Genomic Characterization Provides New Insights for Detailed Phage-Resistant Mechanism for Brucella abortus

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As the causative agent of cattle brucellosis, Brucella abortus commonly exhibits smooth phenotype (by virtue of colony morphology) that is characteristically sensitive to specific Brucella phages, playing until recently a major role in taxonomical classification of the Brucella species by the phage typing approach. We previously reported the discrepancy between traditional phenotypic typing and MLVA results of a smooth phage-resistant (SPR) strain Bab8416 isolated from a 45-year-old custodial worker with brucellosis in a cattle farm. Here, we performed whole genome sequencing and further obtained a complete genome sequence of strain Bab8416 by a combination of multiple NGS technologies and routine PCR sequencing. The detailed genetic differences between B. abortus SPR Bab8416 and large smooth phage-sensitive (SPS) strains were investigated in a comprehensively comparative genomic study. The large indels between B. abortus SPS strains and Bab8416 showed possible divergence between two evolutionary branches at a far phylogenetic node. Compared to B. abortus SPS strain 9-941 (Bab9-941), the specific re-arrangement event in Bab8416 displaying a closer linear relationship with B. melitensis 16M than other B. abortus strains resulted in the truncation of c-di-GMP synthesis, and 3 c-di-GMP-metabolizing genes, were present in Bab8416 and B. melitensis 16M, but absent in Bab9-941 and other B. abortus strains, indicating potential SPR-associated key determinants and novel molecular mechanisms. Moreover, despite almost completely intact smooth LPS related genes, only one mutated OmpA family protein of Bab8416, functionally related to flagellar and efflux pump, was newly identified. Several point mutations were identified to be Bab8416 specific while a majority of them were verified to be B. abortus ST2 characteristic. In conclusion, our study therefore identifies new SPR-associated factors that could play a role in refining and updating Brucella taxonomic schemes and provides resources for further detailed analysis of mechanism for Brucella phage resistance.

Keywords: Brucella abortus, phage resistance, comparative genomics, genome typing, phylogentic analysis
INTRODUCTION

Brucellosis is one of the most serious zoonotic infectious diseases worldwide, and is caused by pathogenic species of Brucella genus. Up to now, 12 species were defined into the genus Brucella (Godfroid et al., 2013). Six of them, including B. melitensis, B. abortus, B. suis, B. canis, B. ovis, and B. neotomae, belong to the “classical” or “traditional” Brucella species1. Generally, all Brucella species with nucleotide similarities > 90% are genetically closely related (Al Dahouk et al., 2010).

Traditional Brucella typing is primarily based on different phenotypic characteristics (Garcia et al., 1988; Jahans et al., 1997; Moreno et al., 2002; Sanogo et al., 2013), including colony morphology, CO2 requirement, H2S production, substrate utilization, growth on serum dextrose agar dye plate, agglutination with monospecific sera, Brucella phage lysis profiles at routine test dilution (RTD) and host preference (Jones et al., 1968; Morris et al., 1973; Rigby et al., 1989). The three major species in terms of disease and economic impact for man, B. melitensis, B. abortus and B. suis are further subdivided into multiple biovars (bv) based on a range of phenotypic and serological characteristics. For example, B. abortus is subdivided into bv 1–6 and 9 (Pappas et al., 2006). Furthermore, despite the close genetic relationship of several genetic loci (e.g., 16S rRNA, 98.7%) and a biochemical profile similar to Ochrobactrum spp., several non-classical Brucella species like B. microti and B. inopinata are often easily misidentified using traditional biochemical typing methods (Scholz et al., 2008a,b). Among these routine phenotypic characterizations, B. abortus with smooth Lipopolysaccharide (LPS) was identified to be sensitive to Brucella phages like Berkeley2 (BK2), Tbilisi (Tb), Weybridge (Wb), and Izatnagar (Iz) (FAO/WHO, 1986). This useful test is significant for differentiating B. abortus from other Brucella species (Jones et al., 1968; Morris et al., 1973).

