

Genome Update: annotation quality in sequenced microbial genomes

Genomes of the month

There are five new microbial genomes described in this month's Genome Update, four from bacterial species and one from a fungus. The bacterial genomes are those of *Desulfovibrio vulgaris*, *Listeria monocytogenes*, *Mycobacterium avium* and a Mediaevalis strain of *Yersinia pestis*. The fungal genome is that of *Phanerochaete chrysosporium*, the fungus responsible for 'white rot' in decaying trees.

D. vulgaris is a sulfate-reducing bacterium found almost everywhere in nature, and is responsible for biocorrosion of metal infrastructures (e.g. oil drilling and pumping machinery); it can be also used for bioremediation of toxic metal ions such as cadmium and uranium. *D. vulgaris* subsp. *vulgaris* strain Hildenborough is the third genome of the δ -*Proteobacteria* to be published. Its genome is GC-rich and encodes about 3400 genes (see Table 1). The main chromosome is 3.5 Mbp long, and the genome also includes a 0.2 Mbp plasmid (Heidelberg *et al.*, 2004).

Listeria monocytogenes is a common environmental bacterium which can cause food poisoning. Three different strains of *Listeria monocytogenes* associated with food-borne infections have been sequenced: the genome of strain F2365 (serotype 4b, cheese isolate) was fully sequenced, while the genomes of strains F6854 (serotype 1/2a, frankfurter isolate) and H7858 (serotype 4b, meat isolate) were sequenced to give about 8 \times coverage, although for these two chromosomes the gaps were not closed (Nelson *et al.*, 2004). This report is a nice example of the power of comparative genomics – by comparing the relatively small number of genes unique to each genome (e.g. one genome contained only 51 unique genes), it is possible to model strain-specific and serotype-specific differences. One of the conclusions of the authors of this report

is that '*L. monocytogenes* strains prevalent in human and animal illness have surprisingly high genomic stability, and rely on a relatively small number of unique regions for antigenic diversity and epidemiologically relevant attributes' (Nelson *et al.*, 2004).

The sequences of two other bacterial genomes listed in Table 1 have been deposited in the EMBL/GenBank libraries, but have not been published yet. The sequenced genome of *Mycobacterium avium* subsp. *paratuberculosis* strain k10 was searched for short sequence repeats (SSRs), which were used to design probes that were tested for discrimination amongst 33 different strains of the same species (Amonsin *et al.*, 2004). The genome sequence of *Y. pestis* bv. Mediaevalis str. 91001 (see Table 1) has also been deposited recently in the EMBL/GenBank libraries, and seems similar in size and many characteristics to the other two *Y. pestis* strains sequenced to date.

Finally, the 30 Mbp genome of the white rot-causing basidiomycete *P. chrysosporium* RP78 has been published recently (Martinez *et al.*, 2004); it is organized into ten chromosomes and encodes about 11 800 genes. This organism secretes enzymes into its environment which allow it to efficiently degrade lignin. The number of rRNA operons in the genome of this organism is not given, although the

authors state, with admirable truthfulness and clarity, that '*Typical of shotgun sequencing of eukaryotes, extended repeats, telomeres and rRNA clusters were excluded from the assembly. Nevertheless, substantial numbers of noncoding repetitive sequences and putative mobile elements were assembled*' (Martinez *et al.*, 2004). This is the first genome to be sequenced from a member of the fungal phylum Basidiomycota.

Method of the month – comparison of genome annotation

For the past several months, we have been systematically going through the columns of genomic data shown in Table 1. Thus, for example, last month's Genome Update included a discussion of comparison of tRNAs and codon usage in various sequenced genomes. This month we come to the last column, which is the number of genes annotated in a given genome. For sequenced bacterial genomes, the range is from a mere 480 genes in *Mycoplasma genitalium* (Fraser *et al.*, 1995) to 8317 genes in *Bradyrhizobium japonicum* (Kaneko *et al.*, 2002); the upper limit is already known to be a bit larger than this, as the genome of the myxobacterium '*Sorangium cellulosum*' is about 12 Mbp long, and is likely to contain more than 10 000 genes (Pradella *et al.*, 2002). Thus, the number of genes in bacterial genomes varies by more than 20-fold. Furthermore, only a small

Microbiology Comment provides a platform for readers of *Microbiology* to communicate their personal observations and opinions in a more informal way than through the submission of papers.

Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others, or we may have a useful piece of methodology that we would like to share.

Guidelines on how to submit a *Microbiology* Comment article can be found in the Instructions for Authors at <http://mic.sgmjournals.org>

It should be noted that the Editors of *Microbiology* do not necessarily agree with the views expressed in *Microbiology* Comment.

Chris Thomas, Editor-in-Chief

Table 1. Summary of the published genomes discussed in this Update

Note that the accession number for each chromosome is the same for GenBank, EMBL and the DNA DataBase of Japan (DDBJ).

