The Journal of Microbiology (2012) Vol. 50, No. 4, pp. 567–574
DOI 10.1007/s12275-012-2022-8

Parallel Gene Loss and Acquisition Among Strains of Different *Brucella* Species and Biovars[§]

Zhijun Zhong[1,4†], Yufei Wang[1†], Jie Xu[1†], Yanfen Chen[1†], Yuehua Ke[1†], Xiaoyan Zhou[3†], Xitong Yuan[1], Dongsheng Zhou[2], Yi Yang[1], Ruifu Yang[2], Guangneng Peng[4], Hai Jiang[3], Jing Yuan[1], Hongbin Song[1], Buyun Cui[3*], Liuyu Huang[1*], and Zeliang Chen[1*]

[1]*Department of Infectious Disease Control, Beijing Institute of Disease Control and Prevention, Beijing 100071, P. R. China*
[2]*Laboratory of Analytical Microbiology, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, P. R. China*
[3]*National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, P.O. Box 5, Changping, Beijing 102206, P.R. China*
[4]*College of Veterinary Medicine, Sichuan Agricultural University, Ya'an 625014, P. R. China*

(Received January 13, 2012 / Accepted May 10, 2012)

The genus *Brucella* is divided into six species; of these, *B. melitensis* and *B. abortus* are pathogenic to humans, and *B. ovis* and *B. neotomae* are nonpathogenic to humans. The definition of gene loss and acquisition is essential for understanding *Brucella*'s ecology, evolutionary history, and host relationships. A DNA microarray containing unique genes of *B. melitensis* Type strain 16MT and *B. abortus* 9-941 was constructed and used to determine the gene contents of the representative strains of *Brucella*. Phylogenetic relationships were inferred from sequences of housekeeping genes. Gene loss and acquisition of different *Brucella* species were inferred. A total of 214 genes were found to be differentially distributed, and 173 of them were clustered into 15 genomic islands (GIs). Evidence of horizontal gene transfer was observed for 10 GIs. Phylogenetic analysis indicated that the 19 strains formed five clades, and some of the GIs had been lost or acquired independently among the different lineages. The derivation of *Brucella* lineages is concomitant with the parallel loss or acquisition of GIs, indicating a complex interaction between various *Brucella* species and hosts.

Keywords: *Brucella*, DNA microarray, comparative genome hybridization, parallel loss and acquisition

[†]These authors contributed equally to this work
*For correspondence. (L. Huang) E-mail: huangly@nic.bmi.ac.cn; Tel.: +86-10-66948301; Fax: +86-10-66948301 / (Z. Chen) E-mail: zeliangchen@yahoo.com; Tel.: +86-10-66948434; Fax: +86-10-66948434; (B. Cui) E-mail: cuibuyun@icdc.cn
[§]Supplemental material for this article may be found at http://www.springer.com/content/120956

Introduction

Brucellae are a group of facultative intracellular bacteria that infect a range of mammalian livestock and wildlife, with most *Brucella* species occurring primarily in one or a few hosts (Moreno *et al*., 1990). *Brucella* was conventionally divided into six classical species based on subtle phenotypic and antigenic differences, and differential host specificity. Due to the limited genetic diversity, the *Brucella* genus was for a time classified as containing only one species with a series of biovars (VERGER *et al*., 1985). However, DNA fragment analysis and sequencing demonstrated that *Brucella* typically contains distinct species-specific lineages, which upheld the traditional division of *Brucella* species and the readoption of the classical species with a series of biovars (Moreno *et al*., 2002; Gargani and Lopez-Merino, 2006). Rooted with the close relative *Ochrobactrum anthropi*, it was found that the *B. ovis* lineage is basal to the rest of the *Brucella* lineage, and *B. suis* is a highly divergent clade with extensive intraspecific genetic diversity (Foster *et al*., 2009).

Changes in genome repertoire, occurring through gene loss and acquisition, are the major events underlying the emergence and evolution of bacterial pathogens. Comparative genome hybridization (CGH) with a whole genome microarray of *B. melitensis* 16MT showed that a number of genes were deleted among different *Brucella* species and clinical isolates (Rajashekara *et al*., 2004). These differentially distributed genes are defined as gene islands. Examination of the genomic context of these islands suggests that many of them were horizontally acquired. Analysis of genome sequences reveals evidence of horizontal gene transfer among *Brucella* species. Some of the genes indicated as having been acquired by lateral transfer play an important role in the survival of this pathogen in its hosts (Wattam *et al*., 2009).

To further evaluate the genetic diversity among different *Brucella* species, and understand the evolutionary framework of the *Brucella* genus, a DNA microarray containing unique genes of *B. melitensis* 16MT and *B. abortus* 9-941 was constructed and used in the present study to probe gene contents of 19 representative strains of the classical six species. Then the relationships and phylogeny of the representative strains were inferred from sequences of housekeeping genes. The putative gene loss and acquisition during derivation of different lineages were identified.