Since SPR B. abortus was initially reported in Corbel and Morris (1974), there have been few studies on this distinct phenotype over the last four decades. The susceptibility of smooth B. abortus strains to lysis by Brucella phages is commonly used to type various Brucella species. We have recently reported the identification of the first SPR B. abortus strain Bab8416 from a brucellosis patient in China (Kang et al., 2015). The phage activity of Bab8416 is similar to that of B. melitensis bv 1 strain 16M and showed special biochemical characteristics distinct from that of all B. abortus biovars. It was not lysed by Tb, Iz, and Wb phage in 1 × RTD and 10^4 × RTD, but lysed by BK2 phage in 1 × RTD and 10^2 × RTD. Due to the unusual discrepancy between phenotypic profiles, Bab8416 could not be precisely classified to any of the existing B. abortus biovars. In this study, we completed the genome sequence of Bab8416 through a combination of next-generation sequencing (NGS) and common PCR-based gap closure and investigated genomic differences between Bab8416 and other Brucella strains for gene association in corresponding biochemical or physiological profiles.

1http://www.bacterio.net/brucella.html

MATERIALS AND METHODS

Ethics Statement

This study and the protocol were carried out in accordance with the recommendations of ethics committee of the local disease control and Prevention Research Center of the Inner Mongolia Autonomous Region and Baotou City. The patient gave written informed consent for participation in this study and publication of his identifiable information, in accordance with the Declaration of Helsinki. The detailed information of strain Bab8416 referred to our previous study (Kang et al., 2015).

Genome Sequencing, Assembly and Annotation

Using 454 GS-FLX system, a total of 190,817 reads were obtained with the average length of 566 bp. Twenty-two contigs with lengths more than 500 bp and average coverage of 33.2X were obtained by Newbler using default parameters. Using the genome of B. abortus 9-941 as a reference, the order of the contigs was sorted and gap closure using common PCR was performed with ContigsScape (Tang et al., 2013). To fix the homopolymer sequencing errors systemically caused by 454 GS-FLX sequencing system, another 180 bp Paired End (PE) library was constructed and sequenced by the Illumina Hiseq 2000 system. Genome sequencing results were refined by short reads using Pilon with default parameters (Walker et al., 2014). The coding genes were predicted by Prodigal (Hyatt et al., 2010) and these genes were annotated by BLAST against NCBI non-redundant (NR), COG, KEGG, TrEMBL, Swissprot databases with e value cutoff of 1e-5 and GO terms assigned to the annotated genes using BLAST2GO pipeline (Conesa et al., 2005). The tRNAs were detected by tRNAscan-SE (v1.23) (Schattner et al., 2005) and rRNAs were identified by blasting homologous rRNA sequences against the Bab8416 genome.

Whole Genome Collinear Analysis

Firstly, oriC site was identified in both references and Bab8416 genome using Ori-Finder 2 and was set to be the first base of Bab8416 genome (Luo et al., 2014). Then, whole genome sequence alignments between these two genomes were processed by MUMmer 3.23 package (Kurtz et al., 2004).

Brucella MLVA Typing and MLST Typing

Multiple-locus variable number tandem repeat analysis (MLVA) assay was employed and the markers were obtained by PCR (Jiang et al., 2013b). The MLVA markers of Bab8416 were compared to the MLVA database2. The multilocus sequence typing (MLST) schemes of Brucella species using 9 conserved housekeeping genes were performed as previously described (Whatmore et al., 2007).

SNP Calling

All the draft genomes were linked to be two pseudo chromosomes by taking B. abortus 9–941 genome as a reference and the

2http://mlva.u-psud.fr/brucella/
sequences were gaped with ‘NNNNN.’ The SNPs were firstly identified by Mauve (Darling et al., 2010) using the genome sequences in this study and after "N" removed, the remaining SNP were finally exported for further analysis.

