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MBGD update 2010: toward a comprehensive resource for exploring microbial genome diversity

Ikuo Uchiyama[1,*], Toshio Higuchi[2] and Mikihiko Kawai[1]

[1]National Institute for Basic Biology, National Institutes of Natural Sciences, Nishigonaka 38, Myodaiji, Okazaki, Aichi 444-8585, Japan and [2]Intec Systems Institute, Inc., 1-3-3 Shinsuna, Koto-ku, Tokyo 136-0075, Japan

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ABSTRACT

The microbial genome database (MBGD) for comparative analysis is a platform for microbial comparative genomics based on automated ortholog group identification. A prominent feature of MBGD is that it allows users to create ortholog groups using a specified subgroup of organisms. The database is constantly updated and now contains almost 1000 genomes. To utilize the MBGD database as a comprehensive resource for investigating microbial genome diversity, we have developed the following advanced functionalities: (i) enhanced assignment of functional annotation, including external database links to each orthologous group, (ii) interface for choosing a set of genomes to compare based on phenotypic properties, (iii) the addition of more eukaryotic microbial genomes (fungi and protists) and some higher eukaryotes as references and (iv) enhancement of the MyMBGD mode, which allows users to add their own genomes to MBGD and now accepts raw genomic sequences without any annotation (in such a case, it runs a gene-finding procedure before identifying the orthologs). Some analysis functions, such as the function to find orthologs with similar phylogenetic patterns, have also been improved. MBGD is accessible at http://mbgd.genome.ad.jp/.

INTRODUCTION

Nearly 1000 microbial genomes have been completely sequenced, and the number of sequences is still growing exponentially. The growth of this number will be even further accelerated by the recent advancement of next-generation sequencing technologies. Thanks to this vast amount of information, much progress has been made in genomics studies toward understanding microbial diversity. One of the promising approaches is the comparison of dozens of closely related or moderately related genomes, which is effective for analyzing critical differences among organisms and understanding the evolutionary process generating such diversity. Another important new advancement is the metagenomic approach, by which researchers can investigate the community structures of microbes and their gene contents in various environmental samples. To facilitate genomic diversity studies, however, effective utilization of the existing genomic data in terms of comparative genomics is crucial, although this becomes more difficult as the size of the genomic database increases.

MBGD (1,2) is a microbial genome database for large-scale comparative genomics based on comprehensive ortholog classification generated by a hierarchical clustering method, DomClust (3). As compared to other comparative genomics resources covering complete microbial genomes, such as CMR (4), MicrobesOnline (5), IMG (6), eggNOG (7) and OMA (8), a prominent feature of MBGD is that it allows users to create ortholog groups using a specified subgroup of organisms. This feature is useful for various types of comparative analysis, including comparisons among closely related as well as among distantly related organisms (1).

In addition to the flexible ortholog analysis functionality, we have recently enhanced the database content by incorporating various types of information regarding gene function and organism phenotype, adding more eukaryotic genomes and implementing several additional functionalities to facilitate large-scale comparative genome analysis. Here, we describe the recent enhancement of MBGD.

DEFAULT ORTHOLOG TABLE

Although one of the significant features of MBGD is that it allows users to create their own ortholog tables by specifying any set of organisms, MBGD also holds a precalculated 'default ortholog table', which is now extended to cover all the organisms stored in the database. Actually, the default ortholog table is created using the default set of organisms that contains one representative genome from each genus, but in the 'extended'

*To whom correspondence should be addressed. Tel: +81 564 55 7629; Fax: +81 564 55 7625; Email: uchiyama@nibb.ac.jp

table, genes of unselected genomes are also classified into an appropriate ortholog group as follows: each gene is classified into the ortholog group giving the best average similarity score if (i) that score is better than the smallest within-cluster score (i.e. the score assigned at the cluster root node) or (ii) that gene is also the most similar to that ortholog group in that genome (i.e. they are in a bidirectional best-hit relationship). Based on this extended default ortholog table, users can now access the ortholog cluster information from any gene information page. Users can also download the entire default ortholog table as a flat text file.

