Genome Rearrangements of Completely Sequenced Strains of *Yersinia pestis*

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Abstract

Yersinia pestis has caused three worldwide plagues in human history that have led to innumerable deaths. We have completely sequenced the genomes of 2 strains (D106004 and D182038) of Y. pestis isolated from Yunnan Province of China. The most striking finding of our study is that large amounts of genome rearrangement events exist between the genomes of two Yunnan strains despite being isolated from two foci only 50 kilometers apart. When we compared the genome sequences of the Yunnan strains with 6 strains (CO92, KIM, 91001, Antiqua, Nepal 516, and Pestoides F) of Y. pestis sequenced previously, we found the genomes of Y. pestis were divided into 61 relatively independent segments. Pair-wise comparisons of all 61 segments among 8 strains showed that the Yunnan strains were most closely related to strain CO92. We concluded that Y. pestis genomes consist of segments that can change their positions and directions within the genomes caused by genome rearrangements and our study confirmed the inference that the third plague pandemic originated in Yunnan since the genome sequences of Yunnan strains were closest to the strain CO92 isolated from the USA.
Introduction

*Yersinia pestis*, a Gram-negative bacterium, is one of the three pathogenic species in the genus *Yersinia*. Unlike the other two human pathogenic species (*Y. pseudotuberculosis* and *Y. enterocolitica*), *Y. pestis* causes infectious bubonic and pneumonic plague in humans that are of great importance to public health and biodefense. It is thought that *Y. pestis* was responsible for three major pandemics throughout history, taking tens of thousands of lives (7, 8, 9). Plague is a zoonotic disease spread by rodents and their fleas, and has already been classified as a reemerging disease. Plague has been controlled well in modern times, nevertheless, human plague is sporadic in some places. During the last 15 years of the 20th century, 36,876 plague cases with 2,847 deaths were reported to the WHO (4).

Currently, there are active plague foci on all continents except Australia and Antarctica (8). There are complex and diverse natural plague foci in China, and plague among animals is still prevalent in several of these foci, occasionally causing human infections. Furthermore, plague cases reported in 2005 in Yulong, Yunnan, where plague has never been recorded before. Epidemiological studies found high ratios and high titers of anti-F1 antibodies in sera of domestic animals such as cats and dogs. *Y. pestis* was then isolated from dead wild *Rattus nitidus*, *Apodemus chevrieri* and their fleas, proving the existence of a natural plague focus in this region (10). The newly discovered Yulong natural plague focus is located in the northwest of Yunnan province, between the *Marmota himalayana* plague focus on the Qinghai-Tibet Plateau and the *Rattus flavipes* plague focus in the southern Yunnan province. In addition, there is another natural plague focus less than 50 km apart from the Yulong focus in Jianchuan, Yunnan that was determined to be the Apodemus chevrieri-Eothenomys miletus plague focus in 1974. So far, no human plague case has been reported there. Natural environments and host-vector compositions are very similar between the Yulong focus and the Jianchuan focus; however, the *Y. pestis* strains isolated from these two regions have distinct biological characteristics. To determine the characteristic of this new plague focus and its potential threat to humans, we chose two representative *Y. pestis* strains, D106004 isolated from Apodemus.
chevrieri in the Yulong focus and D182038 isolated from Apodemus chevrieri in the
Jianchuan focus, and sequenced their whole genomes.

Previous studies showed that there are a large number of insertion sequence (IS)
elements in the *Y. pestis* genome. These IS elements give rise to recombination events
that lead to genome rearrangements (including transpositions and inversions) and
gene deletions (5). Thus, genome rearrangement is one of the most important genetic
features of *Y. pestis*. Deng et al. (11) compared the genome sequence of *Y. pestis* strain
KIM with that of CO92 by dividing both genomes into 27 segments to calculate the
evolutionary processes between the two strains by tracking transpositions and
inversions of large DNA segments. The increasing availability of sequences from
various *Y. pestis* strains in NCBI provides the opportunity for an in-depth
understanding of the genome organization of *Y. pestis*. Our study compared the
genomes of eight completely sequenced *Y. pestis* strains (CO92, KIM, 91001, Antiqua,
Nepal 516, Pestoides F, D106004 and D182038) in an attempt to deduce their genome
rearrangement patterns and to assess their potential use for strain identification and
phylogenetic relationship determination, especially between D106004 and D182038.
Materials and Methods

Identification of genome plates and their boundaries

Preliminary comparison of the eight *Y. pestis* genomes showed a high degree of gene synteny in certain regions. Large numbers of IS elements that can cause frequent intragenomic transpositions and inversions of large DNA segments are located among these synteny regions, even within them. This phenomenon resembles the Earth’s plate tectonics: each plate is highly stable inside while plates can move in relation to one another. Thus, we used the geological term “plate” to describe those DNA segments with similar characteristics in the *Y. pestis* genome and named them genome plates.

