Prokaryotic Virus Orthologous Groups (pVOGs): a resource for comparative genomics and protein family annotation

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ABSTRACT

Viruses are the most abundant and diverse biological entities on earth, and while most of this diversity remains completely unexplored, advances in genome sequencing have provided unprecedented glimpses into the virosphere. The Prokaryotic Virus Orthologous Groups (pVOGs, formerly called Phage Orthologous Groups, POGs) resource has aided in this task over the past decade by using automated methods to keep pace with the rapid increase in genomic data. The uses of pVOGs include functional annotation of viral proteins, identification of genes and viruses in uncharacterized DNA samples, phylogenetic analysis, large-scale comparative genomics projects, and more. The pVOGs database represents a comprehensive set of orthologous gene families shared across multiple complete genomes of viruses that infect bacterial or archaeal hosts (viruses of eukaryotes will be added at a future date). The pVOGs are constructed within the Clusters of Orthologous Groups (COGs) framework that is widely used for orthology identification in prokaryotes. Since the previous release of the POGs, the size has tripled to nearly 3000 genomes and 300 000 proteins, and the number of conserved orthologous groups doubled to 9518. User-friendly webpages are available, including multiple sequence alignments and HMM profiles for each VOG. These changes provide major improvements to the pVOGs database, at a time of rapid advances in virus genomics. The pVOGs database is hosted jointly at the University of Iowa at http://dmk-brain.ecn.uiowa.edu/pVOGs and the NCBI at ftp://ftp.ncbi.nlm.nih.gov/pub/kristensen/pVOGs/home.html.

INTRODUCTION

Although bacteria are the most plentiful and diverse cellular domain of life on earth, their abundance is vastly outweighed by the staggering number of viruses that infect them (1,2). Despite the presence of many thousands of completely sequenced virus genomes in NCBI sequence databases, the vast majority of viruses still remain to be characterized (3). Even so, the large number of sequences available creates considerable difficulties in navigating through all the data in search of biological meaning (4). The Prokaryotic Virus Orthologous Groups (pVOGs, formerly Phage Orthologous Groups, POGs (5–7)) resource aids researchers by providing clusters of orthologous genes in complete genomes of viruses that infect bacteria or archaea, using the microbial COG framework (8,9)—one of the oldest, most accurate, and most-often-used methods to computationally identify orthologs (10). Analogous to the COG database, each viral COG (VOG) represents all of the descendants of a single ancestral gene (orthologs) within the analyzed set of genomes. The pVOGs can be used for gene prediction and functional annotation in new virus genomes (11–13), metagenomic data (14–21) and host genomes containing proviruses (22,23). In addition to gene prediction and protein function, the pVOGs provide a third form of annotation, namely the distribution (presence or absence) of the constituent genes across viruses and cellular organisms, i.e. a ‘phyletic pattern’ or ‘phylogenetic profile’ (24–27). Beyond various forms of annotation, pVOGs have also been used to construct large-scale datasets for studies of viral phylogenetics (28), comparative genomics (29–33) and mathematical modelling of virus evolution (34–36).

The pVOGs provide evolutionary gene families from nearly 3000 complete genomes of viruses that infect bacteria or archaea as a pre-computed resource, thus providing immediate access to information that would otherwise incur a heavy computational cost. This is triple the size of the previous release (7), in both the number of genomes and genes. Taxonomic coverage of pVOGs database has

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been broadened with the newly available genomes, including virus families not represented in the previous dataset, such as *Sphaerolipoviridae*, *Turriviridae* and *Guttaviridae* (Supplementary Figure S1). The number of pVOGs has also increased to more than double the previous number of 4542, now up to 9518 (Supplementary Figure S2). pVOGs are also now available as user-friendly webpages. Multiple routes of access are provided in the form of a list of all genomes in the database and a list of all pVOGs, from either of which pages showing a list of all pVOGs represented in individual genomes can be easily accessed, or the user can choose from among several forms of bulk downloads. For instance, multiple sequence alignments and HMM profiles for each pVOG are provided, with downloads available either for individual pVOGs, individual virus families, or the entire database in bulk.