Gene Family Identification and Phylogenetic Analysis
Thirty-nine available Brucella reference genomes were utilized to perform comparative genomic and phylogenetic analyses, including all known Brucella species and all of seven biovars of B. abortus. Three strains with lower contig numbers and high coverage in each biovar of B. abortus were selected. All genes of the selected strains were ortholog clustered by PGAP (Zhao et al., 2012), a pipeline for pan-genome analysis, and genes with both coverage and identity higher than 90% were considered to be the same ortholog cluster. Hence, a total of 2,014 single copy gene families were identified and a super gene was constructed for phylogenetic analysis by combining all sequences of these genes into one ortholog cluster. A maximum likelihood phylogenetic tree was constructed by Phylm 3.0 (Guindon et al., 2010) using HKY85 nucleotide substitution model with a bootstrap value of 1000. In addition, in order to investigate the regions of differences (RD) from pan-genome analysis, we further added 200 B. melitensis genomes and 197 B. abortus genomes for detailed screening and characterization by using BLASTN program.

Virulence Factor Screening
We downloaded all the virulence factor from Virulence Factors Database (VFDB) (Chen et al., 2005), and we aligned all the protein sequences of the strain Bab8416 to the VFDB using BLASTP program available at NCBI server (ncbi-blast-2.7.1+) with both coverage and identity higher than 80%.

Data Access
The genome sequence and annotations were submitted to GenBank database with accession number CP008774–CP008775. All the reference genomes used in this paper were obtained from PATRIC (Wattam et al., 2017).

RESULTS AND DISCUSSION
Genome Features
The genome size of strain Bab8416 is 3.2 Mb, and it consists of two circular chromosomes: a large chromosome of 2,116,946 bp and a smaller one of 1,156,123 bp. The average GC content of two chromosomes was 57.22% (Crasta et al., 2008; Tsolis et al., 2009). A total of 3,295 Coding DNA sequences (CDSs) have been computationally predicted. The summarized message of Bab8416 genome is showing in Figure 1. The average length of CDS was 856 bp and 2,272 CDSs (68.95%) were assigned definite biological function as well as 1,023 (31.05%) are hypothetical proteins. Figure 2 is showing GO function class of the annotated genes.
Inconsistent Phenotypic and Molecular Typing Results

Except for resistance to phage Iz, Tb, and Wb shown in Table 1, the physiological and biochemical profiles of strain Bab8416 was more closely related to smooth \textit{B. abortus} bv 9 (Morris et al., 1973). In addition, electron microscopy was used to investigate phage Tb/Bab8416 interaction (Figure 3); absorption but no lysis of host bacteria was observed. Here, we performed additional MLVA typing (Le Fleche et al., 2006; Al Dahouk et al., 2007; Van Belkum, 2007; Valdezate et al., 2009). While no 100% match could be found in MLVA database, the top 20 matches consistently with \textit{B. abortus} bv. 3 (Figure 4).

Without coincident results in both traditional phenotyping and modern MLVA genotyping, we further employed MLST method (Whatmore et al., 2007). Twenty-seven \textit{Brucella} sequence types (STs) were initially identified and more STs have been found (Whatmore et al., 2016). Bab8416 was identified as an ST2 in this study.

Phylogenetic Analysis

Determining the evolutionary context of Bab8416 is essential for a detailed comparative genomic analysis and to account for the inconformity of the former two typing results from different strains and isolates of \textit{Brucella} (Crasta et al., 2008). A total of 2,014 single copy genes were identified within 25 \textit{B. abortus} strains with three strains in each biovar and \textit{B. melitensis} str. 16M as one outgroup being used to build a maximum likelihood phylogenetic tree (Figure 5). Many strains within the same biovar are not closely genetically related; conversely, several strains in different biovars have been shown to be closely related. This finding indicates that traditional physiological and biochemical typing designations of biovars within \textit{B. abortus} do not reflect genetic linkage patterns.