To identify the genome plates, a table of similar CDSs was first assembled (supplemental material S1). All CDSs of CO92 were listed in numerical order in the same column of the table. A BLAST search was performed using each CDS of strain CO92 as a query against the DNA sequences of the seven other strains. The gene with the highest percentage identity to the query CDS was retrieved from each strain and added to the table on the same row as the query CDS. It was obvious that except CO92, whose CDSs were completely in numerical order, the CDSs of all seven other strains were arranged in clusters. According to the table of orthologs, a DNA segment with at least ten continuous sequential CDSs was defined as an independent genome plate. Insertion and deletion of less than ten continuous genes within an independent genome plate were allowed in our study.

The above method can only determine the bases between the first and last CDSs of each genome plate. There are gaps between the adjacent plates that should also be included in the plates. Therefore, the boundaries of the genome plates needed to be redefined. In a previously published paper studying genome rearrangement (1), the authors divided each gap regions in half and assigned each half to a neighboring locally collinear block (LCB). In this study, The MegAlign module of DNAStar was used for pair wise alignment of the gap sequences. The assignment of the gap sequence between the second plate (plate No.2) and the third plate (plate No.3) of CO92 is illustrated below as an example (See Fig 1). Plate No.2 of CO92 whose start/end location on the chromosome is 19172th-39262th bp and plate No.56 of KIM...
whose start/end location on the chromosome of KIM is 4219550\textsuperscript{th}-4239651\textsuperscript{st} bp are very similar to each other, but opposite in direction; Plate No.3 of CO92 whose start/end location on the chromosome is 41407\textsuperscript{th}-102380\textsuperscript{th} bp and plate No.3 of KIM whose start/end location on the chromosome of KIM is 58060\textsuperscript{th}-119973\textsuperscript{rd} bp are of a high degree similarity and opposite in direction as well. The gap sequence (from 39263\textsuperscript{rd} bp to 41406\textsuperscript{th} bp on the chromosome of CO92) between plate No.2 and plate No.3 of CO92 was aligned with the gap sequence (from 4217545\textsuperscript{th} bp to 4219549\textsuperscript{th} bp on the chromosome of KIM) between plate No.56 and plate No.55 of KIM. The identical sequence that is 1962bp long was assigned to plate No.2 of CO92. Then the gap sequence between plate No.2 and plate No.3 of CO92 was aligned once again with the gap (from 119974\textsuperscript{th} bp to 122055\textsuperscript{th} bp on the chromosome of KIM) between plate No.3 and plate No.4 of KIM. The segment with identical sequence that is 2087bp long was assigned to plate No.3 of CO92. Thus, the lower boundary for plate No.2 of CO92 and the upper boundary for plate No.3 of CO92 were determined. Because there was overlap between the two boundaries, the overlapped region was assigned to the lower numbered plate, plate No.2. All other gaps of CO92 and seven other strains were assigned following the above procedure. So the genome position of every plate of each strain was finally determined (data shown in supplemental material S2).

**Similarity analysis of related genome plates**

The related genome plates of each strain were pair wise aligned using BLAST. The mean and standard deviations of the sequence identities were calculated for each genome plate of the eight completely sequenced *Y. pestis* strains. In addition, the values of sequence identity of all of the genome plates from each strain, obtained by pair wise comparison with related genome plates from the other seven, were summed up respectively to generate a sequence identity matrix for each strain versus the other seven. Phylogenic relationship analysis of the eight strains was performed by BioNumerics v4.0 software based on the identity matrix.