Materials and Methods

Brucella strains and culture conditions

Nineteen representative strains of different biovars of the

Table 1. Representative strains used in this study

Species	Biovar	Strains	ATCC
B. melitensis	1	16MT	23456
B. melitensis	2	63/9	23457
B. melitensis	3	Ether	23458
B. abortus	1	544A	23448
B. abortus	2	86/8/59	23449
B. abortus	3a	Tulya	23450
B. abortus	4	292	23451
B. abortus	5	B3196	23452
B. abortus	6a	870	23453
B. abortus	7	63/75	23454
B. abortus	9	C68	23455
B. suis	1	1330S	23444
B. suis	2	Thomsen	23445
B. suis	3	686	23446
B. suis	4	40/67	23447
B. suis	5	513	-
B. neotomae	-	5K33	23459
B. ovis	-	63/290	25840
B. canis	-	RM6/66	23365

six *Brucella* species were preserved and supplied by the Chinese Center for Disease Control and Prevention (Table 1). All the strains used for the present study were preserved without extensive laboratory passages. *Brucella* strains were grown to stationary phase at 37°C in Tryptic Soy Broth (TSB) or Tryptic Soy Agar (TSA) with or without 5% CO_2. Genomic DNA was isolated from *Brucella* cultures using a Promga genomic DNA Isolation Kit as recommended by the manufacturer.

DNA microarray development, comparative genome hybridization, PCR verification and data analysis

The development of the DNA microarray and the comparative genomic hybridization were carried out essentially as described (Zhou *et al*., 2004). Briefly, A total of 3218 annotated open reading frames (ORFs), including all the unique genes from *B. melitensis* 16MT and the *B. abortus* 9-941, were chosen for DNA microarray construction. 3212 genes were successfully amplified and the PCR products were purified. PCR products were spotted in duplicate on VAL S25 glass slides (CEL, USA) to generate the DNA microarray. For comparative genome hybridization, a mixture of equal quantities of *B. melitensis* 16MT and *B. abortus* 544A genomic DNAs was used as reference DNA, and genomic DNA from representative strains as test DNA. Cy3- or Cy5-labeled probes were generated by priming of the reference or test DNA with random hexamers and extension with Klenow polymerase. The labeled reference and test DNAs were combined to hybridize with the microarrays by dual-fluorescence hybridization. The hybridized slides were scanned, the scanning images were processed, and the data were further analyzed by using GenePix Pro 4.1 software (Axon Instruments). An intensity ratio was recorded for each spot and then was converted to $\log_2$ values. The mean value of the log ratios for each gene was calculated. Log values ≤ -1 were taken as defining the absence of a gene in the relevant strains. The absence of the genes generated by microarray analysis was verified by PCR. For single genes, primers for probe amplification were used for verification. For a differential genomic region with 3 or more sequential genes, two genes locate separately were selected to represent the region.

Housekeeping gene sequencing and analysis

Seven housekeeping genes, including *aroA*, *cobQ*, *dnaK*, *gap*, *glk*, *gyrB*, and *trpE*, were PCR amplified and sequenced, essentially as described previously (Whatmore *et al*., 2007). The sequence data was edited using the Lasergene package. Each distinct allele at each of the seven loci was given an individual, arbitrary, numerical designation and each unique allelic pattern over all seven loci was identified as a sequence type (ST). Allelic profiles and sequence data were imported into the START package to determine mean % GC content. The same package was used to calculate the average frequencies of synonymous substitutions per potential synonymous site (dS) and nonsynonymous substitutions per potential nonsynonymous site (dN) by the method of Nei and Gojobori in order to test the degree of selection on a locus. Split decomposition analysis of allelic profile data was performed using a web-based version of the SplitsTree program. ST Lineages were analyzed by using the eBURST algorithm, available at http://eburst.mlst.net/. Clonal complexes were defined by using the default setting, in which all STs within a clonal complex differ by no more than one allele from at least one other ST in the clonal complex.

Phylogenetic analysis

The coding sequences of the seven housekeeping genes are concatenated and used to infer phylogentic relationships of the representative strains. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al*., 2007). The evolutionary relationship was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method and are given as the number of base substitutions per site.

Results

Overview of the microarray analysis

To identify genetic components that are differentially distributed among the representative strains of *Brucella*, a whole genome microarray containing unique genes of 16MT and 9-941, representative strains of *B. melitensis* and *B. abortus* respectively, was constructed and used to determine the presence or absence of these genes. A total of 3212 probes representing the unique genes of 16MT and 9-941 were included in the microarray. After filtering spots with bad signals, a total of 3015 (93.8% of the unique probes) were included for data analysis. Control hybridizations showed that the 16MT or 9-941 unique genes could be differentiated by the microarray, indicating that the microarray could detect the presence or absence of a gene (data not

