >99.5%, >79.5%, >96.6%, >93.2% and >94.5% respectively) and an OrthoANI value lower than 95% with the phylogenetically closest species with standing in nomenclature, we consequently propose strain OS09\textsuperscript{T} as the type strain of *Bartonella massiliensis* sp. nov., a new bacterial species within the family *Bartonellaceae*. The strain was isolated from rodent ticks, *O. sonrai*, collected in rural areas of Senegal (West Africa).

**Description of Bartonella massiliensis sp. nov.**

*Bartonella massiliensis* sp. nov. (mas.si.li.en’sis, L. masc. adj. *massiliensis*, ‘of Massilia,’ the ancient Roman name of Marseille, where the strain was isolated) is a nonmotile, Gram-negative rod. Optimal growth is observed at 32°C in an aerobic atmosphere. Colonies are opaque and grey, with a diameter of 0.3 to 1 mm on Columbia blood-enriched agar. Length and width are 1.34 ± 0.26 μm and 0.49 ± 0.13 μm respectively. Cells are rod shaped without flagella or pili. *Bartonella massiliensis* sp. nov., strain OS09\textsuperscript{T} exhibits neither biochemical nor enzymatic activities. The genome size and GC content are 2.22 Mb and 37.76 mol% respectively. The type strain OS09\textsuperscript{T} (= CSUR B624T = DSM 23169\textsuperscript{T}) was isolated from the rodent tick, *Ornithodoros sonrai*, collected in a rural area named Maka Gouye (14°03′N, 15°31′W) located in Senegal.

**Nucleotide sequence accession number**

The 16S rRNA, ITS, ftsZ, rpoB and gltA gene sequences and genome sequences of *Bartonella massiliensis* sp. nov., strain OS09\textsuperscript{T}, are deposited in GenBank under accession numbers HM636440, HM636449, HM636443, HM636452 and HM636446 and CABFVS010000001 to CABFVS010000091 respectively.

**Deposit in culture collection**

Strain OS09\textsuperscript{T} was deposited in two different strain collections under accession numbers CSUR B624\textsuperscript{T} and DSM 23169\textsuperscript{T}.

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Conflict of Interest

None declared.

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