**Genome rearrangement diversity of *Y. pestis* strains**

The order of the genomic plates was compared pair wise among strains, and a
breakpoint was defined wherever there was a difference. The number of breakpoints between two strains may reflect the phylogenic relationships of *Y. pestis* with respect to genome rearrangement. A dendrogram was generated by UPGMA (unweighted pair-group method using arithmetic averages) clustering using BioNumerics v4.0 software according to the breakpoints matrix.
Results
Genome plates of *Y. pestis*

All plate boundaries in the genome of eight strains were assigned according to the procedure described in *Methods*. The defined genome plates were continuous with no intervening gap sequences (supplemental material S2). It was demonstrated that the genomes of all eight *Y. pestis* strains could be divided into 61 plates, 58 of which were shared by all strains. Plates No.11 and No.16 existed in all strains except Pestoides F. Plate No.30 was only absent from Nepal 516.

Generally, the *Y. pestis* genome carries three different plasmids: pPCP, pCD and pMT. Among the eight strains examined in our study, Pestoides F lacks pPCP. The sequence of Nepal516 plasmid pCD is not available on NCBI. In addition, 91001 has an extra copy of pCRY. Sequence analyses of the CDSs showed that the gene order in pPCP was identical in all tested *Y. pestis* strains except Pestoides F and belonged to the same plate. pCD was divided into three (supplemental material S2) and pMT into four plates (supplemental material S2), respectively.

**Sequence identity of genome plates among *Y. pestis* strains**

On the genomic level, *Y. pestis* strains from different sources and hosts share very high percentage identity. However, the level of sequence identity is not consistent among different genome regions. A pairwise comparison of all 61 genome plates of the eight *Y. pestis* strains was performed using BLAST to determine the sequence identity defined by the percentage of identical nucleotides in the longer plate. The mean sequence identity and standard deviation of each corresponding genome plate from all strains were determined (Table 1).

Data in Table 1 implied close phylogenetic relationships among *Y. pestis* species. Each genome plate can change its location and orientation in the genomes of *Y. pestis*. However, the gene content between the plate and its counterpart is almost consistent. Most plates have identity values above 0.9. The most stable plates are Plates No.48 and No.53 with sequence identity values both of 0.993. According to gene functional analyses using the COG database, these two plates are composed mainly of genes involved in inorganic ion transport and metabolism, cell envelope biogenesis,
transcription and carbohydrate transport and metabolism, which are presumably critical for *Y. pestis* survival and pathogenicity. Plates No.19 and No.28 are the least stable, with sequence identity values less than 0.8. These two plates contain genes related to coenzyme metabolism and DNA replication and recombination or are annotated as function unknown. Their instability was probably caused by gene mutation or deletion under selective pressure for *Y. pestis* to adapt to different niches.

It was noticed that there are different degrees of similarity among the eight sequenced *Y. pestis* strains. All of the plate sequence identity values of each strain compared with those of the seven other strains were summed up first, then the sum was divided by 61 (Table 2) and a dendrogram was generated accordingly (Figure 2).

**Genome rearrangement diversity among *Y. pestis* strains**

Using *Y. pestis* CO92 as a reference strain, the genome plate order and orientation of the other seven strains were listed in Figure 3. Many inversion and transposition events occurred in the genome of *Y. pestis* strains. It is to be noted that each chromosome represented by plate of CO92 could not include all the genes of the corresponding strain except CO92, because some genes in other strains could not be found in CO92. When the genome plate order of CO92 was compared with that of KIM, 25 breakpoints were identified. When 91001 was included in the comparison, the number of breakpoints increased to 45. If the other six strains were all considered, the total breakpoints number increased to 60. Based on this tendency, we proposed that the genome plates in *Y. pestis* are relatively stable. The genome cannot be divided unrestrictedly, forming unlimited plates.

Among all 60 breakpoints, 21 belonged exclusively to a single *Y. pestis* strain (35%). Seven were shared by two strains (11.67%). Ten were shared by three strains (16.67%). Six were shared by four strains (10%). Seven were shared by five strains (11.67%). One was shared by six strains (1.67%) and eight were shared by all strains except CO92 which acted as the reference strain (13.33%).

