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Short Communication

First genome sequence of Chilean *Brucella canis* SCL strain provides insights on the epidemiology and virulence factors, explaining differences between geographical origins

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ABSTRACT

Background: *Brucella canis* is the etiological agent of canine brucellosis, a worldwide neglected zoonosis that constitutes one of the major infectious causes of infertility and reproductive failure in dogs. Although genomic information available for this pathogen has increased in recent years, here we report the first genome sequencing of a *B. canis* strain in Chile, and the differences in virulence genes with other *B. canis* strains.

Results: Genome assembly produced a total length of 3,289,216 bp, N50 of 95,163 and GC% of 57.27, organized in 54 contigs in chromosome I, and 21 contigs in chromosome II. The genome annotation identified a total of 1981 CDS, 3 rRNA and 36 tRNA in chromosome I, and 1113 CDS and 10 tRNA in chromosome II. There is little variation between the different strains and the SCL isolate. Phylogenetic analysis showed that the Chilean SCL strain is closely related to *B. canis* and *B. suis* strains. Small differences were found when compared to the Serbian isolate, but all strains shared the same recent common ancestor. Finally, changes in the sequence of some virulence factors showed that the SCL strain is similar to other South American *B. canis* strains.

Conclusions: This work sequenced and characterized the complete genome of *B. canis* strain SCL, evidencing the complete presence of all the genes of the *vir*B operon, and minor changes in outer membrane proteins and in the urease operon. Our data suggest that *B. canis* was introduced from North America and then spread throughout the South American continent.

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1. Introduction

Canine brucellosis, caused specifically by *Brucella canis* constitutes a worldwide urban neglected zoonosis that is considered the main cause of reproductive failure in dogs [1]. *B. canis* is a small Gram-negative, coccobacillus, non-motile, and facultative intracellular bacterium [2]. Brucellosis generates clinical signs associated to reproductive failure in dogs, and although carrier individuals may not show clinical signs, they are able to spread the bacteria to other uninfected animals and humans [3]. The pathogen is transmitted orally, sexually, and transplacentally. It was first recognized in dogs in 1966 and is being continually surveyed in breeding kennels, representing a major source of economic loss due to abortions, stillbirths, and sperm abnormalities [4].

Another problem associated with brucellosis in dogs is that no antibiotic treatment has been shown to eliminate the infection in all treated animals, and to date there are no commercially available vaccines [5]. In people, *B. canis* is considered of low virulence when compared to other zoonotic species of the same genus, and causes nonspecific symptoms such as fever, chills, malaise, peripheral lymphadenopathy and splenomegaly, which may explain why it is underdiagnosed [1,3,5]. Despite its importance to both animal and public health, epidemiological data and strains assessment are scarce in Chile [6]. The limited isolation of *B. canis* strains has

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prevented detailed analysis of their population dynamics in different geographical areas, where characteristics of virulence and phylogeography could be compared. Here, we report the first complete genome sequence of the Chilean *B. canis* SCL strain and the differences of virulence-related factors at the genomic level in different strains of this pathogen.

2. Methods

2.1. SCL strain

The strain was isolated from the urine of an entire 5-year-old male boxer, with signs of hematuria and lumbar pain. Urine samples were plated onto *Brucella* agar (Difco®) plates supplemented with cycloheximide (100 mg/L, Merck®), bacitracin (25,000 IU, Merck®), and polymyxin B (6000 IU, Merck®), and incubated at 37 °C under aerobic conditions [7]. Bacterial growth was observed after 72 h of incubation, with typical macromorphology and Gram staining. The isolate tested positive with anti-*B. canis* serum, but negative with anti-*B. abortus* serum. Biochemical characteristics included urease production and growth on agar plates supplemented with thionin but not with basic fuchsine [8]. At necropsy, the same isolate was recovered from kidneys.

2.2. Whole genome sequencing

Whole genome sequencing was performed in a next-generation Illumina MiSeq platform at the FAVET-INBIOGEN laboratory, Universidad de Chile. Paired libraries were prepared with Nextera XT DNA Sample Preparation Kit and MiSeq Reagent Kit V3 (MiSeq, Illumina®) and 4,326,736 paired reads were obtained. Reads quality was analyzed using FASTQC software [9]. After filtering and correcting the reads, *de novo* assembly was performed using SPAdes v3.5.0 [10]. Contigs scaffolding was carried out using ABACAS [11] with *B. canis* ATCC 23,365 chromosomes (GenBank accession no. GCF_000018525.1) as reference. Finally, each chromosome was annotated independently using the rapid prokaryotic genome annotation (Prokka) pipeline [12].