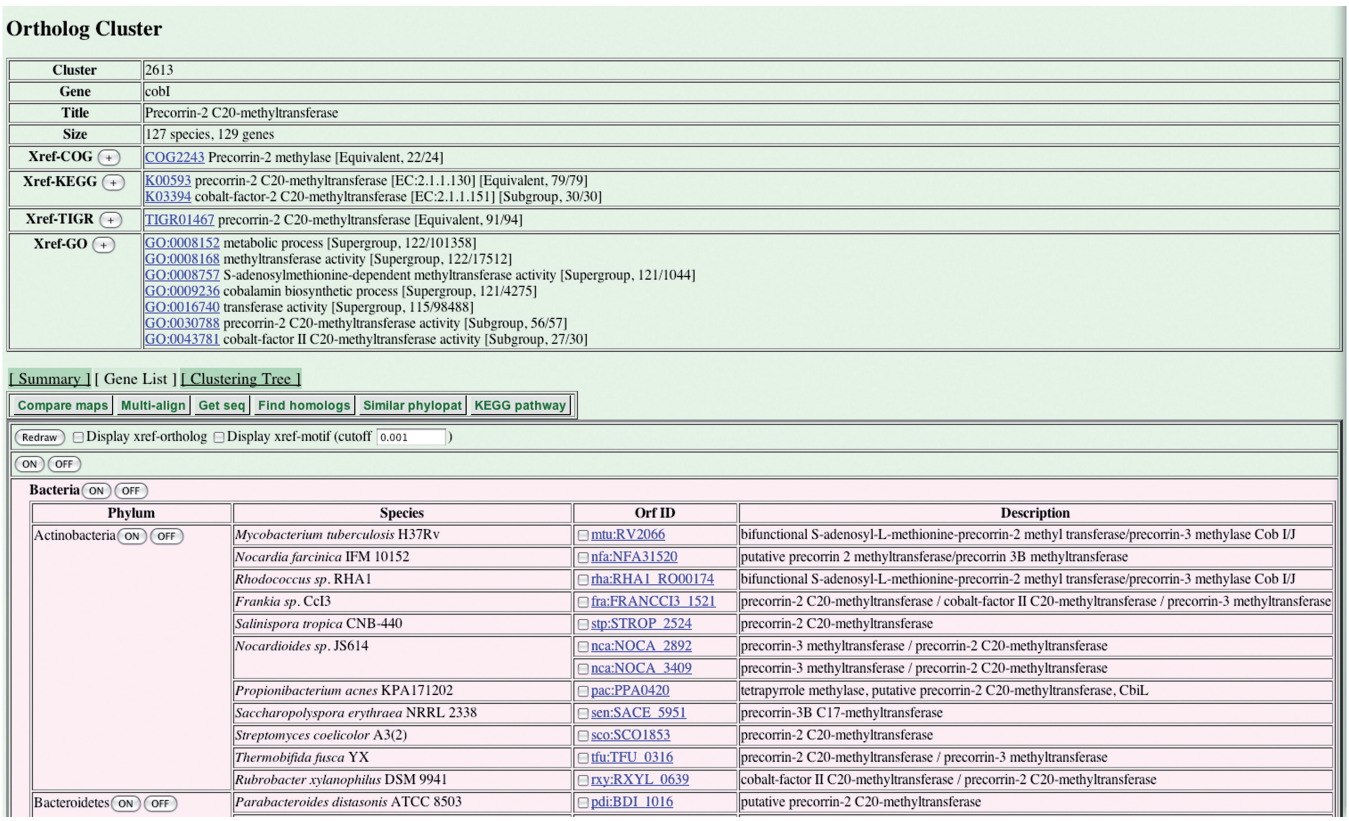
ANNOTATION ASSIGNMENT TO EACH CLUSTER

The ortholog cluster information page has been redesigned (Figure 1). Several types of information are generated from the annotation of its member genes and are added to this page as cluster annotation. The following procedure determines the title of each ortholog cluster: the occurrence of words in the title lines of the member genes are counted, and the words whose occurrence is above or equal to 30% of the most frequent words are retained as frequent words; after scoring each title line based on the frequency of frequent words, the cluster title is constructed by extracting the frequent words from the best-scoring title.

Each cluster entry also contains cross-references to the corresponding entries of COGs, KEGG Orthology, TIGRFAMs and Gene Ontology terms. A correspondence between an MBGD group and a group of another database is classified into 'equivalent', 'supergroup' and 'subgroup', which are defined based on the following set-comparison procedure: let A and B be the MBGD group and the other group, respectively, containing only organisms commonly included in both sets, and let $\alpha = |A \cap B|/|A|$, $\beta = |A \cap B|/|B|$ and $F = 2\alpha\beta/(\alpha + \beta)$; we defined B as being equivalent to A if $F \geq 0.7$; otherwise, B is a supergroup of A if $\alpha \geq 0.7$ or a subgroup of A if $\beta \geq 0.7$. In these cases, B is assigned as a cross-reference entry of A (Figure 1).