The number of breakpoints indicates the frequency of genome rearrangement. It is characteristic for a strain and an indication of the phylogenetic relationship among species. Based on the breakpoint matrix obtained by pair wise comparison, CO92 is
closest to D182038 and D106004 and they cluster together in the tree. KIM and Nepal516 are on the same branch, while 91001 is not as close to them (Figure 4).
Discussion

*Y. pestis* cannot be subclassified based on serotype and phage type. Thus, according to the ability to ferment glycerol and to reduce nitrate, they are classified into three biotypes, Antiqua, Mediaevalis and Orientalis, which are believed to be responsible for the three plague pandemics in history. However, current studies show that this classification system is insufficient to reflect phylogenetic relationships among *Y. pestis* species, which also rouse skepticism about the previous hypothesis. Mark Achtman *et al.* (3) have determined an evolutionary branch order within *Y. pestis* using three different multilocus molecular methods. The result was that there are three major branches on the tree, all of the Orientalis and African Antiqua types belong to Branch 1, All of the Medievalis and Asian Antiqua types belong to Branch 2, All pestoide isolates and the Microtus isolate 91001 belong to Branch 0. Nepal516 and Antiqua are two strains of the classical antiqua biovar and their genomes were completely sequenced in 2006 (5). It has been noticed that although strains Antiqua and Nepal516 are grouped into the same biovar, they represent different lineages by SNP analysis (Strains Antiqua and CO92 belong to one branch, strains Nepal516 and KIM belong to another branch). Darling *et al.* (1) compared the genomes of eight *Yersinia* stains (six *Y. pestis* strains and two *Y. pseudotuberculosis* strains) using the Mauve software and determined 78 locally collinear blocks. They analyzed the phylogenetic relationships of the eight strains based on inversion rates and reported that KIM and Nepal516 belong to the same branch. In our study, we analyzed genome rearrangements of eight *Y. pestis* strains, six of which have genome sequences available in public databases and two of which were recently sequenced in our lab. Among the eight strains, CO92 belongs to Orientalis, KIM belongs to Mediaevalis, 91001 belongs to Microtus and the other five belong to Antiqua. According to sequence identity and rearrangement diversity analyses, we obtained similar results: KIM and Nepal516 have the closest phylogenetic relationship; CO92, D182038 and D106004 belong to the same branch; 91001 and the Antiqua strains are not closely related to the other strains. It has been postulated that the third plague pandemic at the end of the nineteenth century originated in Yunnan, China and then spread to other
countries through Hong Kong (3). Our result confirms this inference, providing that CO92 is more closely related to Yunnan strains D182038 and D106004 than to the others.

*Y. pestis* is a very young species. It evolved from the enteric pathogen *Yersinia pseudotuberculosis* serotype O:1b around 1,500-20,000 years ago according to conventional microbiology, bacterial population genetics and genome sequence data (2,4). One important characteristic that distinguishes *Y. pestis* from its ancestor is the large number of insertion sequence elements, which account for 3.7% of its genome. IS elements can induce transpositions, inversions and deletions of large DNA segments and lead to different gene orders among strains. Though sequence similarity among *Y. pestis* strains is high, the frequent occurrence of genome rearrangement indicates intense gene flow (6). With more genome sequences available, large amounts of genome rearrangements are also observed in other prokaryotes and eukaryotes (1). However, the relationships between genome rearrangement and biological characteristics and virulence of pathogens remain unclear. In this study, we analyzed the genome rearrangements of *Y. pestis* and determined 61 genome plates that can shift relative to each other. We concluded that the plate number and patterns are characteristic for *Y. pestis*. The arrangement of the plates can be random, which does not affect *Y. pestis* survival but could possibly affect its pathogenic characteristics. Rearrangements in the *Y. pestis* genome accumulate during evolution, as other mutations do. The evolutionary distance can be reliably determined through genome rearrangement. So it can be utilized in systemic classification and phylogenetic analysis of *Y. pestis* species if the genome plate composition of a *Y. pestis* strain can be easily deduced without genome sequencing when the specific sequences at the junctions of two neighboring plates can be amplified. The recently discovered Yunnan Yulong natural plague focus is located close to the Yunnan Jianchuan plague focus. Both foci share similar natural environments and host-vector compositions. However, the *Y. pestis* strains isolated from the two foci show very different genome rearrangement patterns, indicating the relative independence of these two plague foci. The genotype analyses of D182038 and D106004 by PFGE (pulse
field gel electrophoresis) indicated that the genomic variability of the *Y. pestis* strains from different foci were caused by genome rearrangement, which may provide a positive selective advantage for *Y. pestis* to adapt to its host environments (12). The two strains are possibly the remnants of the *Y. pestis* that formed the Yunnan *Xenopsylla Cheopis* plague focus when it traveled south from the Qinghai-Tibet Plateau. The two strains generally do not frequently spread between the two foci. According to our analyses, though the genomes of the two strains have very different syntenic structures due to rearrangement, they share high similarity between plates, which may be an indication of similar pathogenicity. It has been proven that *Y. pestis* of the Yunnan Yulong focus can infect humans, causing severe and lethal plague. No plague cases have been reported in Jianchuan so far. Factors other than the pathogen may play a role. Nevertheless, vigilance should be maintained with tightened surveillance in the Jianchuan focus in case of human infection.
Acknowledgments

