

Complete Genome Sequences of *Yersinia pestis* from Natural Foci in China[∇]

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***Yersinia pestis*, the causative agent of plague, is a deadly bacterium that affects humans. Strain D106004 was isolated from a new plague focus in Yulong County, China, in 2006. To gain insights into the epidemic origin, we have sequenced the genomes of D106004 and strains Z176003 and D182038, isolated from neighboring regions.**

This article describes genomic comparisons between three respective *Yersinia pestis* strains isolated from new natural plague foci in China. *Y. pestis* strain D106004 was isolated from *Apodemus chevrieri* in Yulong County in 2006, and its genome was compared to those of strain D182038 (isolated from *A. chevrieri* in 1982 from Jianchuan County) and strain Z176003 (isolated from *Marmota himalayana* in 1976 in Naqu [Tibet] County).

Between 25 October 2005 and 2 November 2005, there was an outbreak of pneumonic plague in Yulong, which was identified as a new natural plague focus (13). The primary *Y. pestis* reservoirs associated with this outbreak were *A. chevrieri*, *Eothenomys miletus*, and *Apodemus latronum*, and the primary vectors associated with plague transmission were also identified as similar to what was observed in neighboring Jianchuan County (7). However, the *Y. pestis* strain identified metabolized maltose significantly differently than the previously described strains (6).

Whole-genome shotgun and solexa methods were used, as previously described (3), to compare the *Y. pestis* D106004, D182038, and Z176003 sequences, which consisted of 475, 385, and 413 contigs, respectively, resulting in an average 9-fold coverage across the genomes. All isolates examined possessed a single circular chromosome with the three virulence plasmids (pMT, pCD, and pPCP) associated with classical *Y. pestis* strains. Automated gene modeling was carried out using the Glimmer3 software program (11) in addition to comparing the respective gene products using the Nt, Nr, KEGG, Swissprot, and COG databases using the basic local alignment search tool for proteins (BLASTP). Open reading frames (ORFs) in the

respective 4,626,944-bp, 4,640,720-bp, and 4,553,586-bp genomes of strains D182038, D106004, and Z176003 were predicted to be of 3,642, 3,636, 3,543, and more than 300 bp in length. Strains D182038, D106004, and Z176003 each had six rRNA (16S-23S-5S) genes and 73 (D182038), 70 (D106004), or 68 (Z176003) tRNA genes predicted by the tRNAScan-SE server (9).

Comparison of *Y. pestis* strains 91001 and KIM to *Y. pestis* strain CO92 identified genetic rearrangements (5, 10, 12) resulting from insertion sequences (2), and pulsed-field gel electrophoresis (PFGE) profile comparisons between D182038 and D106004 suggested that genomic variability of the *Y. pestis* strains from different foci was caused by genome rearrangement (16). According to our analyses, the *Y. pestis* strains isolated from the two foci have very different syntenic structures due to rearrangement, but they share high similarity between plates (8). In addition, a unique multiple-locus variable-number tandem repeat analysis (MLVA) type was defined for the strains isolated from Yulong, indicating a new clonal group. These results also suggested that the Yulong strains were closely related to the strains from the Qinghai-Tibet Plateau plague foci (15). Analysis of *Y. pestis* microevolution has been made possible by comparing single-nucleotide polymorphism (SNP) profiles as previously described (1, 4, 14).

The availability of high-quality sequences is crucial in order to resolve the origins of the new strains isolated from natural plague foci.

Nucleotide sequence accession numbers. The complete genome sequences of *Y. pestis* strains D182038, D106004, and Z176003 were deposited in GenBank as follows: for D182038, accession no. CP001589 to CP001592; for D106004, accession no. CP001585 to CP001588; for Z176003, accession no. CP001593 to CP001596.