Genome	Size (bp)	AT content (%)	rRNA operons	tRNAs	CDS	Accession no.
<i>Desulfovibrio vulgaris</i> subsp. <i>vulgaris</i> Hildenborough	3 570 858	36.9	5	68	3 395	AE017285
<i>Listeria monocytogenes</i> F2365 (serotype 4b, cheese isolate)	2 905 310	62.0	6	67	2 847	AE017262
<i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> k10	4 829 781	30.7	1	46	4 350	AE016958
<i>Yersinia pestis</i> bv. <i>Mediaevalis</i> str. 91001	4 595 065	52.3	7	72	3 895	AE017042
<i>Phanerochaete chrysosporium</i> RP78	~29 900 000	48.5	?	199	11 777	AADS00000000

fraction of the 480 genes in *Mycoplasma genitalium* are well-conserved in other bacteria. (We find less than 10%, although the number depends, of course, on what threshold one chooses; with an e-value cut-off of about 1×10^{-10} , we find only about 40 genes that are conserved throughout the sequenced bacterial genomes).

We had originally intended to make a plot of the number of genes in bacterial genomes, broken down into phyla, but since the coding density for most bacteria is quite high and roughly the same, this plot looks essentially the same as the one shown a few months ago when the length of genomes was discussed [see Fig. 1 in Ussery & Hallin (2004)]. Instead, this month we will briefly discuss how we can estimate the quality of genome annotation.

Many people assume that, since gene-finding is relatively easy in bacterial and archaeal genomes, the genes reported in EMBL or GenBank files have THE correct annotation. However, in writing these Genome Update articles over the past several months, it has become clear that the genomes are not all annotated to the same standards. For example, about 10% of the bacterial genomes (e.g. 15 genomes out of 150 published) do not have the rRNA gene sequences annotated in their GenBank files. It is clear that there can be occasional large differences in the quality of annotation of the genes as well. For example, consider the *Leptospira interrogans* Copenhageni strain Fiocruz L1-130 genome (Nascimento *et al.*, 2004). As mentioned in last month's Genome Update, this is nearly identical in size to another *Leptospira interrogans* genome (Ren *et al.*, 2003), which has nearly 1000 extra genes (3728 vs 4727 genes, for two bacterial genomes of the same species,

both about 4.7 Mbp in length). It seems to be a general rule of thumb that there is very roughly one gene every 1000 bp in many bacterial genomes. Using this criterion, one might expect there to be about 4700 genes encoded by the *Leptospira interrogans* genomes – so perhaps the earlier estimate is closer to what is expected. But what if a genome has undergone some sort of decay, or perhaps had a large insertion of non-coding regions? Is there a practical way for estimating how many genes there should be in a given bacterial genome? A few years ago, we utilized three different statistical measures to estimate the expected number of genes (Skovgaard *et al.*, 2001), and found that most of the

28 bacterial genomes examined at that time were over-annotated by about 20%, compared to our estimates (which, admittedly, could be conservative). The problem is separating the 'ORFs from the ELF's' (Lawrence, 2003; Ochman, 2002) – i.e. trying to accurately determine the small open reading frames (ORFs) which truly encode proteins, versus random ORFs which occur by chance and might not reflect genes which are ever expressed. It is difficult to prove that a given sequence is not a gene – just that it is not expressed under a given set of experimental conditions. However, the problem of over-annotation is unfortunately not one that can be easily

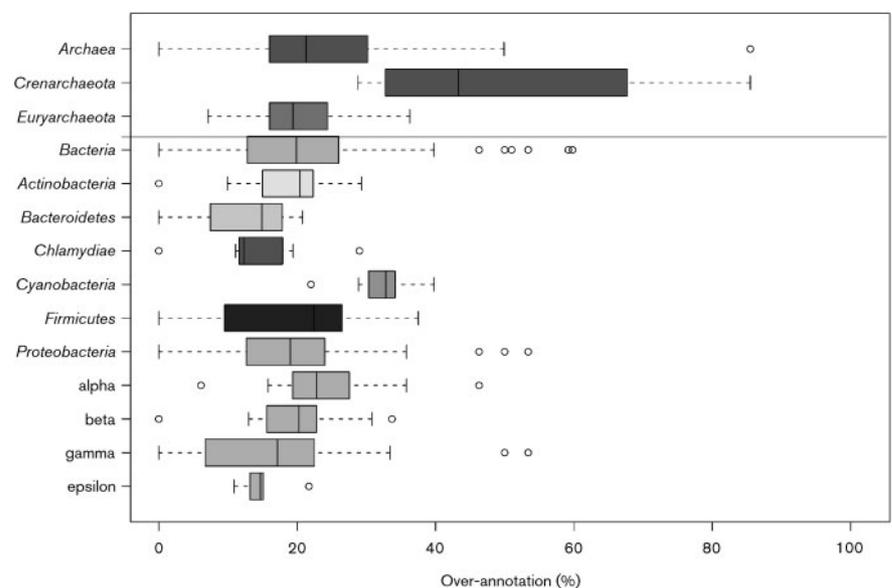


Fig. 1. Over-annotation of archaeal and bacterial genomes. Box-and-whiskers plots show data from the superkingdoms *Bacteria* and *Archaea* and phyla within these groups; more information, including the values for each genome, can be found in the supplemental web pages associated with this article. Note that alpha, beta, gamma and epsilon refer to classes within the *Proteobacteria*.

ignored, if one wants to compare bacterial proteomes. For example, is it REALLY likely to be true that one *Leptospira interrogans* genome encodes an extra 1000 proteins compared to another?

