Published in final edited form as:
*Curr Opin Biotechnol*. 2006 October ; 17(5): 440–447.

# New metrics for comparative genomics

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## Abstract

The availability of genome sequences from a variety of organisms presents an opportunity to apply this sequence information to solving the key problems of molecular biology. One of the principal roadblocks on this path is the lack of appropriate descriptors and metrics that could succinctly represent the new knowledge stemming from the genomic data. Several new metrics have recently been used in comparative genome analysis, yet challenges remain in finding an appropriate language for the emerging discipline of systems biology.

## Introduction

The genomic revolution, which started 11 years ago with the completion of the genome sequence of the bacterium *Haemophilus influenzae* and culminated in 2004 with the sequencing of the human genome, had an enormous impact on the very nature of life science research. Genome-derived information is now paving the way to a consistent quantitative description of all processes in a living cell, the systems biology of the cell. The influx of genomic data has been most profound in prokaryotic genomics, which has experienced exponential growth with the number of sequenced genomes doubling every 16 months [1]. The advantages of having a complete genome sequence are well-known and have been repeatedly highlighted in the past (e.g. [2]). However, these benefits are often limited by the inability of standard (automated) methods of genome analysis to perform three tasks: to assign function to as much as one-third of the genes; to reliably predict existing and missing metabolic pathways and environmental sensing mechanisms; and to highlight unexpected discoveries and suggest practical uses for the sequenced genome [3,4]. The overwhelming diversity of life, revealed by the genome and metagenome sequencing projects, calls for new approaches to comparative genomics, which will require the introduction of appropriate new descriptors. These new metrics — integral parameters that could be used to describe newly sequenced genomes and put them into a proper framework — will ultimately play a major role in defining the discipline of systems biology. Here, we briefly outline recent trends in comparative genome analysis and discuss some new metrics that have been used in the past few years. As most eukaryotic genomes remain only partly finished, we primarily focus on the analysis of prokaryotic genomes.

## Emerging standards in genome description

In the past 11 years, close to 400 complete bacterial, archaeal and eukaryotic genomes have been sequenced, resulting in more than 200 published papers. After a brief initial period, there emerged a clear pattern whereby nearly every paper describing a newly sequenced genome

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This review comes from a themed issue on Systems biology

Edited by Michael Y Galperin and Michael J Ellison

presented a standard list of genome parameters, followed by a brief discussion of the distinctive features of the sequenced organism. These standard parameters typically include genome size, GC content, the number of stable RNA- and protein-coding genes, the fraction of the genes with a functional annotation, and several others (Box 1). Obviously, genome sequences allow ample opportunities for more in-depth analyses. Recent examples of these include the use of whole-genome data to assemble an improved tree of life [5,6,7•,8•], to reconstruct metabolic pathways in terms of existing and 'missing' genes [9,10], to evaluate the number and distribution of various protein folds [11,12] and to determine the fraction of laterally transferred genes [13–15]. At the same time, some of the more ambitious goals of genome analysis remain to be achieved. For example, despite intensive research efforts, the mechanisms of microbial thermophily, psychrophily, halophily, alkalophily, and resistance to radiation and dessication still remain obscure, which makes it impossible at this time to predict these traits from genomic sequences. Likewise, there has been only limited success in using genomic information to design synthetic growth media for fastidious pathogens, which would have helped in fighting syphilis, Lyme disease, spotted fever and other bacterial infections. Up until now, all completely sequenced genomes came from cultivated microorganisms, the growth conditions of which, even if suboptimal, were known. This is not the case for sequences coming from metagenomic projects, which will force us to describe properties of the source organisms solely on the basis of sequence data.

**Box 1 Standard parameters of genome description**

**General features of the genome:** DNA: genome size, G+C content, GC skew

Insertion sequences: number, fraction of the genome

RNA: tRNA genes, rRNA genes, small nucleolar RNAs, riboswitches

Proteins: total number of proteins, percentage with known function, percentage 'hypothetical', phylogenetic distribution of the best hits, percentage transmembrane proteins, percentage transporters

**Deduced metabolic properties:** Aerobic/anaerobic, obligate/facultative, Gram-positive or Gram-negative, spore-forming or not