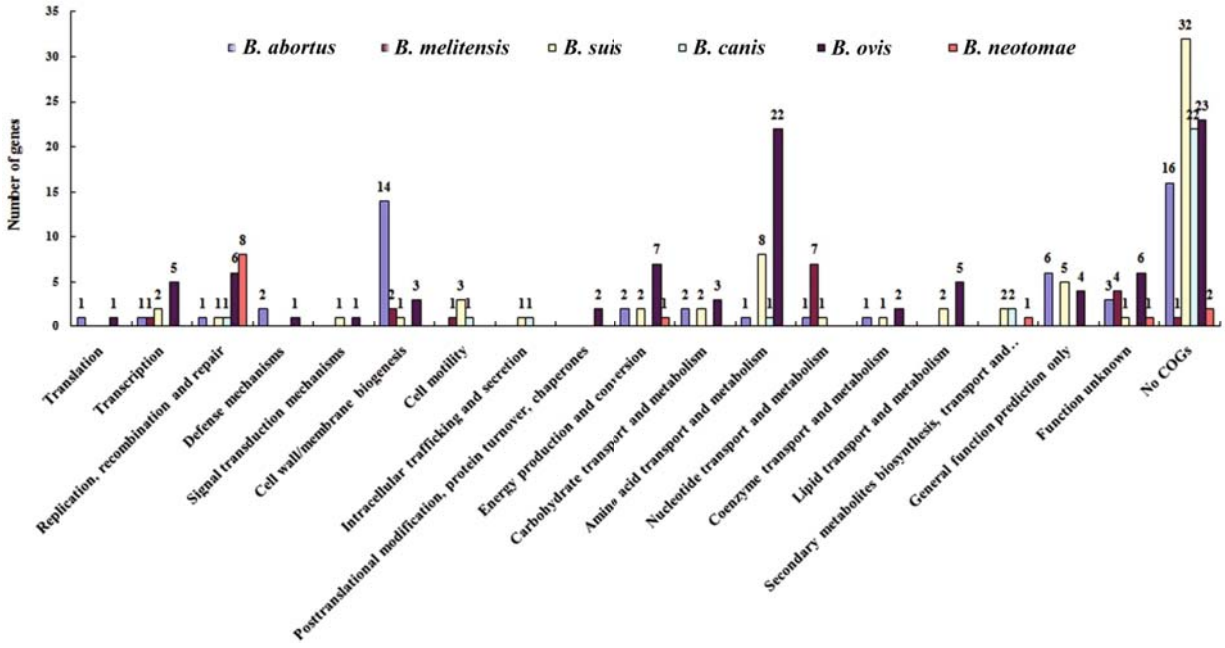

Fig. 1. **Functional categories of differentially distributed genes.**

shown). Then, genomic DNAs of the representative strains were hybridized to the microarrays and the presence or absence of these genes was determined. Hybridization results revealed that the majority of these genes were likely present across all representative strains tested as shown by the majority of genes having a signal intensity ratio close to 1 relative to reference DNA, which is consistent with the notion that *Brucella* is a monospecific genus with limited genetic diversity. Putative deletions were verified by PCR amplification with gene specific primers. A total of 214 genes were identified to be absent in one or more of the representative strains. The predicted product of each such gene and its distribution across representative *Brucella* strains are listed in Supplementary data Table S1. Of these genes, 85 are located on chromosome I and 129 on chromosome II. These genes belong to different functional categories, including transport and metabolism, energy production, and conversion, cell wall/membrane biogenesis, translation and transcription (Fig. 1).

Most of the differentially distributed genes are clustered into GIs with evidence of horizontal gene transfer

Of the 214 genes identified to be differentially distributed

Table 2. **Characteristics of GIs**

GI	GI[a]	Locus range	Gene numbers	Size (bp)	tRNA relation	GC content (%)	Transposation or recombinase	Homologue in *O. anthropi*
GI01	GI-1	BMEI0899-0907	9	3657	tRNA (Leu), (BMEI0889, 907)	53.25	resolvase, recombinase	-
GI02	GI-2	BMEI0994-1012	19	10533	tRNA (Gly), (BMEI1012)	51.4	transposase	BMEI1000, 1003
GI03		BMEI1656-1658	3	785	tRNA (Arg), (BMEI1649, 1664)	52.69	recombinase	-
GI04	GI-3	BMEI1674-1702	24	13774	tRNA (Phe), (BMEI1674)	52.4	transposase	-
GI05		BMEI1819-1822	4	2884		58.32	transposase	BMEI1819
GI06	GI-4	BMEII0185-0226	42	39041	tRNA (Met, Ser), (BMEII0183, 0228)	57.07	transposase	-
GI07		BMEII0438-0442	5	5134	tRNA (Thr), (BMEII0458)	56.71	transposase	-
GI08		BMEII0628-0634	7	4949	tRNA (Asn), (BMEII0649)	59.15	-	BMEII0628, 632, 633
GI09		BMEII0639-0645	6	4594	tRNA (Asn), (BMEII0649)	57.57	-	-
GI10	GI-6	BMEII0710-0719	10	5941	tRNA (Ser), (BMEII0709)	53.44	transposase	-
GI11	GI-7	BMEII0811-0815	5	4880	-	56.91	-	BMEII0813
GI12	GI-8	BMEII0827-0849	23	20533	-	58.12	-	BMEII0827, 828, 832, 834, 848
GI13	GI-9	BMEII0875-0878	4	2936	-	56.77	-	-
GI14		BruAb1_0609-0613	5	3063	-	59.26	-	-
GI15		BruAb2_0590-0596	7	6223	tRNA (Asn), (BruAb2_0599)	56.84	-	BruAb2_0590, 593