| No | CO\textsubscript{2} requirement | H\textsubscript{2}S production | TH | BF | Monospecific sera | Phages at RTD | Interpretation |
|----|------------------|------------------|----|----|-----------------|---------------|----------------|
| 1  | –                | +                | +  | +  | A               | Tb            | B. abortus 8416 |
| 2  | ±                | +                | +  | +  | M               | Wb            | B. abortus 3a  |
| 3  | –                | +                | +  | +  | –               | Iz            | B. abortus 9   |
| 4  | –                | –                | +  | +  | –               | BK2           | B. melitensis 16M |

\(TH, \text{Thionin at 20 } \mu\text{g/ml (1/50,000)}; BF, \text{Basic fuchsin at 20 } \mu\text{g/ml (1/50,000)}; \text{Phages: Tb, Tbilisi; Wb, weybridge; BK2, Berkeley type 2; Fi, Firenze; RTD, Routine test dilution; +, positive; –, negative.}
FIGURE 4 | MLVA typing results of *B. abortus* strain Bab8416.

FIGURE 5 | Phylogenetic tree of *Brucellae*. The phylogenetic tree was based on the 601 core genes of strains used in this analysis and it was constructed by using the maximum likelihood method with bootstrap value 1000. Black arrow is showing the phylogenetic cluster of *B. abortus* strain 8416.
TABLE 2 | Genome features of these strains used in comparative analysis.

| Strains              | Genome status | Biovar | Contig | CDS |
|----------------------|---------------|--------|--------|-----|
| B. abortus 8416      | Complete      | –      | 2      | 3295|
| B. abortus 9-941     | Complete      | 1      | 2      | 3085|
| B. abortus 104M      | WGS           | –      | 92     | 3303|
| B. abortus 2308-A    | WGS           | 1      | 9      | 3072|
| B. abortus 544       | WGS           | –      | 9      | 3120|
| B. abortus NCTC 8038 | WGS           | –      | 10     | 3044|
| B. abortus Tulya     | WGS           | 3      | 10     | 3261|

Comparative Genomics

As draft genomes often generate low resolution results in studies measuring genetic variation, we conducted a comparative genomic analysis using complete genomes as previously described (Ricker et al., 2012; Zhang et al., 2012). B. abortus strain 9-941 (Bab9-941) was a typical SPS strain with the complete genome published. Here, we chose Bab9-941 as a reference for comparative genomic analysis and the genome features of B. abortus used and were listed in Table 2.

Chromosome Arrangement

In comparison with Bab9-941, a large fragment (420 kb) re-arrangement in small chromosome of Bab8416 was found by using MUMmer (Figure 6). Re-arrangements in Brucella species have been previously reported (Sieira et al., 2000; Jiang et al., 2013a), however, this one proved to be exceptional. Compared with other B. abortus genomes observed here, the re-arrangement in Bab8416 was specific and displayed a closer linear relationship with B. melitensis 16M than the other B. abortus genomes. As mentioned above, Bab8416 shared the same phage typing status with B. melitensis bv 1 strain 16M; strongly similar genomic structures were also shown to exist between these two strains.

Nevertheless, neither IS elements nor tRNA operons usually responsible for genome re-arrangement were detected in the terminal region of the Bab8416 re-arrangement sequence. Three genes, BMEI10292, BMEI10293, and BMEI11009, were truncated or incomplete at the terminal fragment in other B. abortus strains. Both BMEI10292 and BMEI11009 contain a GGDEF domain that enables them to generate the cyclic di-GMP (c-di-GMP), a kind of secondary messenger central in regulating bacteria adaptive responses. In addition, analysis of protein-protein interactions using STRING database (Franceschini et al., 2013) indicated that BMEI10293 encodes a hypothetical protein that is tightly associated with the synthesis and degradation of c-di-GMP. In B. melitensis, 11 c-di-GMP-metabolizing proteins had been inferred to regulate c-di-GMP metabolism (Petersen et al., 2011). The structure of these 11 genes were verified to be intact in Bab8416, but BMEI10929, BMEI10292 and BMEI11009, were found absent in Bab941 and some B. abortus strains.
TABLE 3 | ORFs related to deletions in B. abortus str.8416 compared to B. abortus 9–941 genome.