As previously, each cluster entry is assigned functional categories, but the definition of functional category has been extended: in addition to the original definition (1), users can now choose a functional category system from among those defined in other databases (COG, KEGG and TIGR); category assignment to each cluster is based on a majority vote of categories assigned to individual genes referring to the cross-reference data.

In the cluster entry page, several comparison functions are available, such as multiple map comparison and multiple sequence alignment (Figure 1). In addition, a function to search for clusters with similar phylogenetic patterns is now available. Here, the cluster table is ordered



Ortholog Cluster

Cluster	2613
Gene	cobI
Title	Precorrin-2 C20-methyltransferase
Size	127 species, 129 genes
Xref-COG (+)	COG2243 Precorrin-2 methylase [Equivalent, 22/24]
Xref-KEGG (+)	K00593 precorrin-2 C20-methyltransferase [EC:2.1.1.130] [Equivalent, 79/79] K03394 cobalt-factor-2 C20-methyltransferase [EC:2.1.1.151] [Subgroup, 30/30]
Xref-TIGR (+)	TIGR01467 precorrin-2 C20-methyltransferase [Equivalent, 91/94]
Xref-GO (+)	GO:0008152 metabolic process [Supergroup, 122/101358] GO:0008168 methyltransferase activity [Supergroup, 122/17512] GO:0008757 S-adenosylmethionine-dependent methyltransferase activity [Supergroup, 121/1044] GO:0009236 cobalamin biosynthetic process [Supergroup, 121/4275] GO:0016740 transferase activity [Supergroup, 115/98488] GO:0030788 precorrin-2 C20-methyltransferase activity [Subgroup, 56/57] GO:0043781 cobalt-factor II C20-methyltransferase activity [Subgroup, 27/30]

[Summary] [Gene List] [Clustering Tree]

Compare maps | Multi-align | Get seq | Find homologs | Similar phylopat | KEGG pathway

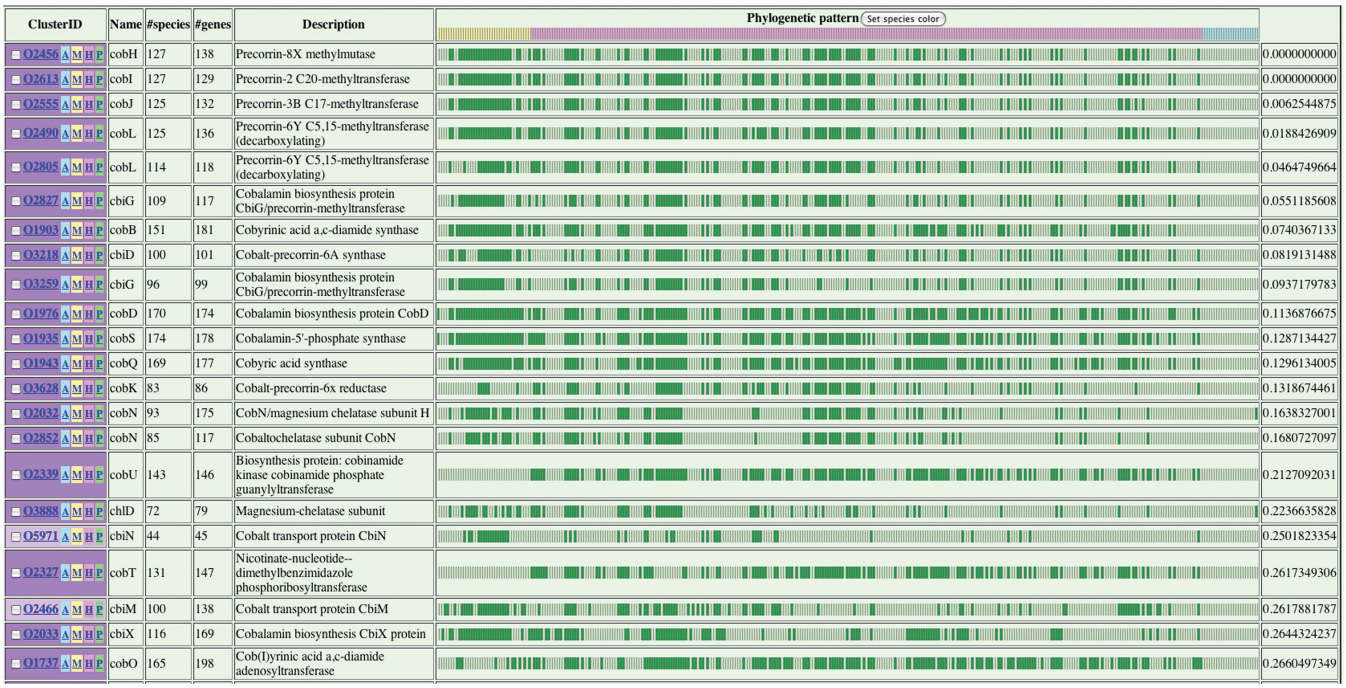
Redraw ☐ Display xref-ortholog ☐ Display xref-motif (cutoff 0.001)

