## Genome Sequence of the Zoonotic Pathogen *Chlamydophila psittaci*<sup>∇</sup>

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We present the first genome sequence of *Chlamydophila psittaci*, an intracellular pathogen of birds and a human zoonotic pathogen. A comparison with previously sequenced *Chlamydophila* genomes shows that, as in other chlamydiae, most of the genome diversity is restricted to the plasticity zone. The *C. psittaci* plasmid was also sequenced.

Avian *Chlamydophila psittaci* infections range from chronic and symptomless to acute, with various mortality rates (11, 12). Epidemic outbreaks of *C. psittaci* have been reported among wild birds and commercially farmed poultry, causing significant economic losses (11, 12). Although it primarily infects birds, *C. psittaci* is a human zoonotic pathogen causing pneumonia or fever following close contact with infected birds (12).

We sequenced C. psittaci strain RD1, which was isolated from a mixed culture with Chlamydia trachomatis serovar L2b. Although human mixed ocular infections with these two species have been reported (7), C. psittaci strain RD1 is thought to derive from a laboratory-based cross-contamination event from an undetermined source soon after C. trachomatis strain isolation. DNA was prepared (17), sequenced using 454 pyrosequencing on a GS20 machine with an average read length of 100 bp, and assembled using Newbler (Roche). The contigs were ordered using the Chlamydophila abortus genome as a reference (18) and manually finished to produce an improved high-quality draft genome sequence (6) of six contigs, with approximately  $40.7 \times$  coverage. The five unspanned gaps are clearly marked in the genome annotation (size estimates based on comparison with C. abortus: 3,592 bp between *pmp11G* and *pmp13G* [*pmp12G* is absent], 116 bp at the 3' end of pmp16G, and three gaps of 887, 797, and 1,481 bp within the rRNA operon). Annotation and comparative analysis with the closely related species C. abortus, Chlamydophila caviae, and Chlamydophila felis (1, 8, 14, 18) were performed using Artemis (16) and ACT (5).

The draft genome sequence of *C. psittaci* comprises 1,156,417 bp, showing average nucleotide identities of 91.3, 85.9, and 84.8% with *C. abortus*, *C. caviae*, and *C. felis*, respec-

\* Corresponding author. Mailing address: Pathogen Genomics, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire CB10 1SA, United Kingdom. Phone: 011-1223 494 761. Fax: 011-1223 494 919. E-mail: nrt@sanger.ac.uk. tively. The *C. psittaci* genome is predicted to encode 959 coding sequences (CDSs). Analysis of *ompA* indicates that *C. psittaci* strain RD1 belongs to genotype A (9, 13). Like other *Chlamy-dophila* genomes, that of *C. psittaci* carries 36 tRNA genes and one rRNA operon and shows high conservation of gene content and order with other members of its genus. A total of 16 pseudogenes were detected within the genome, including two polymorphic membrane protein (*pmp*) genes (*cpsi\_2861* and *cpsi\_2911*) (10) and one transmembrane head/inclusion membrane family (TMH/Inc) gene (*cpsi\_7871*) (2, 15). The *C. psittaci* strain RD1 plasmid, designated pRD1, is 7,553 bp long, encodes 8 CDSs, and differs by only four single-nucleotide polymorphisms from *C. psittaci* plasmid pCpA1 (NC\_002117).

Most of the *C. psittaci*-specific sequences are located in the plasticity zone (PZ), which carries an additional 18,139 bp of sequence compared to *C. abortus*, including a 9,762-bp CDS predicted to encode a cytotoxin (*cpsi\_5561*) which shows 44% identity with cytotoxins found at the same locus in *C. felis* and *C. caviae*. The PZ also contains the intact *guaBA-add* operon (*cpsi\_5591-5611*) encoding proteins thought to be involved in purine nucleotide interconversion (1, 14, 18). *C. psittaci* lacks the tryptophan biosynthesis operon (3, 4).

**Nucleotide sequence accession numbers.** The sequences determined in this study have been deposited in the EMBL database under accession numbers FQ482149 (chromosome) and FQ482150 (plasmid).

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## REFERENCES

- Azuma, Y., et al. 2006. Genome sequence of the cat pathogen, *Chlamy-dophila felis*. DNA Res. 13:15–23.
- Bannantine, J. P., D. D. Rockey, and T. Hackstadt. 1998. Tandem genes of *Chlamydia psittaci* that encode proteins localized to the inclusion membrane. Mol. Microbiol. 28:1017–1026.
- Beatty, W. L., T. A. Belanger, A. A. Desai, R. P. Morrison, and G. I. Byrne. 1994. Tryptophan depletion as a mechanism of gamma interferon-mediated chlamydial persistence. Infect. Immun. 62:3705–3711.
- 4. Caldwell, H. D., et al. 2003. Polymorphisms in Chlamydia trachomatis tryp-

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tophan synthase genes differentiate between genital and ocular isolates. J. Clin. Invest. **111**:1757–1769.

- Carver, T. J., et al. 2005. ACT: the Artemis comparison tool. Bioinformatics 21:3422–3423.
- Chain, P. S. G., et al. 2009. Genome project standards in a new era of sequencing. Science 326:236–237.
- Dean, D., R. P. Kandel, H. K. Adhikari, and T. Hessel. 2008. Multiple Chlamydiaceae species in trachoma: implications for disease pathogenesis and control. PLoS Med. 5:e14.
- Everett, K. D. E., R. M. Bush, and A. A. Andersen. 1999. Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each one containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. Int. J. Sys. Bacteriol. 49:415–440.
- Geens, T., et al. 2005. Sequencing of the *Chlamydophila psittaci ompA* gene reveals a new genotype, E/B, and the need for a rapid discriminatory genotyping method. J. Clin. Microbiol. 43:2456–2461.
- Grimwood, J., and R. S. Stephens. 1999. Computational analysis of the polymorphic membrane protein superfamily of *Chlamydia trachomatis* and *Chlamydia pneumoniae*. Microb. Comp. Genomics 4(3):187–201.

- Harkinezhad, T., T. Geens, and D. Vanrompay. 2009. *Chlamydophila psittaci* infections in birds: a review with emphasis on zoonotic consequences. Vet. Microbiol. 135:68–77.
- Longbottom, D., and L. J. Coulter. 2003. Animal chlamydioses and zoonotic implications. J. Comp. Pathol. 128:217–244.
- Mitchell, S. L., et al. 2009. Genotyping of *Chlamydophila psittaci* by real-time PCR and high-resolution melt analysis. J. Clin. Microbiol. 47:175–181.
- Read, T. D., et al. 2003. Genome sequence of *Chlamydophila caviae* (*Chlamydia psittaci* GPIC): examining the role of niche-specific genes in the evolution of the Chlamydiaceae. Nucleic Acids Res. 31:2134–2147.
- Rockey, D. D., M. A. Scidmore, J. P. Bannantine, and W. J. Brown. 2002. Proteins in the chlamydial inclusion membrane. Microbes Infect. 4:333– 340.
- Rutherford, K., et al. 2000. Artemis: sequence visualization and annotation. Bioinformatics 16:944–945.
- Skipp, P., J. Robinson, C. D. O'Connor, and I. N. Clarke. 2005. Shotgun proteomic analysis of *Chlamydia trachomatis*. Proteomics 5:1558–1573.
- Thomson, N. R., et al. 2005. The *Chlamydophila abortus* genome sequence reveals an array of variable proteins that contribute to interspecies variation. Genome Res. 15:629–640.