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Short communication

Differences in two-component signal transduction proteins among the genus *Brucella*: Implications for host preference and pathogenesis

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ABSTRACT

Two-component systems (TCSs) are the predominant bacterial signal transduction mechanisms. Species of the genus *Brucella* are genetically highly related and differ mainly in mammalian host adaptation and pathogenesis. In this study, TCS proteins encoded in the available genome sequences of *Brucella* species have been identified using bioinformatic methods. All the *Brucella* species share an identical set of TCS proteins, and the number of TCS proteins in the closely related opportunistic human pathogen *Ochrobactrum anthropi* was higher than in *Brucella* species as expected from its lifestyle. *O. anthropi* lacks orthologs of the *Brucella* TCSs NodVW, TceSR and TcfSR, suggesting that these TCS proteins could be necessary for the adaptation of *Brucella* as an intracellular pathogen. This genomic analysis revealed the presence of a differential distribution of TCS pseudogenes among *Brucella* species. Moreover, there were also differences in TCS pseudogenes between strains belonging to the same *Brucella* species, and in particular between *B. suis* biovars 1 and 2.

1. Introduction

Brucella species are facultative intracellular pathogens that establish and maintain chronic infections in a wide variety of mammalian hosts. During the infection cycle, *Brucella* species have to rapidly adapt to the intracellular niche to resist the harsh conditions generated by the immune system (Moreno and Moriyón, 2002). The genus *Brucella* is divided in several species that are genetically highly related and differ mainly in pathogenicity and preferential host specificity (Godfroid et al., 2005). In contrast, the closely related *Ochrobactrum* species are members of the soil microbiota and potential human pathogens unable to establish chronic infections (Leal-Klevezas et al., 2005). *Brucella* species and the type species *Ochrobactrum anthropi* present a complex genome with two chromosomes (chromosomes I and II) of unequal size (Jumas-Bilak et al., 1998). The complete genome sequences of 10 *Brucella* isolates of different species have become available (DelVecchio et al., 2002; Paulsen et al., 2002; Chain et al., 2005; Halling et al., 2005; Crasta et al., 2008; Audic et al., 2009; Tsolis et al., 2009; Wattam et al., 2009). Comparative genomics of *Brucella* species reveals extensive similarity and conserved genetic organization.

Pathogen–host interactions during bacterial infection expose bacteria to multiple physiological and biological stresses (Dorrell et al., 1998). Two-component systems (TCSs) are the predominant signal transduction mechanism by which bacteria sense and respond to intracellular and extracellular signals mainly, through regulation of gene expression. Typically these signalling systems are composed of a sensor histidine kinase (HK) and a response

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regulator (RR) (Stock et al., 2000). The total number of TCS and other signalling proteins encoded in a bacterial genome can be used as a measure of the adaptative potential of the organism (Galperin, 2005).

2. Materials and methods

2.1. Identification of TCS proteins in the Brucella species

The computational domain analysis of protein sequences was used to search for TCS proteins encoded in the genomes of *Brucella* species and *O. anthropi*. In the Pfam database of protein families (http://pfam.sanger.ac.uk), five different Hidden Markov Models (HMMs) profiles (accession numbers PF00512, PF07568, PF07730, PF07536 and PF06580) target different HK families (HisKA, HisKA_2, HisKA_3, HWE_HK and His_kinase), and one HMM profile targets the RR receiver (REC) domain (accession number PF00072). These HMM profiles were used to identify the putative HKs and RRs, and hits with an *E*-value below a selected cut-off (10^{-5}) were extracted. Hybrid HKs (REC-HKs) were determined by the presence of complete HK transmitter and REC domains in a single protein. Orthologs of the identified HKs and RRs between these genomes were determined by BLAST searches based on the reciprocal best hits against the other genomes. Finally, functional domains of the HKs and RRs were identified by search the Conserved Domain Database (CDD) (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) with Reverse Specific Position BLAST.

2.2. Construction of genome BLAST Atlases

The genome BLAST Atlas is a visualization method to show a vast amount of data in one plot by comparing a reference genome against a set of other genomes. Among other things, this can provide an overview of intraspecies genomic diversity (Hallin et al., 2008). The two BLAST atlases were constructed by using the *Brucella melitensis* chromosomes I and II as reference genomes, compared to the other public available sequenced genomes of *Brucella* species and *O. anthropi* (Fig. 1).