[a] GI indicates the GI was also identified by Rajashekara *et al.* (2004)

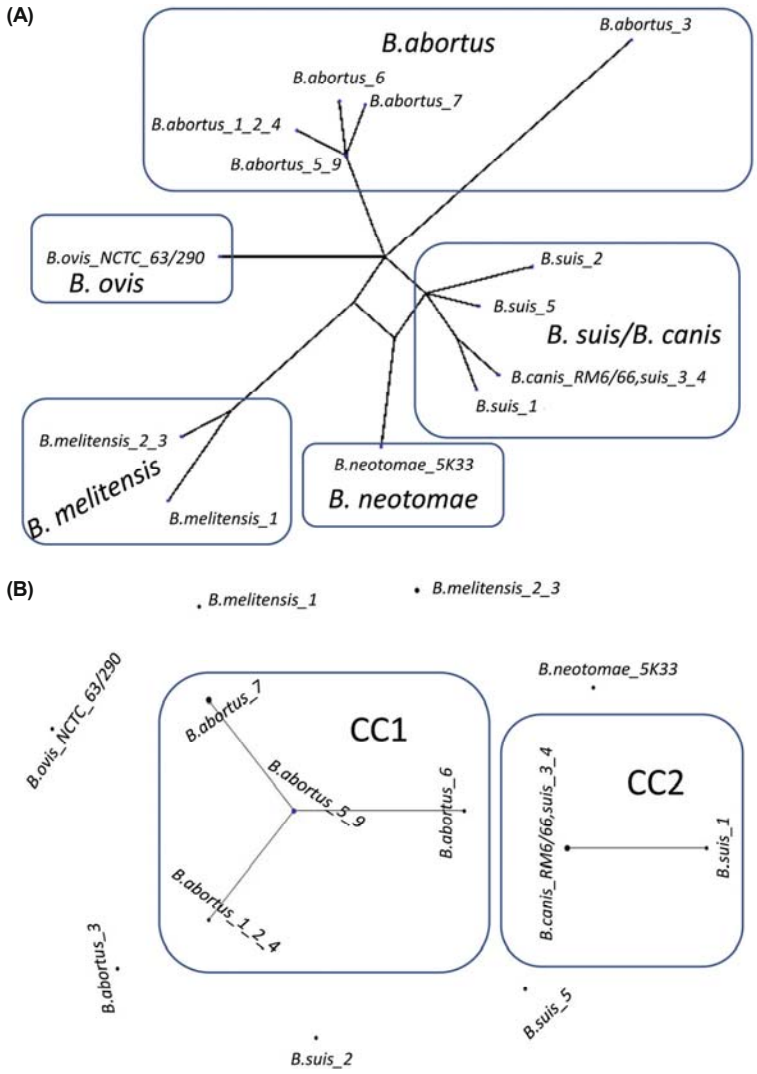

Fig. 2. Recombination history in sequences and clonal complex relationships of different *Brucella*. (A) Split tree decomposition analysis of the sequence type of the representative strains. (B) Clonal complexes of the representative strains.

among representative strains, 173 genes were found to be clustered. A gene cluster with 3 or more sequential genes was designated as a genomic island (GI), and 15 GIs were identified among the 214 genes. These GIs were designated based on their location in the genome of 16MT or 9-941. As shown in Table 2, these GIs contained 3 to 42 genes, with a length range of 0.78–39.0 kb. We analyzed the distribution of the 15 GIs on the two chromosomes. As shown in Table 2, the numbers of GIs on the two chromosomes were different, with 6 GIs on chromosome I and 9 on chromosome II. The GI gene distribution showed that 64 genes locate on chromosome I and 109 on chromosome II, indicating that chromosome II had a higher percentage of variable genes than chromosome I.

GIs that are acquired or lost through horizontal transfer usually have connected genetic evidence. Typical characteristics of a transferrable GI include integration adjacent to or within tRNA genes, with an integrase or insertion sequence flanking the ends. Analysis of the surrounding sequences of the GIs in 16MT or 9-941 showed that adjacent tRNA or recombinase genes were found for 10 of 15 GIs (Table 2). Some of these GIs showed dissimilar GC content compared to the remaining genome. Of the 6 GIs located on chromosome I, 4 have lower GC content (52.4%) than the average GC content (58.2%) of the chromosome. However, for GIs located on the chromosome II, only one GI (GI10) showed decreased GC content (Table 2). These characteristics indicated that these GIs might be acquired or lost by horizontal gene transfer.