**CHANGES IN THE pVOGs DATABASE**

Previous versions of the pVOGs resource have been described under the name POGs (Phage Orthologous Groups (5–7)). Later, after expanding beyond the original content of dsDNA tailed bacteriophages to also include archaeal viruses, and then even further to also include bacterial and archaeal viruses with ssDNA, dsRNA, or ssRNA genomes, this name was changed to Prokaryotic virus Orthologous Groups, but the acronym POGs was kept for historical continuity. However, that is no longer possible because another, unrelated database published in this journal very shortly after the initial POGs publication has already registered the POG name (37). Thus, we rename the individual POGs as VOGs (for viral COGs, similar to another database that existed previously at NCBI many years ago (38)), and the database as a whole as pVOGs (Prokaryotic Virus Orthologous Groups), to clearly indicate that eukaryotic viruses are not (yet) included.

Although the names of individual POGs were changed to VOGs, the numeric portion of the name was kept intact. For example, the old POG00001 is now re-named to VOG00001, and still represents the same V protein family present in the *Inoviridae* family of filamentous phages and a few other viruses (with appropriate updates—e.g. some genomes that were previously unclassified have since been assigned to the *T7likevirus* genus).

Since the previous release of this data set, the number of genomes and proteins has tripled, and the number of VOGs has doubled (Supplementary Figure S2). The coverage of virus types in the pVOGs database has also been both broadened and deepened with increased coverage of previously included viral groups (families, genera and species), as well as the addition of representatives from several new viral groups that were not present in the previous POGs dataset (Supplementary Table S1, Supplementary Table S2, and Supplementary Table S3).

The basis of forming VOGs has changed from using domain-based orthology assignments to full-length proteins. All other COG-based resources currently use full-length proteins rather than domains (e.g. COGs (8,9), KOGs (39), arCOGs (40), NCVOGs (41), mimiCOGs (42), etc.), although those resources use manual curation to provide ‘domain-aware’ orthology predictions, avoiding grouping together of COGs due to domain recombination (10). This curation step was not implemented for pVOGs because multidomain proteins are rare in viruses, and the number of instances of domain recombination that would cause erroneous joining of multiple COGs is accordingly negligible (6). As an added bonus to consistency across COG resources, removal of the extremely computationally expensive domain splitting step, in conjunction with the automated procedure to map new viral COGs to existing groups, will allow for more frequent future updates of the pVOGs database.

**STATISTICS OF THE pVOGs DATABASE**

The pVOGs resource encompasses all publicly available complete genome assemblies of viruses that infect bacteria and archaea. As of May 2016, NCBI’s RefSeq database contained nearly 2000 complete genomes of prokaryotic viruses, which we then supplemented with a manually curated list of an additional 1000 complete genome entries from the Nucleotide (GenBank) database when a RefSeq entry was not available for a particular virus. Altogether then, the pVOGs database encompasses a comprehensive set of nearly 3000 complete genomes of viruses with prokaryotic hosts that are currently available. Most (>97%) represent phages that infect bacteria, although 77 complete genomes of archaeal viruses and four high-quality complete genome entries from uncultured viruses (e.g. crAssphage (43) and experimentally verified oceanic ssDNA phages (44)) are included as well. In total, these genomes contain nearly 300 000 protein-coding genes. To fill gaps left by automated annotation pipelines, we conservatively added >10 000 automated gene predictions (see below). These genes were then clustered into 9518 orthologous groups using the latest version of the COG algorithm (7,9,45,46). On average, about two-thirds of the proteins encoded in a virus genome are conserved in a VOG. The remaining one-third represents novel genes shared by fewer than three viruses, or cases of extreme evolutionary divergence where our automated detection procedure (see below) was unable to detect the low level of similarity. This coverage is not evenly distributed (Figure 1), but rather, the best-characterized viral families display a higher proportion of gene coverage than the poorly characterized families (especially archaeal viruses with few members). Some individual viruses even display coverage as high as 100%, e.g. *Mycobacterium phage* Ramsey for which every one of its genes is also found in at least two other distinct viruses. At the other end of the spectrum, some viruses do not have even a single gene that is shared with other viruses, such as the tiny *Leuconostoc phage* L5 with only five genes, none of which were annotated in GenBank.