3. Results and discussion

3.1. TCS proteins in Brucella species

The genomes of *Brucella* species contain 22 HKs and 24 RRs (Table 1), two of which were hybrid HKs (CckA and PrlS) located on chromosome I. Recent surveys of the *B. melitensis* genome identified only 20 HKs and 21 RRs (Letesson and De Bolle, 2004; Hallez et al., 2007). HKs and RRs encoded by clusters of adjacent genes or involved in an identified phosphotransfer system were considered as putative TCS pairs (15 HK-RR pairs in all *Brucella* species) (Table 2). TCS genes not clustering in HK-RR gene pairs nor being part of an identified phosphorelay system were considered orphan genes (Table 3). Several of these *Brucella* TCSs have been investigated: BvrSR (Sola-Landa et al., 1998), NtrBC (Dorrell et al., 1999), NtrYX (Foulongne et al., 2000), FeuQP (Dorrell et al., 1998), the flagellar master regulator FtcR (Leonard et al., 2007), the DivK/CtrA regulatory pathway (Hallez et al., 2007), the blue light-activated LOV (light, oxygen, or voltage) HKs (LOV-HKs) (Swartz et al., 2007), and the BMEI0066 (CenR) RR (Zhang et al., 2009). The TCS BvrSR is essential for *Brucella* virulence and regulates the expression of outer membrane proteins (Sola-Landa et al., 1998). In *B. abortus*, the BA-LOV-HK appears to function as a photoreceptor regulating virulence (Swartz et al., 2007). The number of TCS proteins

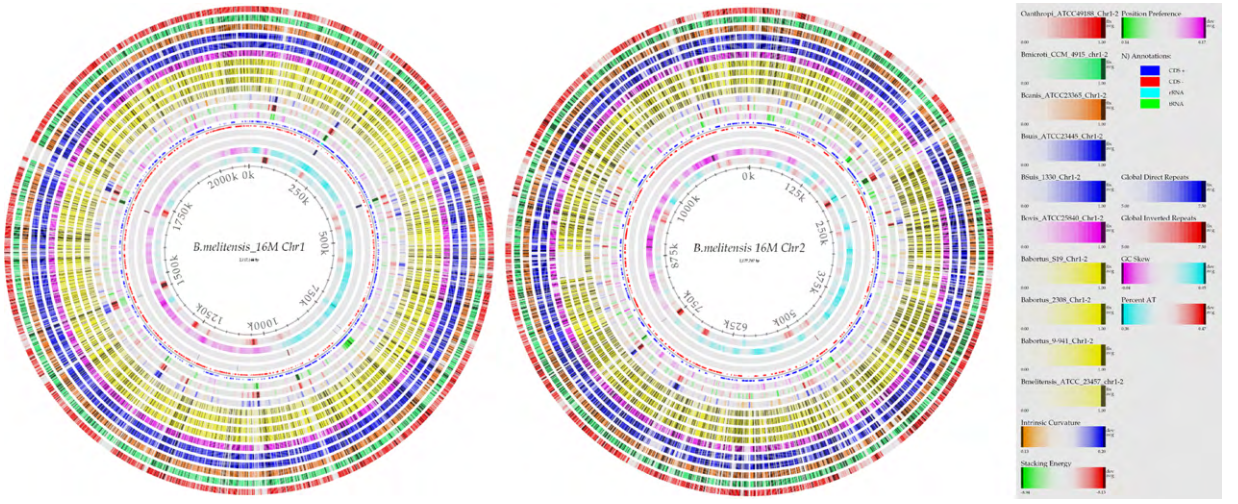

Fig. 1. Genome BLAST Atlases of *B. melitensis* 16 M chromosomes I and II. *B. melitensis* 16 M chromosomes I and II are the reference genome and are compared to other sequenced and public available genomes of *Brucella* species and *O. anthropi*. The two BLAST atlases were constructed using the "50/50" rule: at least 50% of the length of the protein, and a minimum of 50% sequence identity (Hallin et al., 2008). Each of the concentric circles represents different genomic properties mapped along the chromosome. The outer circles represent BLAST hits of a given genome (named in the legend) to the *B. melitensis* 16 M chromosome I or II. For the BLAST comparisons, regions with strong matches are darkly coloured and regions with weak or no matches are not coloured, as described previously (Hallin et al., 2008). BLAST alignments are performed at the amino acid level and only for proteins. A larger and zoomable version of the BLAST atlas is available online: http://www.cbs.dtu.dk/services/GenomeAtlas/suppl/zoomatlas/.