| BAB9-941 coordinate | Region length | Associated genes | Gene length | Gene length % in RD region | Gene functions |
|---------------------|---------------|------------------|-------------|----------------------------|---------------|
| Chr1 Deletion        | 80475..80812  | BruAb1_0072      | 2,271       | 14.88%                     | Hypothetical protein |
| Deletion             | 85269..85303  | BruAb1_0075      | 750         | 4.82%                      | Amino acid efflux LysE family protein |
| Deletion             | 88412..88434  | BruAb1_0079      | 384         | 0.52%                      | Hypothetical protein |
| RD1 287585..295517   | 7,933         | BruAb1_0284      | 1,767       | 100.00%                    | Phage integrase family site specific recombinase |
| Deletion             | 375984..376015| BruAb1_0371      | 1,128       | 2.84%                      | ABC transporter substrate-binding protein |
| Deletion             | 1040055..1040246| BruAb1_1057     | 1,596       | 4.04%                      | DEAD/DEAH box helicase |
| Deletion             | 1774700..1774731| BruAb1_1803     | 405         | 8.21%                      | 3OS ribosomal protein S16 |
| Deletion             | 1795037..1795098| BruAb1_1825     | 711         | 8.90%                      | Hypothetical protein |
| Chr2 Deletion        | 156432..156703| BruAb2_0168      | 5,052       | 17.87%                     | Outer membrane transporter |
| RD2 158847..159637   | 791           | BruAb2_0377      | 1,335       | 62.76%                     | FAD-binding oxidoreductase |
| RD3 376963..382403   | 4,088         | BruAb2_0378      | 420         | 100.00%                    | Hypothetical protein |
| Deletion             | 620905..620941| BruAb2_0616      | 1,143       | 3.25%                      | Major facilitator family transporter |
| RD4 701966..701998   | 843           | BruAb2_0690      | 477         | 100.00%                    | IS711, transposase orfB |
| Deletion             | 711185..711248| BruAb2_0698      | 1,296       | 7.27%                      | Transposase orfA |

TABLE 4 | ORFs in insertions in B. abortus 8416 compared to B. abortus 9-941 genome.