Energy source: autotrophic or heterotrophic, lithotrophic or phototrophic

Carbon source: CO, $CO_2$, methylotrophy, saccharolysis, proteolytic

Sources of nitrogen, phosphorus, sulfur, iron and uptake mechanisms

Biosynthetic pathways: nucleotide, amino acid, cofactor, lipid Secondary metabolism, xenobiotics

**Organism-level adaptations:** Signaling systems: number of histidine kinases, response regulators

Intracellular organelles: thylakoids, heterocysts, spores, storage granules

Pathogenic and defense mechanisms

To measure how much is known about an organism with a completely sequenced genome, Janssen and colleagues introduced a novel metric, termed the 'species knowledge index' (SKI). It is calculated simply as the number of PubMed abstracts mentioning the given species divided by the number of predicted protein-encoding genes [16•]. According to the authors, the average number of abstracts per gene for the first 200 genomes is somewhere between 0.9 and 5, with the obvious exception of human, mouse, well-studied model organisms such as *Escherichia coli*, *Bacillus subtilis* and yeast, and some important pathogens. Naturally, some organisms are covered very poorly or not covered at all. For example, Acidobacteria bacterium Ellin345, the first sequenced representative of the phylum Acidobacteria, has been previously mentioned in a single publication [17] and never described in any detail.

## Genome-based phylogeny

Phylogeny is one of the areas that benefited most from genome sequencing. It can now rely on whole-genome data to build a better tree of life than was afforded by 16S rRNA sequences. Konstantinides and Tiedje introduced two simple integral parameters: ‘average nucleotide identity’ (ANI), as a measure of genome relatedness at the species level, and ‘average amino acid identity’ (AAI), as a measure of phylogenetic proximity for higher taxa [18•,19•]. The phylogenetic tree built on the basis of AAI, as well as those based on the alignment of universally conserved protein sequences (e.g. ribosomal proteins, RNA polymerase subunits and such), proved to be remarkably similar to the 16S rRNA-based tree [5,6,7•,8•,19•], suggesting that all these trees actually reflect the evolutionary history of the prokaryotic world.

However, genomic comparisons revealed a significant variance (up to tenfold in some phyla) in genome sizes and the numbers of encoded proteins of supposedly closely related organisms, illuminating the need for new measures of genome relatedness that would take into account the differences in gene content. Even at the species level, several strains of *Prochlorococcus marinus*, with 97–99% rRNA identity, were found to encode dramatically different protein sets [20]. For pairwise comparisons, genome relatedness can be described with an ‘orthology coefficient’, the fraction of genes that form orthologous pairs in the two genomes [21]. For closely related genomes, the degree of synteny, the fraction of genes that are orthologous and located in co-linear fragments of the genome, can be used as well [22,23]. Another recently introduced metric is ‘genome conservation’, a measure of evolutionary distance between species that takes into account both gene content and sequence similarity at the whole-genome level and allows for apparently accurate phylogenetic reconstructions [7•]. In addition, a measure of the degree of horizontal gene transfer (HGT) between different nodes of the phylogenetic tree, ‘HGT vine width’ was introduced, and a three-dimensional plot (Figure 1) suggested as a better representation of the tree of life than the traditional two-dimensional tree [8•]. For comparing several related genomes, it proved very useful to divide all genes into two categories, those shared by every organism in the given set (referred to as a ‘conserved gene core’ or ‘genome signature’), and those found only in some genomes (referred to as a ‘variable shell’) and to compare the number of the genes in each category [20,24–27].

## Defining protein function on a genome scale

One of the intrinsic problems illuminated by comparative genome analysis is the paucity of appropriate descriptors for protein function and the degree of confidence that the ascribed function is correct. The Gene Ontology (GO) project [28] was extremely successful in categorizing proteins with known — and relatively simple — functions (e.g. enzymes and transcriptional regulators). At the same time, categorization of poorly characterized proteins that fall into the ‘twilight zone’ or ‘grey area’ of sequence similarity [29,30] and are often referred to as ‘hypothetical’ remains a difficult task. To differentiate between proteins that can and cannot be assigned a general biochemical function, the terms ‘known unknowns’ and ‘unknown unknowns’ have been introduced [4]. Recent analysis of the *Shewanella* genome introduced a seven-category scheme that ranks newly sequenced open reading frames by the confidence and precision of their functional annotation all the way from ‘exact biochemical function’ down to ‘general biological function’ and ‘certain functional insights’ [31•]. These rankings are based on the degree of sequence similarity between the protein in question and its experimentally characterized homologs, if any, and the availability of supplementary information [31•]. This approach provided descriptors to distinguish various shades of grey in the grey area of functional annotation of new genomes.