Clonal lineage relationships among the representative strains

To analyze the relative phylogenetic relationships of different *Brucella* species, seven housekeeping genes were amplified and sequenced. The sequences of internal fragments of the seven housekeeping genes were determined for the 19 representative strains. The molecular characterization of the 19 representative strains by MLST identified 13 different STs, indicating a high genotypic diversity (Supplementary data Table S2). Three (*trpE*) and 7 (*glk*) alleles were found for these loci, and the number of polymorphic nucleotide sites at the seven loci also varied between 3 (*trpE*) and 7 (*glk*)

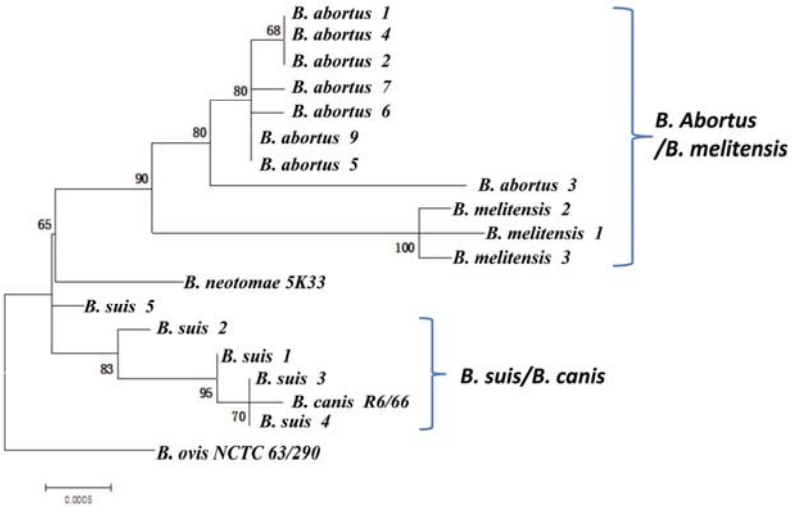

Fig. 3. Neighbor-joining tree of 19 representative *Brucella* strains based on concatenated sequences. Coding sequence of housekeeping genes were concatenated and a neighbor-joining tree of the concatenated sequences was reconstructed by MEGA4. 1,000 bootstrap replicates were used to examine the confidence in the tree as described in the 'Materials and Methods' section.

(Supplementary data Table S3). The most represented STs were ST1 and ST6, which each comprised 3 of the 19 strains. Split decomposition analysis on allelic profile data using SplitsTree showed that STs of *B. suis* were highly divergent and *B. canis* formed a complex with *B. suis* (Fig. 2A). The 13 STs were divided by eBURST into 1 major clonal complex (CC1), 1 minor clonal complex and 7 singletons (Fig. 2B). The major clonal complex, CC1, comprised 4 different STs and included 7 representative strains of *B. abortus*. The minor clonal complex CC2 comprised 2 STs and included 4 strains, including *B. suis* 1, 3, 4, and *B. canis* RM6/66. All the other strains were singletons.

Phylogenetic relationship among representative *Brucella* strains

The coding sequences of the seven housekeeping genes were concatenated and a total of 3,468 bp sequences were included in the final dataset. The phylogenetic tree was constructed based on sequence similarity using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary relationship of the strains. When rooted with *B. ovis*, the 19 strains formed five clades: *B. abortus*, *B. melitensis*, *B. suis-B. canis*, *B. ovis*, and *B. neotomae*, being consistent with what was observed by using SNPs from whole genome sequences. As shown in Fig. 3, *B. suis* was the most diverse species within *Brucella*. A close relationship of *B. abortus* and *B. melitensis* and a more distant grouping of *B. suis* was observed. There was a close relationship of *B. suis* biovar 3 and 4 to *B. canis* and a close but more distant relationship of *B. suis* biovar 1. The species *B. canis* appears to have arisen directly from a *B. suis* ancestor, making currently defined *B. suis* isolates paraphyletic.

Parallel gene loss and acquisition during *Brucella* lineage evolution

To further analyze the gene loss or acquisition among the representative strains, the GIs were mapped on the phylo-

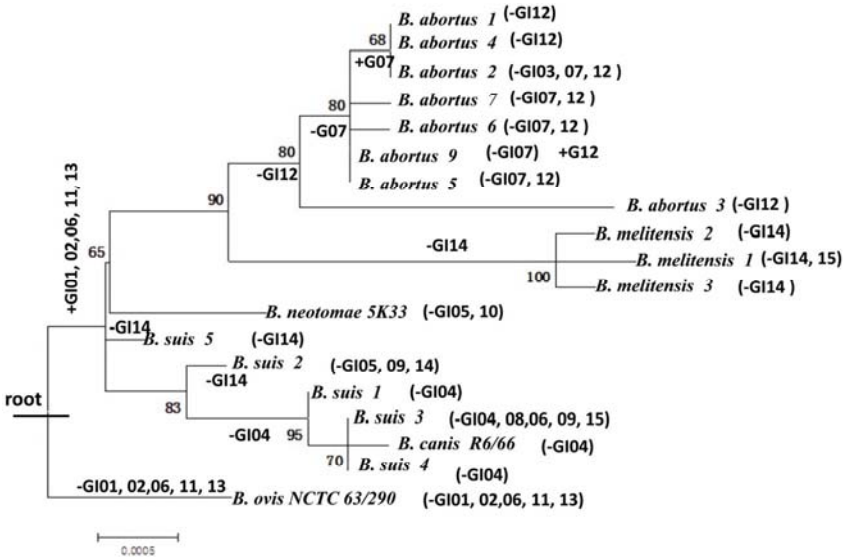

Fig. 4. Parallel GI loss and acquisition for 19 representative *Brucella* strains.