| BAB8416 coordinate | Region length | Associated ORFs | Gene length | ORF length % in Insertions | Gene Function |
|--------------------|---------------|----------------|-------------|----------------------------|---------------|
| Chr1 15707..15843  | 137           | BAB8416_I0012   | 1155        | 11.86%                     | ABC transporter, substrate-binding protein |
| 374342..374383     | 42            | BAB8416_I0362   | 2019        | 2.08%                      | Xanthine dehydrogenase, molybdenum binding subunit |
| 643653..643790     | 138           | BAB8416_I0630   | 1104        | 12.50%                     | ATP/GTP-binding site motif A |
| 1035800..1037009   | 1210          | BAB8416_I1044   | 651         | 100.00%                    | Diguanylate cyclase/phosphodiesterase domain |
| 1040674..1040705   | 32            | BAB8416_I1049   | 480         | 6.67%                      | Multidrug resistance protein A |
| 1409719..1409750   | 32            | BAB8416_I1431   | 196         | 16.41%                     | FIG00450652: hypothetical protein |
| Chr2 4629..4760     | 132           | BAB8416_I0007   | 912         | 14.47%                     | Nucleoside ABC transporter, permease protein 2 |
| 10010..10058       | 49            | BAB8416_I0012   | 726         | 6.75%                      | 4’-phosphopantetheinyl transferase entD |
| 231155..231181     | 27            | BAB8416_I0234   | 570         | 4.74%                      | Nitric oxide reductase activation protein NorE |
| 248732..248754     | 23            | BAB8416_I0253   | 828         | 2.78%                      | Various polyls ABC transporter, ATP-binding component |
| 459512..459556     | 45            | BAB8416_I10463  | 1335        | 3.37%                      | Branched-chain alpha-keto acid dehydrogenase, E1 component, alpha subunit |
| 572539..572616     | 78            | BAB8416_I0069   | 1203        | 6.48%                      | Acetyl-CoA acetyltransferase |
| 849448..849532     | 87            | BAB8416_I0843   | 1107        | 7.86%                      | RND efflux membrane fusion protein |
| 842069..942905     | 837           | BAB8416_I0941   | 1047        | 46.51%                     | Putative Heme-regulated two-component response regulator |
| Deletions and Insertions
Compared with SPS Bab9-941, 49 indels (≥ 20 bps), including 25 deletions and 24 insertions, were found in the Bab8416 genome (Tables 3, 4). A 16.5 kb region is absent from Bab8416 genome and a 3.2 kb region appears to be unique. Only four large regions (> 500 bp) were represented by deletions, one Region of Differences (RD) 1 in chromosome 1 and three (RD2–RD4) in small chromosome. Genes lost in these regions are determined by referencing the annotation of B. abortus 9–941. The details of deletions and associated ORFs are shown in Table 3.
Eight genes, BruAb1_0284-0292, were located in RD1 region. BruAb1_0284 and BruAb1_0287 are specific recombinases, belonging to phage integrase and resolvase families, respectively. BruAb1_0285 and BruAb1_0288 were annotated as pseudo genes and the others were labeled hypothetical proteins. In addition, we further detected the RD1 region in 200 B. melitensis genomes and 197 B. abortus genomes by using BLASTn. In all of B. melitensis 200 strains we could not find any sequence similar with RD1. While 127 out of 197 B. abortus strains could be found the sequences with identity higher than 99% and coverage over 90% (Supplementary Table S4). These evidences above showed that RD1 was exclusively specific to B. abortus and the insert event should occur after the differentiation of the most recent common ancestor of B. abortus 9–941 and Bab8416. RD2 and another small deletion are involved in the locus of an outer membrane transporter, BruAb2_0168. An earlier study confirmed that this locus was conserved between B. abortus (Halling et al., 2005), but variation is present in Bab8416. RD3 contains four genes, BruAb2_0377 to BruAb2_0380. BruAb2_0377 encodes FAD-binding oxoreductase. BruAb2_0378 was defined as a hypothetical protein. BruAb2_0379 encodes an epimerase that catalyzes the transformation of dTDP-glucose to dTDP-4-oxo-6-deoxy-β-glucose. BruAb2_0380 encodes an amino transferase that participates in arginine and proline metabolism, metabolic pathways and biosynthesis of secondary metabolites. Two intact genes and one partial gene are encoded by RD4. The two complete genes, BruAb2_0690 and BruAb2_069, encode transposase.

Inserted regions specific to Bab8416 are shown in Table 4. Among the 20 Bab8416 specific regions, six regions are located at intergenic spacer (IGS) and fifteen ORFs are involved in the other 14 insertions. All of these ORFs are annotated with known functions.

**Variant ORFs**

The variant ORFs were identified by BLASTn method. The results are shown in Table 5. In consideration of the prediction discrepancy and the restriction of software, we searched these ORFs within these five genomes. BLASTn results showed that 144 Bab9-941 ORFs were found deleted or incomplete in Bab8416 and 129 Bab8416 ORFs were found to be Bab8416 specific. These deletions may be partly responsible for the unusual Brucella phage status of Bab8416.

**SNPs**

A total of 1,373 SNPs were identified between Bab8416 and Bab9-941. Using B. abortus 9–941 as a reference, 336 SNPs were intergenic and 1,036 SNPs were located in the ORFs. In addition, 518 genes-encoding proteins showed amino acid changes caused by 632 non-synonymous SNPs. As the SNP number was large, we inferred that these markers appeared in Bab8416 could be the characteristics of ST2. Since no other complete genomes of ST2 were available, we chose to utilize the existing draft genomes. In consideration of insuring the quality of sequencing and assembly, only the draft genomes with contig numbers less than 12 were selected. The MLST typing results of these genomes are shown in Supplementary Table S1. Fifteen out of 95 genomes were identified to be ST2. We tested the SNPs between the 16 ST2 genomes and found that overwhelming majority (95.05%) of former identified SNPs were verified to be potential markers of ST2 strains and only 68 SNPs appeared to be Bab8416 specific. The detailed SNP annotations are present in Supplementary Table S2 and the Bab8416 specific SNP involved genes are presented in Supplementary Table S3.