Most of the individual VOGs in the pVOGs database are small, with 69% containing ≤10 members (Supplementary Figure S3). Furthermore, most VOGs are simple families of orthologs, with barely any paralogs: 95% of VOGs contain none, and <3% contain more than one. However, several notable exceptions exist for both features, such as several families that include typical mobile elements such as HNH endonucleases (47,48), and several very large families of VOGs shared across many viruses, such as the large
**Figure 1.** Coverage of protein sequences in VOGs for each viral family. The number of genomes in each family is shown in parentheses. Families with representatives covered by VOGs are shown. **Globuviridae** is not shown since its representative viruses had no proteins present in VOGs.

terminase subunit, a DNA packaging ATPase family found in about fourth fifths (83%) of the viruses in the order **Caudovirales** (tailed bacteriophages). VOG4543 uniquely represents an exception to both features at once, with 3.5-fold more proteins and a nearly 5-fold higher level of paralogy than any other VOG, in a group of helix-turn-helix DNA binding proteins found in an extremely diverse array of viruses (Figure 2). This VOG contains members of several protein families—including HNH endonucleases, transcriptional regulators such as cro/cI repressor, etc.—and altogether encompasses more than four fifths of the 3000 genomes, including both dsDNA and ssDNA, and with both bacterial and archaeal hosts. As such, from the standpoint of utility for the purposes of functional annotation, this VOG might better have been treated as a result of over-lumping of too many distinctly related proteins. However, we refrained from manually splitting this group up, as was done for the microbial COG database, for two reasons: (i) consistency: although this group seems an obvious outlier, similar situations could lay hidden among the >9000 VOGs in the pVOGs database, especially among the many groups that, large or small, exclusively have annotations of 'hypothetical protein' and (ii) ease of future updates, especially because the vast majority of the genetic diversity present in the virosphere has yet to be explored, and therefore any procedure that includes manual annotation as a requirement for future progress will be difficult to keep up to date.

**pVOGs DATABASE ACCESS AND WEB INTERFACE**

The pVOGs database is primarily accessed through the homepage at the University of Iowa at [http://dmk-brain.ecn.uiowa.edu/pVOGs](http://dmk-brain.ecn.uiowa.edu/pVOGs) or the NCBI at [ftp://ftp.ncbi.nlm.nih.gov/pub/kristensen/pVOGs/home.html](ftp://ftp.ncbi.nlm.nih.gov/pub/kristensen/pVOGs/home.html). Access to the pVOGs database is provided via two main interactive avenues: a list of all genomes (Figure 3A) and all VOGs (Figure 3B) in the pVOGs database. In the former, a user can choose a particular virus genome to investigate (Figure 4A), examine the conserved gene families it shares with other viruses, and choose an individual VOG (Figure 4B) to find
more information; via the latter avenue, the user can choose an individual VOG directly by name or protein annotation. From an individual VOG page (Figure 4B), links to the other VOGs conserved in each of its member genomes are provided. A third, non-interactive avenue of access is provided in the form of bulk data files accessible from the downloads page, available for the pVOGs database in its entirety as well as for each viral family. All data available on the webpages are also provided as text files for programmatic access (for large-scale studies), and can be accessed either from individual VOG pages or the downloads page. A tutorial page is also available with screenshots that further illustrate each type of webpage and helps users to navigate the pVOGs database.

**pVOGs DATABASE CONSTRUCTION**

**Genomes**

All complete genome assemblies of viruses that infect bacteria were downloaded as of May 2016 from NCBI’s Nucleotide database (GenBank), as described previously (7). For example, for phages, the following Entrez query was used to obtain RefSeq entries: `Viruses[Organism] NOT cellular organisms[ORGN] AND srcdb_refseq[PROP] AND vhost bacteria[filter] AND 'complete genome' [All Fields]`. A similar query was used for archaeal viruses; for non-RefSeq entries, the query was modified to include NOT srcdb_refseq[PROP] NOT mRNA[filter]. The resulting set was manually curated to remove records described as clones, mutants, partial sequences, plasmids, CDS, or unverified, as well as prophage or proviral. After further examination, exceptions were made for four uncultured virus genomes obtained from metagenomic studies because these viruseshave been validated experimentally (43, 44, 49). For duplicate virus genomes with identical taxonomy IDs shared between RefSeq and non-RefSeq entries, the former was kept and all of the latter were discarded; when there was no RefSeq record, all of the non-RefSeq entries were retained (no automated way exists of choosing the single ‘best’ representative). This curation process yielded 2912 bacterial viruses, 77 archaeal viruses and the four experimentally-verified uncultured viruses. Of these 2993 entries, 1986 were from RefSeq, and the rest from GenBank entries present in the nucleotide database.
Genes

In addition to the 286,242 protein-coding genes that were already present in the genome records, we conservatively added 10,353 denovo gene predictions made using the non-supervised heuristic GeneMarkS program (50). Genes were only added if they met the following criteria: (i) not overlapping with an existing gene by more than 50 bp and (ii) not oriented in the opposite direction of the three upstream and three downstream gene neighbors. This procedure was manually verified on several tens of well-annotated genomes from curated databases (such as T4, T7 and lambda), as described previously (6,7).