Fig. 1 shows the fraction of genes estimated to be over-annotated for 159 sequenced genomes, sorted by superkingdom and phylum. Nearly all genomes are 'over-annotated' by about 20%. The good news, in a way, is that most of the genomes seem to have roughly the same ratio of genes annotated, compared to our estimates. Note that *Crenarchaeota* genomes seem to be more over-annotated; however, this is based on only four genomes, two of which are by far the most over-annotated, with about twice as many genes predicted as might be expected (e.g. one gene every 500 bp, rather than one gene every 1000 bp as for most other genomes). These were both annotated by the same group, which actually reported all the ORFs over a certain length. It should also be noted that a gene-finder has not been run through the sequences.

For the sequenced bacterial genomes, one of the most over-annotated genomes is that of the above-mentioned *Leptospira interrogans* strain (Copenhagen strain Fiocruz L1-130). We estimate its genome to be over-annotated by about 60%, whilst the genome of *Leptospira interrogans* strain 56601 is about 30% over-annotated, closer to the values for the other spirochaete genomes. It could well be that our estimates for the 'true number of proteins' are too low. However, regardless of this fact, at least this provides us with some measure to allow us to see which genomes differ significantly in their annotation criteria. Full results, including a table of all published archaeal and bacterial genomes, sorted by their 'over-annotation' value, as well as protein length distribution plots for each genome, can be found on our supplemental web pages. Only about ten genomes are significantly different from the average – perhaps these genomes should be treated with caution when doing proteome comparisons based solely on the EMBL or GenBank files.

Next month, the method of genome comparison discussed will be the

Artemis Comparison Tool (ACT) (<http://www.sanger.ac.uk/Software/ACT/>).

Supplemental web pages

Web pages containing supplemental material related to this article can be accessed from the following url: <http://www.cbs.dtu.dk/services/GenomeAtlas/suppl/GenUp006/>

Acknowledgements

This work was supported by a grant from the Danish Center for Scientific Computing.

David W. Ussery and Peter F. Hallin

Center for Biological Sequence Analysis, Department of Biotechnology, Building 208, The Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark

Correspondence: David W. Ussery (dave@cbs.dtu.dk)

Amonsin, A., Li, L. L., Zhang, Q., Bannantine, J. P., Motiwala, A. S., Sreevatsan, S. & Kapur, V. (2004). Multilocus short sequence repeat sequencing approach for differentiating among *Mycobacterium avium* subsp. *paratuberculosis* strains. *J Clin Microbiol* **42**, 1694–1702.

Fraser, C. M., Gocayne, J. D., White, O. & 22 other authors (1995). The minimal gene complement of *Mycoplasma genitalium*. *Science* **270**, 397–403.

Heidelberg, J. F., Seshadri, R., Haveman, S. A. & 32 other authors (2004). The genome sequence of the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. *Nat Biotechnol* **22**, 554–559.

Kaneko, T., Nakamura, Y., Sato, S. & 14 other authors (2002). Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. *DNA Res* **9**, 189–197.

Lawrence, J. (2003). When ELF's are ORFs, but don't act like them. *Trends Genet* **19**, 131–132.

Martinez, D., Larrondo, L. F., Putnam, N. & 12 other authors (2004). Genome sequence of the lignocellulose degrading fungus *Phanerochaete chrysosporium* strain RP78. *Nat Biotechnol* Epub ahead of print, DOI: 10.1038/nbt967

Nascimento, A. L., Verjovski-Almeida, S., Van Sluys, M. A. & 10 other authors (2004). Genome features of *Leptospira interrogans* serovar Copenhageni. *Braz J Med Biol Res* **37**, 459–477.

Nelson, K. E., Fouts, D. E., Mongodin, E. F. & 30 other authors (2004). Whole genome

comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. *Nucleic Acids Res* **32**, 2386–2395.

Ochman, H. (2002). Distinguishing the ORFs from the ELF's: short bacterial genes and the annotation of genomes. *Trends Genet* **18**, 335–337.

Pradella, S., Hans, A., Sproer, C., Reichenbach, H., Gerth, K. & Beyer, S. (2002). Characterisation, genome size and genetic manipulation of the myxobacterium *Sorangium cellulosum* So ce56. *Arch Microbiol* **178**, 484–492.

Ren, S. X., Fu, G., Jiang, X. G. & 36 other authors (2003). Unique physiological and pathogenic features of *Leptospira interrogans* revealed by whole-genome sequencing. *Nature* **422**, 888–893.

Skovgaard, M., Jensen, L. J., Brunak, S., Ussery, D. & Krogh, A. (2001). On the total number of genes and their length distribution in complete microbial genomes. *Trends Genet* **17**, 425–428.

Ussery, D. W. & Hallin, P. F. (2004). Genome update: length distributions of sequenced prokaryotic genomes. *Microbiology* **150**, 513–516.

DOI 10.1099/mic.0.27338-0