**LPS Synthesis**

Lipopolysaccharide is tightly associated with the virulence of pathogens and the efficiency of corresponding vaccines. Brucella with rough lipopolysaccharide (R-LPS) was lysed by Brucella phage R/C, and is host specific (Hammerl et al., 2017). In Brucella, genes essential in synthesizing LPS and developing a smooth phenotype have been located at the Wbk region of chromosome I (Godfroid et al., 2000; Gonzalez et al., 2008; Zygmunt et al., 2009). Inactivation of formyltransferase (wbkC) gene is the significant factor that contributes to rough phenotype (Lacerda et al., 2010). BLASTn results showed that none of these genes were deleted/missing in Bab8416. Four non-synonymous mutations were identified in Bab8416 LPS genes, only one (BruAb1_1699) was found not belonging to ST2. This gene encodes an OmpA family protein, which is tightly related to flagellar protein production and also related to the efflux pump.

**Virulence Factors**

Bab8416 was isolated from a patient with clinical brucellosis, indicating that this strain was virulent. The presence of 23 Brucella virulence factors confirmed by VFDB was tested in the Bab8416 isolate. Bab8416 was found to have a full complement of these loci. BLASTp results showed that eleven genes were 100% identical, eight genes had point mutations, and short deletions were found in the other four genes with only one deletion being present in VFG2217. In addition, compared to BAB1_0069, a putative outer membrane protein considered to be a virulence factor, a 133 amino acid deletion is present in this locus of Bab8416. We inferred these changes might exert some influence.
on the virulence of Bab8416 but not that much to cause high level attenuation as it is still a pathogenic bacterium.

CONCLUSION

Combining NGS sequencing technology and comparative genomics analysis, the complete genome sequence of B. abortus SPR strain Bab8416 was obtained and specific genetic characteristics of B. abortus SPR were comprehensively investigated in this study. Study of smooth LPS related genes showed that Bab8416 does share some LPS key genes with other B. abortus SPR strains, which supported veracity of previous phenotype screening results. The gold standard for Brucella characterization is still based on specific properties of the bacteria. None of the available molecular typing methods covers all currently known species and biovars of the genus Brucella (Hammerl et al., 2017). The difference between biotyping and genotyping of some special strain need further analysis not only on genomic but protein expressive level, because the host strains co-evolve with their special phages. The importance of individual amino acids of the tail collar protein for the host range of the Brucella phages has not yet been investigated. To avoid diverging lysis patterns, examine the phage genomes by sequencing were recommended if the lysis results are inconsistent on the same indicator strains (Hammerl et al., 2017).

Bab8416 has a genetic profile different from that typically found in most B. abortus strains. The arrangement sequences in small chromosome resulted in the truncation of c-di-GMP synthesis. The indels within SPS and SPR B. abortus showed that two evolutionary branches might have diverged at a far phylogenetic node. Plentiful point mutations were identified to be Bab8416 specific while the majority of the point mutations were verified to be ST2 characteristic of B. abortus. While few Bab8416 SNPs were identified, SNPs might still exert a significant influence on phage typing status. Despite the unique genetic characteristics of Bab8416 uncovered in this study, full details of its resistance to phage have not yet been elucidated at the genomic level. Our findings established some novel molecular mechanisms underlying Brucella sensitivity to brucelallass that might contribute to improving our understanding on Brucella phenotyping.

AUTHOR CONTRIBUTIONS

X-ML and Y-XK performed genomic sequencing and comparative genomic analyses and wrote the manuscript. LL, E-HJ, and D-RP performed Brucella MLVA typing and phage typing. HJ, C-CZ, and JH performed Brucella MLST typing and PCR sequencing. Y-FC, X-KG, and YZ designed the whole experiments and revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.00917/full#supplementary-material

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