Orthologous gene clusters

Prior to forming orthologous gene clusters (those shared between at least three ‘distinct’ viruses), closely related genomes that effectively represent multiple isolates of the same virus were merged. This was done to reduce the bias in the dataset caused by the non-uniform representation of different viruses, by preventing the formation of a VOG from only trivially related isolates of the same virus (6,7). In the current dataset, this procedure clustered the 2,993 genomes into 1,795 lineages. Next, an all-against-all BLAST (51) search was performed for all proteins in all genomes, and the standard COGtriangles algorithm was used to construct Clusters of Orthologous Groups (COGs) of the virus proteins (9,45). Briefly, this procedure begins by identifying symmetric best matches shared between three genomes, and merges such triangles into larger groups whenever they share a common ‘side’ (a two-way symmetric best-match). This approach groups together even fast-evolving orthologs as long as sufficient sequence similarity exists to detect the signal, with matches having e-value > 10 or covering < 50% of the query or target length discarded to ensure high quality. In 1.5% of the proteins, this approach failed to distinguish between membership in multiple COGs, which is an indication of unresolved paralogy or domain recombination.

Mapping

To ensure continuity of the updated pVOGs database with the previous dataset of virus orthologs (7) and measure the differences resulting from splitting proteins into domains vs. using full-length proteins, the clustering of viral proteins into orthologous groups in the ‘pVOGs 2016’ dataset was compared to that in the older ‘POGs 2013’ dataset (Table 1) for a reduced dataset shared between the two versions. Given that several virus genome entries that were present in GenBank in the older POGs dataset were now replaced with entries from RefSeq, and during this change several protein annotations were also changed, genomic coordinates shifted, and gene start and stop locations altered, a recip-
Figure 4. Screenshot of pVOGs webpages showing additional types of information available: (A) individual genome table showing a list of VOGs present in a particular genome and respective protein annotations; (B) individual VOG table, describing detailed information about each VOG and protein content. This page also provides tabular files, multiple sequence alignments and HMM profiles for downloading.

A high degree of agreement was found between the datasets (Table 1), with >92% of VOGs belonging to the first two match categories. For these, the numeric portion of the names of the original POGs were assigned to the current VOGs, to ensure consistency between versions. In cases where a current VOG matched multiple older POGs, and did so with 100% identity to the union of those clusters, the name of the largest original cluster was inherited, and a note added describing all of the names of the original clusters. New VOGs not observed in the previous dataset, as well as those with only partial overlap with one or more previous POGs, were sequentially assigned new names, starting at the point where the older POGs left off (i.e. VOG4543 and above).

Availability

The pVOGs database is freely and publicly available at the University of Iowa at http://dmk-brain.ecn.uiowa.edu/pVOGs and the NCBI at ftp://ftp.ncbi.nlm.nih.gov/pub/kristensen/pVOGs/home.html. Both locations will be updated as changes are made in the future, although the latter FTP site will host only simple webpages devoid of active content such as JavaScript and/or CGI whereas the former HTTP server will have active content added as it is developed. The previous version (still called POGs) will remain available at its original location on the NCBI FTP site at ftp://ftp.ncbi.nlm.nih.gov/pub/kristensen/thousandgenomespogs/. All queries and comments regarding the pVOGs database should be directed to DMK.

FUTURE DEVELOPMENTS

In accordance with the name ‘Prokaryotic Virus Orthologous Groups’, only the subset of viruses that infect bacterial and archaeal hosts are currently available in the pVOGs database, although in the future we anticipate the addition of eukaryotic virus families. Algorithmic improvements to orthology identification are also planned to span the some-
times wide distances between VOGs that are homologous but are not detected by simple BLAST searches that lack the increased sensitivity of profile HMM approaches. In the more immediate future, the webpages at the Iowa HTTP webserver will be enhanced to allow for easier interactivity with the large-scale data by using JavaScript functionality (such as sorting, filtering and per-column searching of the large tables). Additional ease-of-use improvements that are currently under development will also continue to be released when they become ready, including additional statistics for each VOG, such as the Viral Quotient as a helpful measure of the ‘virusness’ of a particular gene family (propensity to be found in virus genomes versus non-prophage regions of cellular organisms).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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