2.3. Phylogenetic analysis

In order to understand the evolutionary history of *B. canis*, we analyzed the 16S gene region (624 bp) of the SCL strain, 28 strains available at NCBI (Table 1), and three representative strains of *B. suis* (GenBank accession nos. GCF_000701065.1, GCF_000371185.1, and GCF_000331635.1). The phylogeny was inferred using the Maximum Likelihood method and Tamura-Nei model [13] with MEGA X [14]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A total of 2000 bootstraps were performed.

2.4. Analysis of virulence factors

Coding regions (CDS) for the *vir*B operon (*vir*B1-11), urease operon (*ure*D, *ure*E, *ure*F, *ure*G, and urease subunits alpha, beta, gamma), outer membrane proteins 25 and 31 (*omp*25 and *omp*31), and the *bvf* gene of the 28 *B. canis* strains and the SCL strain were obtained from the annotated genomes, using in-house scripts. Single nucleotide polymorphisms (SNPs) were predicted based on sequence alignments using all the strains available with MEGA X. Coding regions were translated to proteins to char-

Table 1
SNPs and amino acid changes in virulence genes of 29 *B. canis* strains, including SCL.

		Urease operon (alpha subunit)		*omp*31		*omp*25		*vir*B operon	
B. canis strain	Origin	SNP320 (T/C)	AA change	SNP620 (C/A)	AA change	SNP320 T/C)	AA change	SNP8367 (G/A)	AA change
CNGB 1324	Argentina	T	Met	A	STOP	T	Pro	G	Glu
07-2859-6071	Brazil	T	Met	A	STOP	T	Pro	G	Glu
07-2859-6070	Brazil	T	Met	A	STOP	T	Pro	G	Glu
10469	Brazil	T	Met	A	STOP	T	Pro	G	Glu
CNGB 513	Chile	T	Met	A	STOP	T	Pro	G	Glu
SCL	Chile	C	Leu	A	STOP	T	Pro	G	Glu
BCB018	China	T	Met	C	Ser	C	Leu	A	Glu
118	China	T	Met	C	Ser	T	Pro	A	Glu
GB1	China	T	Met	C	Ser	C	Leu	A	Glu
ZJ-2	China	T	Met	C	Ser	C	Leu	A	Glu
ZJ-1	China	T	Met	C	Ser	C	Leu	A	Glu
CNGB 1172	Colombia	T	Met	A	STOP	T	Pro	G	Glu
Oliveri	Colombia	T	Met	A	STOP	T	Pro	G	Glu
09-369-776-1	Finland	T	Met	C	Ser	C	Leu	A	Glu
96-7258	France	T	Met	C	Ser	T	Pro	G	Glu
79/122	Japan	T	Met	C	Ser	C	Leu	A	Glu
HSK A52141	Korea	T	Met	C	Ser	C	Leu	A	Glu
04-2330-1	Serbia	T	Met	C	Ser	T	Pro	A	Glu
F7/05A	South Africa	T	Met	C	Ser	C	Leu	A	Glu
96-9626	Spain	T	Met	C	Ser	T	Pro	G	Glu
SVA10	Sweden	T	Met	C	Ser	C	Leu	A	Glu
SVA13	Sweden	T	Met	A	STOP	T	Pro	G	Glu
UK10/02	UK	T	Met	C	Ser	T	Pro	G	Glu
2010009751	USA	T	Met	C	Ser	C	Leu	A	Glu
ATCC 23365	USA	T	Met	C	Ser	T	Pro	G	Glu
RM66/6	USA	T	Met	C	Ser	T	Pro	G	Glu
2009004498	USA	T	Met	C	Ser	T	Pro	G	Glu
2009013648	USA	T	Met	A	STOP	T	Pro	G	Glu
FDAARGOS_420	USA	T	Met	C	Ser	T	Pro	G	Glu

T = thymine; C = cytosine; A = adenine; G = guanine; STOP = stop codon; Met = methionine; Leu = leucine; Ser = serine; Pro = proline; Glu = glutamate.

acterize possible differences in amino acid sequence given changes in the genomic sequence. A series of exploratory analyzes revealed that diversity was relatively low between strains, for each of the genes analyzed; but particularly for *bvf*, for which no variants were found. Therefore, this gene was excluded from further analysis. All the sequence has been included in Fig. S1.

3. Results and discussion

3.1. Genome assembly and annotation

Genome assembly with SPades v3.5.0 produced 74 contigs with an average coverage of 146X. The total length of sequencing was 3,289,216 bp with an N50 of 95,163 and a GC% of 57.27. After ABACAS reordering and chromosome contig assignation, chromosome I resulted in 54 contigs with a total length of 2,092,334 bp and 57.23 GC%, and chromosome II in 21 contigs with a total length of 1,198,140 and 57.34 GC%. A total of 1981 CDS, 3 rRNA, 36 tRNA, 1113 CDS, and 10 tRNA were annotated in chromosome I and II, respectively.