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References


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**Figure Legends**

Fig 1 Method used for gap sequences assignment. Rectangle indicates the genome plate, and double black line indicates the gap sequence. The number in rectangle indicates the serial number of genome plate and the name of *Y. pestis* strains are placed forward. Two genome plates that have a high degree of similarity are shown by rectangles with the same color. Straight arrow indicates the direction of genome plate. Gap sequences linked by the dotted line are 100% identity between CO92 and KIM. The overlapped region is marked by cross-slash.

Fig 2 Phylogenetic relationships between eight *Y. pestis* genomes inferred by UPGMA using BioNumerics v4.0 software based on the average sequence identity of the genome plates that was listed in table 2.

Fig 3 The arrangement patterns of genome plates of each chromosome from eight completely sequenced *Y. pestis* strains. Each chromosome has been laid out horizontally, and the origin of replication is located in the left-most. Each rectangle indicates a plate (scale is not shown), and the number in the rectangle indicates the serial number of plate of strain CO92, which acts as reference strain. For example, the number “1” in the rectangle represents the plate No. 1 of CO92. Plates that are inverted relative to strain CO92 are shifted below a genome’s center axis shown by a strike-through line.

Fig 4 Phylogenetic relationships among eight *Y. pestis* genomes inferred from the breakpoint distance matrix using BioNumerics v4.0 software.
**TABLE 1** The mean identity of each genome plate among eight *Y.pestis* strains

<table>
<thead>
<tr>
<th>Plate No.</th>
<th>Mean identity ± SD</th>
<th>Plate No.</th>
<th>Mean identity ± SD</th>
<th>Plate No.</th>
<th>Mean identity ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.926 ± 0.043</td>
<td>21</td>
<td>0.966 ± 0.012</td>
<td>41</td>
<td>0.942 ± 0.030</td>
</tr>
<tr>
<td>2</td>
<td>0.866 ± 0.106</td>
<td>22</td>
<td>0.977 ± 0.014</td>
<td>42</td>
<td>0.882 ± 0.150</td>
</tr>
<tr>
<td>3</td>
<td>0.966 ± 0.019</td>
<td>23</td>
<td>0.935 ± 0.035</td>
<td>43</td>
<td>0.977 ± 0.018</td>
</tr>
<tr>
<td>4</td>
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<td>24</td>
<td>0.972 ± 0.047</td>
<td>44</td>
<td>0.909 ± 0.090</td>
</tr>
<tr>
<td>5</td>
<td>0.954 ± 0.024</td>
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<tr>
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<tr>
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<td>27</td>
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<td>47</td>
<td>0.963 ± 0.017</td>
</tr>
<tr>
<td>8</td>
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<td>28</td>
<td>0.799 ± 0.191</td>
<td>48</td>
<td>0.993 ± 0.010</td>
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<td>0.978 ± 0.025</td>
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<td>0.972 ± 0.022</td>
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<tr>
<td>10</td>
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<td>30</td>
<td>0.975 ± 0.017</td>
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<tr>
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<tr>
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<td>0.935 ± 0.045</td>
</tr>
<tr>
<td>20</td>
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<td>0.953 ± 0.064</td>
<td>60</td>
<td>0.919 ± 0.081</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61</td>
<td></td>
<td>61</td>
<td>0.946 ± 0.076</td>
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TABLE 2 The sequence identity matrix among eight *Y. pestis* strains

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<th>2</th>
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<th>5</th>
<th>6</th>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>1.000</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>91001</td>
<td>0.916</td>
<td>0.916</td>
<td>1.000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Nepal516</td>
<td>0.955</td>
<td>0.956</td>
<td>0.908</td>
<td>1.000</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td>Antiqua</td>
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<td>0.939</td>
<td>0.907</td>
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<td>1.000</td>
<td>-</td>
<td>-</td>
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<td>6</td>
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<td>0.914</td>
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<td>0.925</td>
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<td>1.000</td>
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</tr>
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