genetic tree. As shown in Fig. 4, compared with the other strains, *B. ovis* has lost 5 GIs (GI01, GI02, GI06, GI11, and GI13). That is, the five GIs exist in all *Brucella* species except *B. ovis*. A total of 79 genes were contained in the five GIs, and only 3 of them were homologous to *Ochrobactrum anthropi* genes, indicating that most of these genes were acquired by *Brucella* after they diverged from their ancestors. *B. ovis*, which did not acquire these GIs, fell into a different clade and remained nonpathogenic to many hosts. We also analyzed the presence of homologues of all the GI genes in *Ochrobactrum anthropi*. To our surprise, only 14 of them had homologues in *Ochrobactrum anthropi* (Table 2). This implied that most of these genes were acquired after the divergence of *Brucella* from their common ancestors. Consistent with their high degree of homology, all three *B. melitensis* isolates lost GI14. In addition, 7 of the 8 biovars of *B. abortus* had lost GI12. The only exception is *B. abortus* biovar 9, which only lost GI07. It might be inferred that an ancestor of *B. abortus* 9 had lost GI12 and then acquired it again during the course of adaptation or interaction with its host, or that it did not lose this GI. The loss of GIs in different *B. suis* biovars was diverse, as is true for the diversity of the strains themselves. The close relatives *B. suis* 3, 4 and *B. canis* lost GI4. However, *B. suis* 3 also lost another 3 GIs. Together, these findings suggest that there has been parallel gene loss and acquisition among *Brucella* taxa during it evolution and interaction with hosts.

Discussion

The species concept for *Brucella* was a subject of debate for a time, but subsequent genetic analysis has led to the adoption of a conventional genus with various species. The high degree of similarity of all these *Brucella* genomes, in comparison to other bacterial groups, suggests a close phylogenetic relationship. However, clear differences in host preference might still justify separate species designations. For example, although *B. abortus* has also been found in various mammalian species, including humans, it is isolated from cattle at greatly higher frequencies.

The present microarray analysis revealed extensive gene content similarities among *Brucella* species. We discovered that over 93.8% of the 3,212 genes represented on the microarray were present in all of the 19 representative strains. A total of 214 of the unique genes (7.0%) are absent from at least one of the representative strains. Most of these genes are involved in functional categories that might play roles in the interaction with the host or survival under both *in vitro* and *in vivo* conditions. For example, genes involved in membrane structure, metabolism, transport and intracellular trafficking are usually related to *Brucella* survival and virulence. As shown in Fig. 1, the six species showed different deletion profiles in both gene content and functional categories. *B. abortus* lost 22 genes involved in cell wall/membrane biogenesis, implying possible membrane structural differences compared to the other five species. Our results suggest that genomes of *Brucella* species are highly homogenous and imply that a relatively small number of genetic changes may be responsible for differences in host preference and virulence among different *Brucella* species.

As is observed in many pathogenic bacteria, functionally related genes are linked and lost or acquired together. We found that 173 (80%) of the 214 genes were clustered into 15 genomic regions, which were named genomic islands (GI). We compared the GIs with those identified previously. In their CGH analysis with a microarray of 16MT, a total of 16 GIs were identified. Among the 15 GIs identified by our study, 8 were also found to be absent in the relevant *Brucella* strains by Rajashekara *et al.* (2004). The other 7 GIs were newly identified to be absent among the six species. This difference might be mainly due to the different strains and species used in the two studies. In their CGH analysis, more strains of the main species were included, and in our study, all the representative strains for the six species, but only one strain for each of the biovars, were included.

Among the representative strains, *B. ovis* lost the highest number of GIs (GI01, GI02, GI06, GI11, GI13), which is consistent with the loss of virulence of this species for most mammalian hosts. The large number of gene content changes observed in the *B. ovis* genome may have resulted from more active ISs in this species than in other *Brucella* spp. *B. ovis* has more IS6501 copies (about 30 copies) than other *Brucella* species (4 to 10 copies) (Ouahrani *et al.*, 1993). ORFs in GI02 and GI06 encode factors involved in *Brucella* virulence. GI06 has 19 ORFs encoding peptide ABC-type transporters such as Dpp, Opp, and Pot systems. Homologues of these transporters in other bacteria are important for root colonization, intracellular survival, attachment to host cell, and virulence (Borezee *et al.*, 2000; Kuiper *et al.*, 2001). Absence of the Opp system in *B. ovis* may cause increased uptake of peptides due to dysregulation of rate of peptide uptake, thus influencing their intracellular survival (Tsolis *et al.*, 2009). From examining the GIs for potential lateral transfer, we note that most of these GIs are unique to *Brucella* and not shared with *Ochrobactrum*. It is likely that these regions were acquired by *Brucella* after diverging from its ancestor *Ochrobactrum*. This also implies lateral transfer did exist in *Brucella* despite its intracellular lifestyle preferences.