ON OFF

Bacteria ON OFF

Phylum	Species	Orf ID	Description
Actinobacteria ON OFF	*Mycobacterium tuberculosis* H37Rv	mtu:RV2066	bifunctional S-adenosyl-L-methionine-precorrin-2 methyl transferase/precorrin-3 methylase Cob I/J
	Nocardia farcinica IFM 10152	nfa:NFA31520	putative precorrin 2 methyltransferase/precorrin 3B methyltransferase
	Rhodococcus sp. RHA1	rha:RHA1_RO00174	bifunctional S-adenosyl-L-methionine-precorrin-2 methyl transferase/precorrin-3 methylase Cob I/J
	Frankia sp. CcI3	fra:FRANCCI3_1521	precorrin-2 C20-methyltransferase / cobalt-factor II C20-methyltransferase / precorrin-3 methyltransferase
	Salinispora tropica CNB-440	stp:STROP_2524	precorrin-2 C20-methyltransferase
	Nocardioides sp. JS614	nca:NOCA_2892	precorrin-3 methyltransferase / precorrin-2 C20-methyltransferase
		nca:NOCA_3409	precorrin-3 methyltransferase / precorrin-2 C20-methyltransferase
	Propionibacterium acnes KPA171202	pac:PPA0420	tetrapyrrole methylase, putative precorrin-2 C20-methyltransferase, CbiL
	Saccharopolyspora erythraea NRRL 2338	sen:SACE_5951	precorrin-3B C17-methyltransferase
	Streptomyces coelicolor A3(2)	sco:SCO1853	precorrin-2 C20-methyltransferase
	Thermobifida fusca YX	tfu:TFU_0316	precorrin-2 C20-methyltransferase / precorrin-3 methyltransferase
	Rubrobacter xylanophilus DSM 9941	rxy:RXYL_0639	cobalt-factor II C20-methyltransferase / precorrin-2 C20-methyltransferase
Bacteroidetes ON OFF	*Parabacteroides distasonis* ATCC 8503	pdi:BDI_1016	putative precorrin-2 C20-methyltransferase

Figure 1. Ortholog cluster entry page displaying the orthologous group of the cobalamin biosynthetic gene, *cobI,* of the default cluster set. The page contains a cluster annotation table showing the gene name, title and cross-references to other databases, and a table showing the list of member genes.