A further advancement into the twilight zone came from comparing structurally related proteins in terms of the structure of their loop regions. A new parameter, termed the ‘loop-based Hausdorff measure’ (LHM), was used to quantify the structural (dis)similarities in the loop regions, which are often responsible for substrate (or ligand) specificity [32,33•]. In a majority of protein families, structural similarity of the loops, measured by LHM, correlated with sequence similarity [33•,34•], suggesting that this parameter could serve as an indirect indicator of the degree of functional divergence between homologous proteins. A related measure, ‘the evolutionary plasticity of structure’, quantifies the relation between the changes in protein structure and the sequence variation within a protein family in the course of evolution. This parameter reflects the constraints in the sequence-structure relationships and is remarkably uniform across different folds and families of proteins [34•]. These two metrics could be useful in assessing the quality of protein structure-based functional assignments, discussed in detail by Rigden in this issue [35].

Another important development in genome annotation has been the agreement between the three international nucleotide sequence databases — the DNA Data Bank of Japan, the European Molecular biology Laboratory (EMBL) nucleotide sequence database and GenBank — to allow third-party annotation of the existing sequence records, including those derived from genomes. The databases accept two types of third-party annotation: ‘experimental’ and ‘inferential’ [36•] (see http://www.insdc.org/TPA.html for details). However, the latter group does not include functional annotations that are based on superfamily assignment or a solved three-dimensional structure, which means that for the foreseeable future the most complete annotation of the grey area will be available in curated databases, such as UniProt, COGs (Clusters of Orthologous Groups of proteins), and others. In any case, moving protein characterization on the genome-scale beyond the ‘70% hurdle’ [3] will require a concerted community-wide effort at experimental characterization of ‘hypothetical’ proteins [37–39].

## Comparative analysis of metabolic pathways

All (known) living organisms rely on the same core pathways of intermediate metabolism. If similar biochemical reactions were always carried out by related proteins, finding appropriate descriptors for metabolic pathways would be a relatively easy task. In reality, the same key functional roles (‘essential functional niches’ [40]) are often carried out by unrelated proteins [10,41,42], and different organisms have substantially different enzyme sets. These factors limit the usefulness of the Enzyme Commission (EC) numbers, which have been routinely used as identifiers for metabolic reactions. In addition, principles of assigning the EC numbers (http://www.ebi.ac.uk/intenz/rules.html) are not entirely conducive to their use as metabolic pathway markers. For example, two analogous phosphoglycerate mutases that differ, among other traits, in their requirement for bisphosphoglycerate, have the same EC number 1.15.1.1. By contrast, two analogous DNA ligases have two different EC numbers, 6.5.1.1 for the ATP-dependent enzyme and 6.5.1.2 for the NAD-dependent one. Besides, unavoidable delays in manual assignment of the EC numbers lead to the proliferation of incomplete EC numbers and, hence, further confusion [43]. This heightens the need for alternative descriptors of biochemical reactions. Kanehisa and colleagues recently came up with a new classification scheme for enzymatic reactions, which is based on the reaction chemistry rather than just the nature of the substrate and product [44••]. This method offers an entirely new — automated — approach to assigning EC numbers, which are then referred to as the RC (reaction classification) numbers. Given that computationally assigned RC numbers appear to be in general agreement with both the manually assigned EC numbers and protein sequence data, as reflected by the KEGG

(Kyoto Encyclopedia of Genes and Genomes) ortholog clusters [44••], these numbers could serve as useful metabolic markers that would simplify and streamline pathway analysis.

Sometimes alternative forms of enzymes are not limited to variations of a single protein and contain several proteins capable of catalysing the same reaction or even variations of a complete pathway [10,42]. The recent discovery of the third pathway of pyrimidine catabolism [45], as well as the very fact that it has remained hidden for so many years [46], show that the degree of functional variance among metabolic pathways is much greater than previously recognized. The redundancy of pathways for formaldehyde oxidation [47] and benzoate catabolism [48, 49] in *Burkholderia xenovorans* LB400 is just one example of metabolic diversity that is relevant to biotechnology. There is a clear need for the appropriate language to describe pathway diversity — and redundancy — among different organisms and even within a single organism. The most promising approach appears to be subdivision of the metabolic network into 'subsystems' [42,50•] or 'phylogenetic network modules' [51•], on the basis of a combination of sequence-based clustering of enzymes and phylogenetic profiles of enzyme distribution. This approach allows one to identify evolutionarily conserved metabolic building blocks that can be acquired, lost, combined and/or exchanged in the course of biochemical evolution in various lineages.