Genome reduction, or reductive evolution, involves gene loss through mutational inactivation and deletion. It has been observed in a number of intracellular pathogenic bacteria, including *Rickettsia prowazekii* (Andersson *et al.*, 1998) and *Mycobacterium leprae* (Cole *et al.*, 2001). *Brucella* genomes are all similar in size, with an average size of 3.29 Mb, markedly smaller than their nearest sequenced relatives *O. anthropi* (5.22 Mb) and *O. intermedium* (4.6 Mb). Accumulation of pseudogenes or complete losses of genes are direct evidence of genome reduction. Using the general estimate that bacterial genomes have about 1–5% pseudogenes (Liu *et al.*, 2004), the 4.6% fraction observed in *Brucella* can be considered relatively high and suggestive of genome degradation. However, no obvious genome reductions were observed among different *Brucella* species in terms of both genome size and gene number. Therefore, the difference in gene content between the different *Brucella* species might mainly have resulted from the different requirements for these genes in the interaction between *Brucella* and its host. More pseudogenes were observed on chromosome II than on chromosome I. Together with the higher degree of gene

content changes observed on chromosome II, it can be concluded that chromosome II is more dynamic, perhaps owing to its hypothesized origin as a plasmid (Slater *et al.*, 2009).

B. suis is the most diverse species within *Brucella* thus far examined. Exceptional diversity in clades of this species, compared to the other clades, was observed (Whatmore *et al.*, 2007; Foster *et al.*, 2009). In the present study, the 19 representative strains are clustered into five clades and *B. suis* forms two clades. A range of genetic analyses have indicated considerable diversification within the *B. suis* clades and have even suggested likely relationships among the biovars. Most studies looking at variation within *B. suis* have difficulties in differentiating isolates from that of *B. canis*, suggesting a close relationship between these two species (Fretin *et al.*, 2008). *B. canis* appears to have arisen directly from a *B. suis* ancestor, making currently defined *B. suis* isolates paraphyletic. Early fragment analysis by Allardet-Servent *et al.* using restriction endonucleases also suggested that *B. canis* likely evolved from a strain of *B. suis* (Allardet-Servent *et al.*, 1988).

Combined with the phylogenetic tree, gene loss and acquisition along the evolutionary lineage could be putatively inferred. As shown in Fig. 4 and Table 2, after derivation from a common ancestor, *B. ovis* and the other strains acquired a number of GIs. Compared with the basal lineage *B. ovis*, all the other species or biovars acquired 4 GIs and lost different sets of GIs. For the *B. melitenesis*/*B. abortus* clades, all the *B. melitensis* representatives lost GI14, and all the *B. abortus* biovars except biovar 9 lost GI12. From the phylogentic tree, it can be inferred that ancestors of *B. abortus* 9 lost and then re-acquired GI12 at least once. The exceptional genetic diversity of *B. suis* was also observed in the GI distribution (Rajashekara *et al.*, 2004). Seven GIs were observed in *B. suis* strains, while none of them was consistently present or absent in all the biovars (Rajashekara *et al.*, 2004; Wattam *et al.*, 2009). From this point of view, the GIs were lost or acquired among the different strains independently. This parallel gene loss and acquisition might have resulted from the complex interaction of *Brucella* with its host and adaptation to different environments (Wattam *et al.*, 2009).

Acknowledgements

This work was supported by National Basic Research Program of China (Grant No. 2009CB522602), National Natural Science Foundation of China (31000548, 31000041, 81071320), National Key Program for Infectious Diseases of China (2008ZX10004-015, 2009ZX10004-103, 2008ZX10004-008).

References

Allardet-Servent, A., Bourg, G., Ramuz, M., Pages, M., Bellis, M., and Roizes, G. 1988. DNA polymorphism in strains of the genus *Brucella*. *J. Bacteriol.* **170**, 4603–4607.

Andersson, S.G., Zomorodipour, A., Andersson, J.O., Sicheritz-Ponten, T., Alsmark, U.C., Podowski, R.M., Naslund, A.K., Eriksson, A.S., Winkler, H.H., and Kurland, C.G. 1998. The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature* **396**, 133–140.

Borezee, E., Pellegrini, E., and Berche, P. 2000. OppA of *Listeria monocytogenes*, an oligopeptide-binding protein required for bacterial growth at low temperature and involved in intracellular survival. *Infect. Immun.* **68**, 7069–7077.