3.2. Genome nucleotide sequence availability

The whole genome of *B. canis* SCL strain was deposited in DDBJ/EMBL/GenBank under accession nos. GCA_001078335.1 and GCF_001078335.1 for GenBank and Refseq, respectively.

3.3. Phylogenetic analysis

The phylogenetic analysis revealed high similarity among different *B. canis* strains (Fig. 1). Interestingly, two *B. suis* isolates clustered together with the *B. canis* isolates, suggesting a degree of interspecies cross-infection [15]. The Serbian isolate of *B. canis* has a SNP (C/T) in position 429, but shares the same recent common ancestor. A common ancestor for all *B. canis* isolates suggests that there might be a single spread route for canine brucellosis in South America, possibly due to dog introductions from North America (probably from USA or Mexico) [16]. This is similar to what was first reported for the isolation of *B. canis* in China [17].

3.4. Analysis of virulence genes of B. canis

A search was made for virulence genes identified in other *B. canis* strains, specifically the *vir*B operon, which encodes a type IV secretion system (TIVSS) and is one of the most important virulence factors in bacteria [18]. The *vir*B operon has been associated with the ability of intracellular replication [19], with a relevant role in the invasive capacity of *Brucella* spp. [20,21], and in the virulence of *B. canis* [22]. This analysis revealed slight changes among the different *B. canis* isolates, as a single nucleotide mutation in position 8367 (A versus G) of the operon, leading to a synonymous mutation at the amino acid level (Table 1). The case of the Omp31 protein is interesting, because a stop codon at position 206 changes protein length (Table 1). In general terms, the stop codon is found in all South American isolates, and in an isolate from Sweden (*B. canis* str. SVA13) and USA (*B. canis* str. 2009013648). A non-synonymous mutation of the Omp25 protein was observed in position 106 of the amino acid chain. Similarly, this mutation is shared by all the South American strains. This is an important result, as it has been previously shown in *Brucella* spp. that minor changes at the nucleotide level in these genes could result in antigenic differences among isolates [23], which could lead to reduced vaccine effectiveness. Therefore, this should be considered for immunization and vaccine development.

Regarding other virulence genes, a single nucleotide difference was found in the urease operon of the SCL strain. This mutation is in the alpha subunit of the urease gene in position 106 of the protein (Table 1), and causes a methionine-to-leucine substitution. Although the actual effect of this change at the amino acid level is unknown, it has been shown that changes in the coding sequence of this operon can lead to the development of new pathogenic routes for specific strains of *Brucella*. For example, it has been previously reported that certain genotypes of this operon show increased survival at the gastrointestinal level [24].

4. Conclusions

This work sequenced and characterized the complete genome of the Chilean *B. canis* SCL strain. It is interesting to note that all *B. canis* strains clustered together when considering the 16S region, except from one isolate, but this one shared the same recent common ancestor. The characterization of changes in the sequence of several virulence factors showed that the Chilean strain is similar

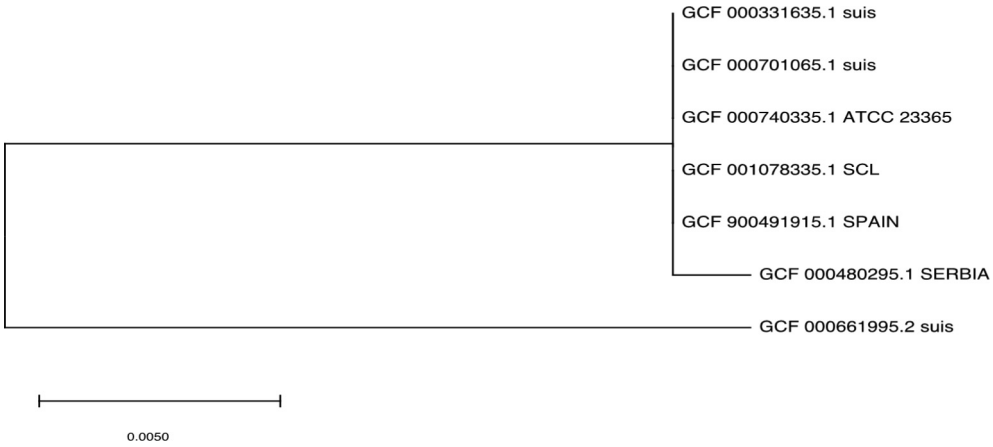

Fig. 1. Phylogenetic analysis of *B. canis* SCL and other *B. canis* reference sequences based on the 16S gene. Names are given following refseq database and include the strain origin. For sake of brevity, the ATCC isolate was plotted in the tree as the sequence of this strain is shared by all *B. canis* strains.

to other South American strains, and also to some Swedish and American strains. Therefore, our data are likely to be consistent with the hypothesis of a major introduction of *B. canis* from North America, and subsequent spread throughout the South American continent.

Conflict of interest

The authors declare that there is no conflict of interest.

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Supplementary material

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