New approaches to the analysis of metabolic pathways include improved pathway visualization and comparison tools, such as those provided by the KEGG and MetaCyc databases [52,53]. Combining such static 'wiring diagrams' with real-time dynamic representations of metabolic fluxes will allow an entirely new level of comprehension of cell metabolism.

## Functional profiling of genomes

Several recently sequenced microbial genomes were found to have lineage-specific gene expansions that could be directly linked to the unique biochemistry of the corresponding organisms. Thus, the two genomes of *Dehalococcoides* species encode numerous copies of reductive dehalogenases, which allow these organisms to use chlorinated hydrocarbons, including the common pollutant perchloroethylene, as terminal electron acceptors [54,55]. By contrast, *Desulfitobacterium hafniense,* a dechlorinating bacterium from a different phylum, had only two genes for reductive dehalogenases but encoded numerous terminal oxidases, similar to the dimethyl sulfoxide reductase [56•]. Owing to the expansion of these and several other protein families, the genome of *D. hafniense* revealed a significantly elevated fraction of genes (COGs) related to energy production and conversion (functional category C in the COG database [57]). This approach to analyzing lineage-specific expansions and losses of metabolic enzymes and whole pathways offers a convenient way of highlighting peculiarities of a given organism or clade. This can be reflected in a new genome-scale metric, which shows the distribution of proteins assigned to each functional category in the COG database as compared with distribution in related organisms [58]. By analogy with the phylogenetic profiles, this distribution can be referred to as a COG profile or, more generally, a 'functional category profile'. Figure 2 shows the results of such an analysis for four organisms with similarly sized genomes. The analysis illustrates the dramatic expansion of proteins involved in DNA replication and recombination (mostly transposases) in *Shigella dysenteriae*, and reveals the unusually high number of enzymes of lipid biosynthesis and secondary metabolism in *Mycobacterium avium* and of signal transduction proteins in *Dechloromonas aromatica*. COG functional category assignments for every sequenced prokaryotic genome are available in the National Center for Biotechnology Information RefSeq database, making such comparisons easy to perform. Furthermore, RefSeq provides tables that show fractions of genes belonging to each functional category per genus, phylum and bacteria in general (see, e.g. http://www.ncbi.nlm.nih.gov/sutils/coxik.cgi?gi=380). Although these are automated assignments that could be prone to certain biases, they provide a convenient birds-eye view of the peculiarities of each given genome. Certainly, such functional profiling could use other functional classifications, such as the one introduced by Riley [59].

## Signal transduction

All organisms adapt to their environment by perceiving environmental signals and modifying their behavior (and/or metabolism) accordingly. Recent attempts to compare microorganisms in terms of their ability to adjust to environmental changes have led to the introduction of two new metrics [60•]. The first, a signaling index, referred to as ‘bacterial IQ’, reflects the abundance of signal transduction components encoded in a given organism as compared with others of a similar genome size. The second parameter, the degree of ‘extrovertness’, reflects the fraction of transmembrane receptors among all sensory proteins encoded in a given genome. The viability of the second parameter was demonstrated by the finding that cyanobacteria, which have a complex system of intracellular membranes, are the most ‘introverted’ bacteria [60•]. The relevance of the first parameter was affirmed by the finding that bacteria with the highest proportion of environmental sensors (‘highest IQ’) encode the most diverse sets of response regulators [61]. Although these parameters still remain to be statistically validated, they seem to offer new ways to describe the complexity of an organism’s signal transduction machinery.