ClusterID	Name	#species	#genes	Description	Phylogenetic pattern (Set species color)	
O2456 A M H P	cobH	127	138	Precorrin-8X methylmutase		0.0000000000
O2613 A M H P	cobI	127	129	Precorrin-2 C20-methyltransferase		0.0000000000
O2555 A M H P	cobJ	125	132	Precorrin-3B C17-methyltransferase		0.0062544875
O2490 A M H P	cobL	125	136	Precorrin-6Y C5,15-methyltransferase (decarboxylating)		0.0188426909
O2805 A M H P	cobL	114	118	Precorrin-6Y C5,15-methyltransferase (decarboxylating)		0.0464749664
O2827 A M H P	cbiG	109	117	Cobalamin biosynthesis protein CbiG/precorrin-methyltransferase		0.0551185608
O1903 A M H P	cobB	151	181	Cobyrinic acid a,c-diamide synthase		0.0740367133
O3218 A M H P	cbiD	100	101	Cobalt-precorrin-6A synthase		0.0819131488
O3259 A M H P	cbiG	96	99	Cobalamin biosynthesis protein CbiG/precorrin-methyltransferase		0.0937179783
O1976 A M H P	cobD	170	174	Cobalamin biosynthesis protein CobD		0.1136876675
O1935 A M H P	cobS	174	178	Cobalamin-5'-phosphate synthase		0.1287134427
O1943 A M H P	cobQ	169	177	Cobyric acid synthase		0.1296134005
O3628 A M H P	cobK	83	86	Cobalt-precorrin-6x reductase		0.1318674461
O2032 A M H P	cobN	93	175	CobN/magnesium chelatase subunit H		0.1638327001
O2852 A M H P	cobN	85	117	Cobaltochelatase subunit CobN		0.1680727097
O2339 A M H P	cobU	143	146	Biosynthesis protein: cobinamide kinase cobinamide phosphate guanylyltransferase		0.2127092031
O3888 A M H P	chlD	72	79	Magnesium-chelatase subunit		0.2236635828
O5971 A M H P	cbiN	44	45	Cobalt transport protein CbiN		0.2501823354
O2327 A M H P	cobT	131	147	Nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase		0.2617349306
O2466 A M H P	cbiM	100	138	Cobalt transport protein CbiM		0.2617881787
O2033 A M H P	cbiX	116	169	Cobalamin biosynthesis CbiX protein		0.2644324237
O1737 A M H P	cobO	165	198	Cob(I)yrinic acid a,c-diamide adenosyltransferase		0.2660497349

Figure 2. Ortholog cluster table containing ortholog clusters with phylogenetic patterns similar to those of the *cobI* orthologs shown in Figure 1. The table is ordered by correlation coefficient against the phylogenetic pattern of the *cobI* ortholog group. The phylogenetic patterns are graphically represented by green bars indicating 'present'. The value shown in the rightmost column is a dissimilarity value, $d=(1-r)/2$, calculated from the correlation coefficient, r.

according to the dissimilarity in phylogenetic pattern between each cluster and the original cluster (Figure 2), where the dissimilarities are calculated based on a correlation coefficient, the hamming distance or mutual information (9). This function is useful for predicting functional linkages (10) and similar functions are implemented in some more specialized databases (11,12). In MBGD, users can combine this type of analysis with more flexible ortholog analysis.

ORGANISM SELECTION BASED ON PHENOTYPIC PROPERTIES

The utilization of information on the phenotypic properties of individual organisms is becoming more important for comparative genomics studies as the number of genomes increases. The current version of MBGD provides an interface for specifying a set of organisms to be analyzed using phenotypic information as well as taxonomic information as a reference, where the phenotypic properties are taken from the organism metadata collection in the GOLD database (13) that includes cell shape, motility, oxygen requirements, temperature range and so on (Figure 3). Users can also use a similar interface to specify a phylogenetic pattern in order to search for orthologous groups having similar phylogenetic patterns or specify species colors to interpret the phylogenetic patterns displayed in the header of the ortholog table (Figure 2).

ADDING MORE EUKARYOTIC GENOMES

MBGD is periodically updated using the complete genome data in the RefSeq database as a data source. Although previous MBGD versions mainly contained prokaryotic genomes (except *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* for reference purposes), we have now added more complete genome sequences of eukaryotic microbes belonging to the fungi and protists such as *Plasmodium falciparum*, *Dictyostelium discoideum*, *Aspergillus nidulans* and *Candida glabrata*. In addition, the complete genome sequences of some higher eukaryotes, including *Caenorhabditis elegans*, *Drosophila melanogaster*, *Arabidopsis thaliana* and *Homo sapiens*, have also been added as a reference. To incorporate the eukaryotic genomic data, we have modified our genome map viewer as well as the database schema to correctly display eukaryotic genomes where the genes are interrupted by introns.

ENHANCEMENT OF THE MyMBGD FUNCTIONALITY

The MyMBGD functionality, which allows users to add their own genome sequences to MBGD, has been enhanced: we now provide the 'gene prediction mode', in which users can submit genomic nucleotide sequences without any annotation and ask the system to predict the genes within them. The gene-finding procedure implemented here uses both the GeneMarkS program (14) and the Glimmer3 program (15) and merges their