Cole, S.T., Eiglmeier, K., Parkhill, J., James, K.D., Thomson, N.R., Wheeler, P.R., Honore, N., Garnier, T., Churcher, C., Harris, D., and *et al.* 2001. Massive gene decay in the leprosy bacillus. *Nature* **409**, 1007–1011.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Foster, J.T., Beckstrom-Sternberg, S.M., Pearson, T., Beckstrom-Sternberg, J.S., Chain, P.S., Roberto, F.F., Hnath, J., Brettin, T., and Keim, P. 2009. Whole-genome-based phylogeny and divergence of the genus *Brucella*. *J. Bacteriol.* **191**, 2864–2870.

Fretin, D., Whatmore, A.M., Al Dahouk, S., Neubauer, H., Garin-Bastuji, B., Albert, D., Van Hessche, M., Menart, M., Godfroid, J., Walravens, K., and *et al.* 2008. *Brucella suis* identification and biovar typing by real-time PCR. *Vet. Microbiol.* **15**; 131(3–4), 376–385.

Gargani, G. and Lopez-Merino, A. 2006. International Committee on Systematic Bacteriology, Subcommittee on the taxonomy of Brucella correspondence report (interim report 1991-1993). *Int. J. Syst. Evol. Microbiol.* **56**, 1167–1168.

Kuiper, I., Bloemberg, G.V., Noreen, S., Thomas-Oates, J.E., and Lugtenberg, B.J. 2001. Increased uptake of putrescine in the rhizosphere inhibits competitive root colonization by *Pseudomonas fluorescens* strain WCS365. *Mol. Plant Microbe Interact.* **14**, 1096–1104.

Liu, Y., Harrison, P.M., Kunin, V., and Gerstein, M. 2004. Comprehensive analysis of pseudogenes in prokaryotes: widespread gene decay and failure of putative horizontally transferred genes. *Genome Biol.* **5**, R64.

Moreno, E., Cloeckaert, A., and Moriyon, I. 2002. *Brucella* evolution and taxonomy. *Vet. Microbiol.* **90**, 209–227.

Moreno, E., Stackebrandt, E., Dorsch, M., Wolters, J., Busch, M., and Mayer, H. 1990. *Brucella abortus* 16S rRNA and lipid A reveal a phylogenetic relationship with members of the alpha-2 subdivision of the class *Proteobacteria*. *J. Bacteriol.* **172**, 3569–3576.

Ouahrani, S., Michaux, S., Widada, J., Bourg, G., Tournebize, R., Ramuz, M., and Liautard, J. 1993. Identification and sequence analysis of IS6501, an insertion sequence in *Brucella* spp.: relationship between genomic structure and the number of IS6501 copies. *Microbiology* **139**, 3265.

Rajashekara, G., Glasner, J.D., Glover, D.A., and Splitter, G.A. 2004. Comparative whole-genome hybridization reveals genomic islands in *Brucella* species. *J. Bacteriol.* **186**, 5040–5051.

Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406.

Slater, S., Goldman, B., Goodner, B., Setubal, J., Farrand, S., Nester, E., Burr, T., Banta, L., Dickerman, A., and Paulsen, I. 2009. Genome sequences of three *Agrobacterium* biovars help elucidate the evolution of multichromosome genomes in bacteria. *J. Bacteriol.* **191**, 2501.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* **24**, 1596.

Tsolis, R.M., Seshadri, R., Santos, R.L., Sangari, F.J., Lobo, J.M., de Jong, M.F., Ren, Q., Myers, G., Brinkac, L.M., Nelson, W.C., and *et al.* 2009. Genome degradation in *Brucella ovis* corresponds with narrowing of its host range and tissue tropism. *PLoS ONE* **4**, e5519.

Verger, J., Grimont, F., Grimont, P., and Grayon, M. 1985. *Brucella*,

a monospecific genus as shown by deoxyribonucleic acid hybridization. *Int. J. Syst. Evol. Microbiol.* **35**, 292.

Wattam, A.R., Williams, K.P., Snyder, E.E., Almeida, N.F., Jr., Shukla, M., Dickerman, A.W., Crasta, O.R., Kenyon, R., Lu, J., Shallom, J.M., and *et al.* 2009. Analysis of ten *Brucella* genomes reveals evidence for horizontal gene transfer despite a preferred intracellular lifestyle. *J. Bacteriol.* **191**, 3569–3579.

Whatmore, A.M., Perrett, L.L., and MacMillan, A.P. 2007. Characterisation of the genetic diversity of *Brucella* by multilocus sequencing. *BMC Microbiol.* **7**, 34.

Zhou, D., Han, Y., Song, Y., Tong, Z., Wang, J., Guo, Z., Pei, D., Pang, X., Zhai, J., Li, M., and *et al.* 2004. DNA microarray analysis of genome dynamics in *Yersinia pestis*: insights into bacterial genome microevolution and niche adaptation. *J. Bacteriol.* **186**, 5138–5146.