## Conclusions: from reading to comprehension and utilisation of genomic information

Genome-based functional profiling, the reconstruction of metabolic pathways and assessment of an organism’s signaling capabilities are only the first, and simplest, ways to describe an organism on the basis of its genome sequence. Better tools, better approaches and better metrics are needed to fully comprehend the information stored in the genomic data and to eventually utilize this information in biotechnology, pharmacology and genomic medicine. The appearance of new descriptors is an important part of developing the new language of systems biology, which will be used to communicate new ideas and the underlying datasets. However, the examples listed in this paper clearly show that new metrics for genome comparisons cannot simply be pulled out of a hat. Each emerges as a result of careful research and painstaking efforts to understand molecular mechanisms of cell life and to find adequate parameters and tools to describe them. Thus, the current lack of high-quality descriptors, for example for microbial adaptations to extreme conditions, is hardly due to the lack of trying: rather it is caused by a lack of understanding of what is and what is not important for the maintenance of a thermophilic (or psychrophilic) lifestyle. Once underlying principles of adaptation to extreme conditions are better understood, genomic determinants for these traits will become additional metrics for genome description. As noted by Kerényi, “languages are not so much a means of expressing truth that has already been established as a means of discovering truth that was previously unknown” (http://www.wikipedia.org/wiki/Sapir-Whorf_Hypothesis), which will remain the task of systems biology for years to come.

### Acknowledgements

We thank Yuri Wolf, Tatiana Tatusova and Natali Kolker for helpful suggestions, and Roger Higdon and Evelyne Kolker for critical reading of the manuscript. Supported by the Intramural Research Program of the NIH, National Library of Medicine (MYG) and the US Department of Energy grant DE-FG08-01ER63218 (EK).

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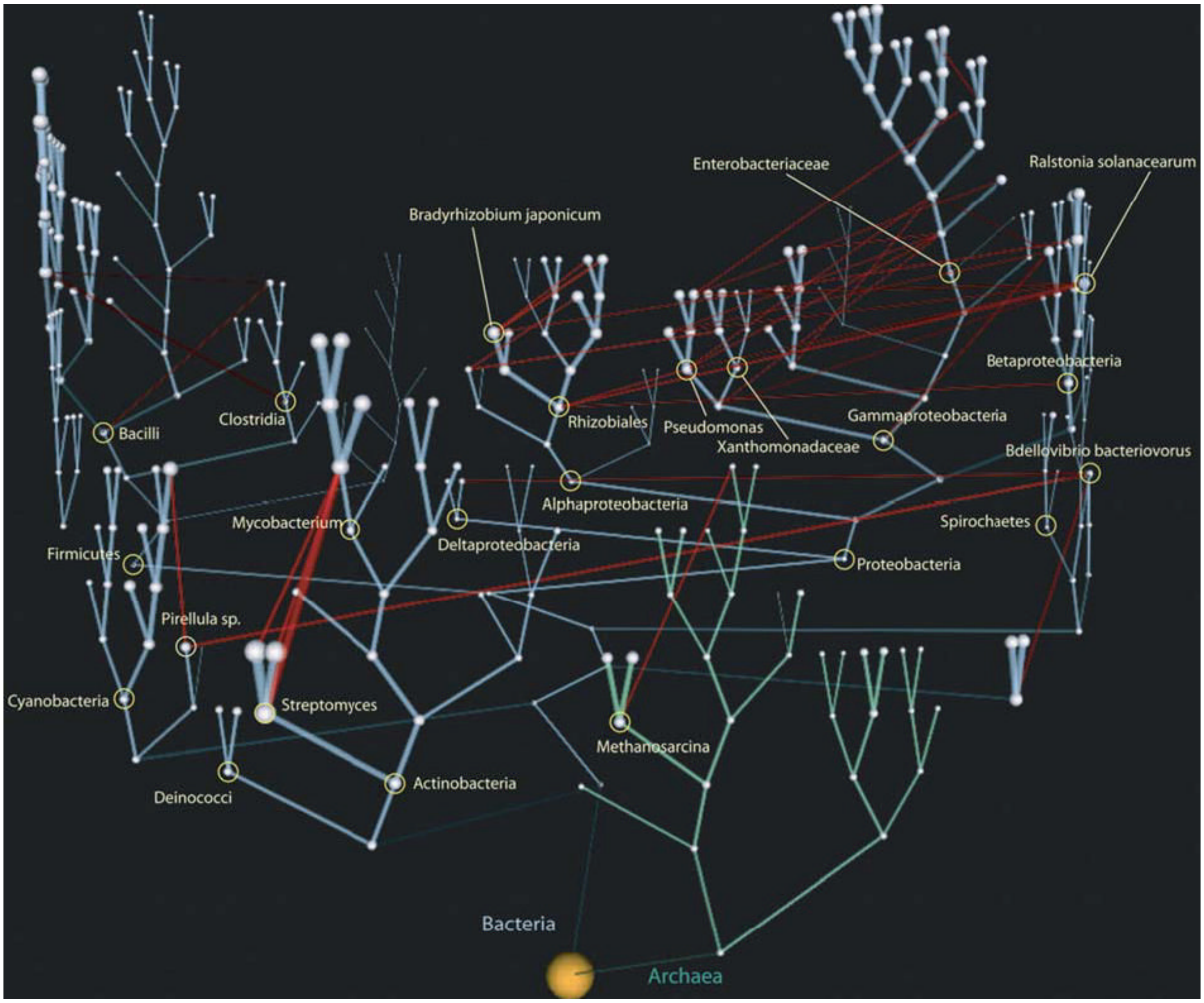