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REFERENCES

- Achtman, M., K. Zurth, G. Morelli, G. Torrea, A. Guiyoule, and E. Carniel. 1999. *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. *Proc. Natl. Acad. Sci. U. S. A.* **96**:14043–14048.
- Auerbach, R. K., A. Tuanyok, W. S. Probert, L. Kenefic, A. J. Vogler, D. C. Bruce, C. Munk, T. S. Brettin, M. Eppinger, J. Ravel, D. M. Wagner, and P. Keim. 2007. *Yersinia pestis* evolution on a small timescale: comparison of whole genome sequences from North America. *PLoS One* **2**:e770.
- Chain, P., J. Lamerdin, F. Larimer, W. Regala, V. Lao, M. Land, L. Hauser, A. Hooper, M. Klotz, J. Norton, L. Sayavedra-Soto, D. Arciero, N. Hommes, M. Whittaker, and D. Arp. 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph *Nitrosomonas europaea*. *J. Bacteriol.* **185**:2759–2773.
- Chain, P. S., P. Hu, S. A. Malfatti, L. Radnedge, F. Larimer, L. M. Vergez, P. Worsham, M. C. Chu, and G. L. Andersen. 2006. Complete genome sequence of *Yersinia pestis* strains Antiqua and Nepal516: evidence of gene reduction in an emerging pathogen. *J. Bacteriol.* **188**:4453–4463.
- Deng, W., V. Burland, G. Plunkett III, A. Boutin, G. F. Mayhew, P. Liss, N. T. Perna, D. J. Rose, B. Mau, S. Zhou, D. C. Schwartz, J. D. Fetherston, L. E. Lindler, R. R. Brubaker, G. V. Plano, S. C. Straley, K. A. McDonough, M. L. Nilles, J. S. Matson, F. R. Blattner, and R. D. Perry. 2002. Genome sequence of *Yersinia pestis* KIM. *J. Bacteriol.* **184**:4601–4611.
- Guo, Y., L. Zhang, L. Xia, Z. Zhang, H. Cai, F. Ma, J. Zhang, R. Hai, and Z. Song. 2008. Biochemical characters of *Yersinia pestis* isolated from Yulong County of Yunnan Province in China. *Endem. Dis. Bull.* **23**:12–14. (In Chinese.)
- Li, G., W. Cai, F. Zhang, L. Lu, Y. Liang, G. Wang, Z. Zhang, Y. He, Z. Chen, Q. Liu, Z. Song, D. Yu, and J. Wang. 2009. Surveillance on the host and vector in Yulong plague focus in Yunnan province. *Dis. Surveillance* **24**:114–117. (In Chinese.)
- Liang, Y., X. Hou, Y. Wang, Z. Cui, Z. Zhang, X. Zhu, L. Xia, X. Shen, H. Cai, J. Wang, D. Xu, E. Zhang, H. Zhang, J. Wei, J. He, Z. Song, X. J. Yu, D. Yu, and R. Hai. 2010. Genome rearrangements of completely sequenced strains of *Yersinia pestis*. *J. Clin. Microbiol.* **48**:1619–1623.
- Lowe, T. M., and S. R. Eddy. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **25**:955–964.
- Parkhill, J., B. W. Wren, N. R. Thomson, R. W. Titball, M. T. Holden, M. B. Prentice, M. Sebaihia, K. D. James, C. Churcher, K. L. Mungall, S. Baker, D. Basham, S. D. Bentley, K. Brooks, A. M. Cerdeno-Tarraga, T. Chillingworth, A. Cronin, R. M. Davies, P. Davis, G. Dougan, T. Feltwell, N. Hamlin, S. Holroyd, K. Jagels, A. V. Karlyshev, S. Leather, S. Moule, P. C. Oyston, M. Quail, K. Rutherford, M. Simmonds, J. Skelton, K. Stevens, S. Whitehead, and B. G. Barrell. 2001. Genome sequence of *Yersinia pestis*, the causative agent of plague. *Nature* **413**:523–527.
- Salzberg, S. L., A. L. Delcher, S. Kasif, and O. White. 1998. Microbial gene identification using interpolated Markov models. *Nucleic Acids Res.* **26**:544–548.
- Song, Y., Z. Tong, J. Wang, L. Wang, Z. Guo, Y. Han, J. Zhang, D. Pei, D. Zhou, H. Qin, X. Pang, Y. Han, J. Zhai, M. Li, B. Cui, Z. Qi, L. Jin, R. Dai, F. Chen, S. Li, C. Ye, Z. Du, W. Lin, J. Wang, J. Yu, H. Yang, J. Wang, P. Huang, and R. Yang. 2004. Complete genome sequence of *Yersinia pestis* strain 91001, an isolate avirulent to humans. *DNA Res.* **11**:179–197.
- Song, Z., L. Xia, Y. Liang, Y. Guo, L. Lu, G. Wang, W. Cai, Z. Zhang, Y. He, F. Zhang, X. Dong, G. Yu, J. Wang, and D. Yu. 2008. Confirmation and study of plague natural foci for Yulong County and Guchengqu in Yunnan Province, China. *J. Control Endem. Dis.* **23**:3–7. (In Chinese.)
- Touchman, J. W., D. M. Wagner, J. Hao, S. D. Mastrian, M. K. Shah, A. J. Vogler, C. J. Allender, E. A. Clark, D. S. Benitez, D. J. Youngkin, J. M. Girard, R. K. Auerbach, S. M. Beckstrom-Sternberg, and P. Keim. 2007. A North American *Yersinia pestis* draft genome sequence: SNPs and phylogenetic analysis. *PLoS One* **2**:e220.
- Zhang, X., R. Hai, J. Wei, Z. Cui, E. Zhang, Z. Song, and D. Yu. 2009. MLVA distribution characteristics of *Yersinia pestis* in China and the correlation analysis. *BMC Microbiol.* **9**:205.
- Zhang, Z., R. Hai, Z. Song, L. Xia, Y. Liang, H. Cai, Y. Liang, X. Shen, E. Zhang, J. Xu, D. Yu, and X. J. Yu. 2009. Spatial variation of *Yersinia pestis* from Yunnan Province of China. *Am. J. Trop. Med. Hyg.* **81**:714–717.