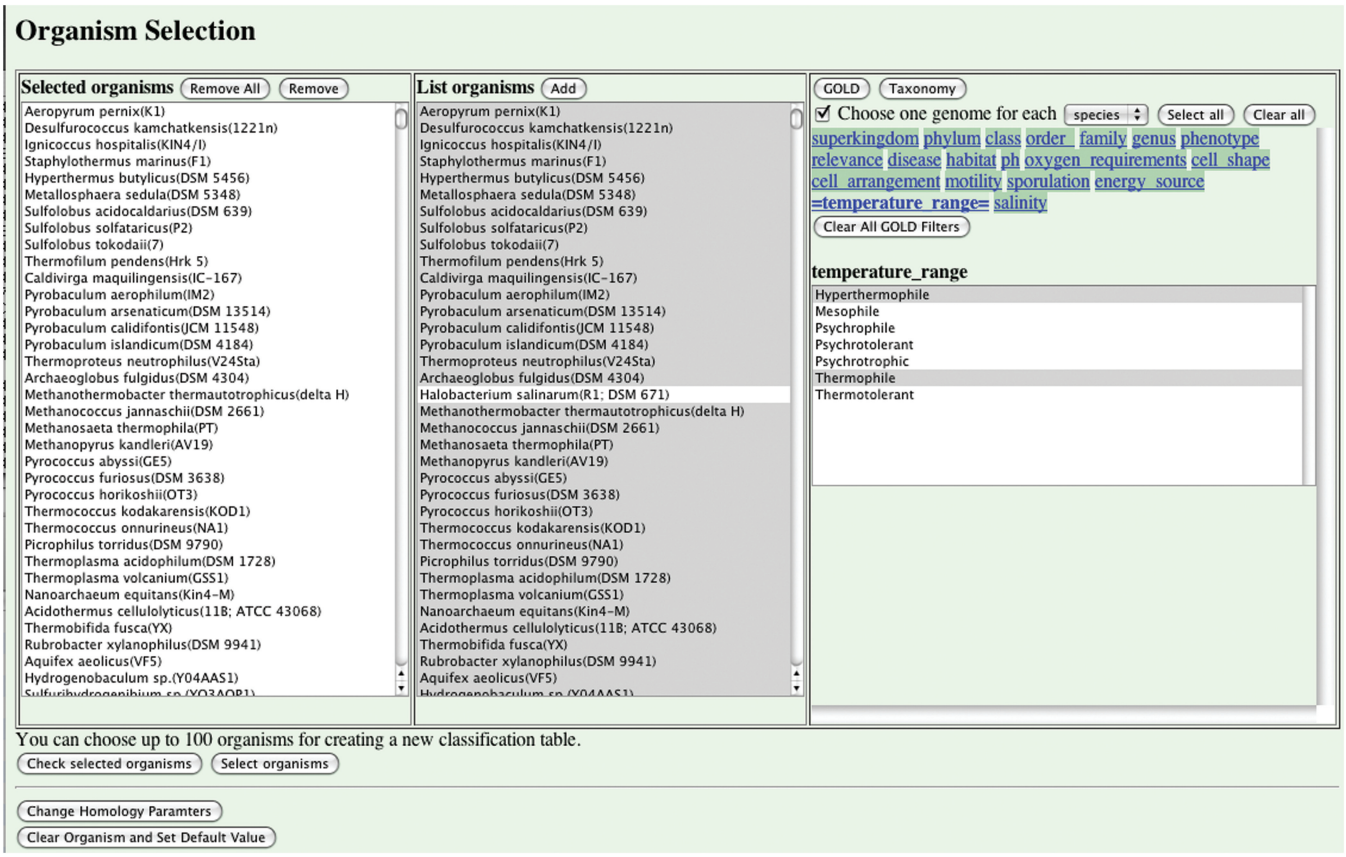


Figure 3. The interface for organism selection. In the right panel, users can specify the conditions on the organism properties taken from the GOLD database to filter the set of organisms. In this example, the condition on the temperature range is specified as 'Either hyperthemophile or themophile', and the organisms satisfying this condition are listed in the middle panel. Upon adding another condition on taxonomy ('Choose one genome for each species' at the top of the right panel), a further selection occurs, and the selected organisms are highlighted in the middle panel and added to the box in the left panel.

outputs by taking the longer region when two programs predict different start positions in the same reading frame. The predicted genes are then subjected to the DomClust procedure (3) after an all-against-all similarity search, which is the usual MyMBGD procedure described previously (2). MyMBGD also provides the 'metagenome mode', which accepts a set of nucleotide or protein sequences from a mixture of genomes and applies an ortholog assignment procedure similar to that for extending the default ortholog table described above, except for omitting the secondary condition for testing the bidirectional best hit. With this enhancement, users can now use the MyMBGD functionality as a tool to annotate a newly sequenced genome or new metagenome data.

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