**Figure 1.**

The tree of life in three dimensions. The plot, taken from [8•], reflects the ‘genome conservation’ and ‘horizontal gene transfer vine width’ data. Individual taxonomic nodes are shown as white spheres, the diameters of which reflect the number of node-specific gene families. The bacterial phylogenetic tree is in cyan and the archaeal tree in light green. Red lines indicate the horizontal gene transfer. The width of each line reflects the number of gene families transferred along that line. Reprinted with permission from *Genome Res* 2005, **15**:954–959.

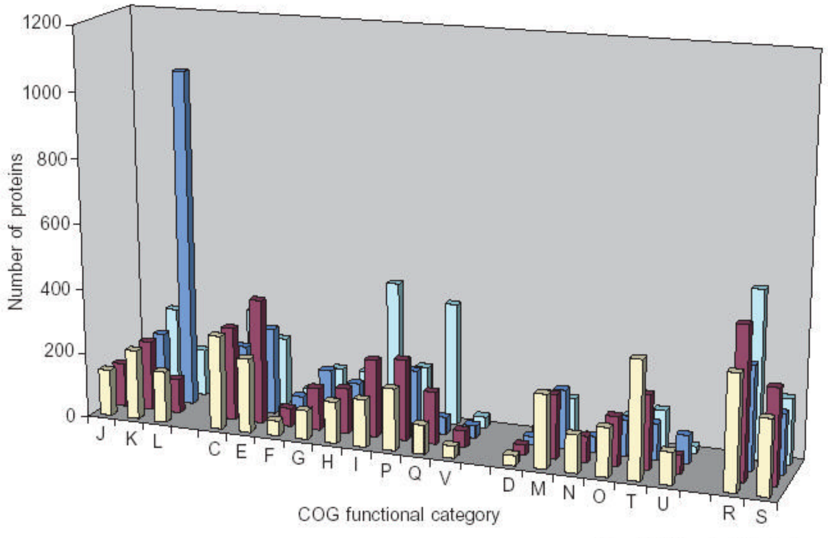

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**Figure 2.**

Functional category profiles of four bacterial genomes. The plot shows the number of proteins from *Dechloromonas aromatica* RCB (front row; cream), *Rhodopseudomonas palustris* HaA2 (second row; magenta), *Shigella dysenteriae* Sd197 (third row; blue) and *Mycobacterium avium* subspecies *paratuberculosis* K-10 (back row; cyan), assigned to COGs in each functional category. COG functional categories are grouped as follows: 1, information storage and processing (J, translation, ribosomal structure and biogenesis; K, transcription; L, DNA replication, recombination and repair); 2, metabolism (C, energy conversion; E, amino acid transport and metabolism; F, nucleotide transport and metabolism; G, carbohydrate transport and metabolism; H, coenzyme transport and metabolism; I, lipid metabolism; P, inorganic ion transport and metabolism; Q, secondary metabolism; V, defense mechanisms); 3, cellular processes (D, cell division; M, cell envelope; N, motility and secretion; O, post-translational modification and protein turnover; T, signal transduction; U, intracellular trafficking); and 4, poorly characterized (R, general function prediction only; S, unknown function). The data are taken from the Entrez Genome database (http://www.ncbi.nlm.nih.gov/sutils/coxik.cgi?gi=18621 and similar entries).