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GenoMycDB: a database for comparative analysis of mycobacterial genes and genomes

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ABSTRACT. Several databases and computational tools have been created with the aim of organizing, integrating and analyzing the wealth of information generated by large-scale sequencing projects of mycobacterial genomes and those of other organisms. However, with very few exceptions, these databases and tools do not allow for massive and/or dynamic comparison of these data. GenoMycDB (http://www.dbbm.fiocruz.br/GenoMycDB) is a relational database built for large-scale comparative analyses of completely sequenced mycobacterial genomes, based on their predicted protein content. Its central structure is composed of the results obtained after pair-wise sequence alignments among all the predicted proteins coded by the genomes of six mycobacteria: *Mycobacterium tuberculosis* (strains H37Rv and CDC1551), *M. bovis* AF2122/97, *M. avium subsp. paratuberculosis* K10, *M. leprae* TN, and *M. smegmatis* MC2 155. The database stores the computed similarity parameters of every aligned pair, providing for each protein sequence the predicted subcellular localization, the assigned cluster of orthologous groups, the features of the corresponding gene, and links to several important databases. Tables containing pairs or groups of potential homologs between selected species/strains can be produced dynamically by user-defined criteria, based on one or multiple sequence similarity parameters. In addition, searches can be restricted according to the predicted subcellular localization of the protein, the DNA strand of the corresponding gene and/or the description of the protein. Massive data search and/or retrieval are available, and different ways of exporting the result are offered. GenoMycDB provides an on-line resource for the functional classification of mycobacterial proteins as well as for the analysis of genome structure, organization, and evolution.

Key words: Mycobacteria, Genome evolution, Perl programming, Functional classification, FASTA, MySQL

INTRODUCTION

Complete genome sequences are a unique source of data, because together with the epigenetic networks and through their interaction with such networks they represent in principle all the necessary information to make an organism. However, it is not immediately obvious what we can do with all this information. For instance, it is believed that the comprehensive analysis of entire genomes has the potential to provide a complete understanding of the genetics, biochemistry, physiology, and pathogenesis of microorganisms (Brosch et al., 2001). In contrast, it is argued that such potential can only be realized by the comparative study of genomes, syntenic regions or genes of two or more species, subspecies or strains, because a genome considered alone, without the phylogenetic framework of the evolutionary process, merely provides an incomplete understanding of those issues (Clark, 1999).

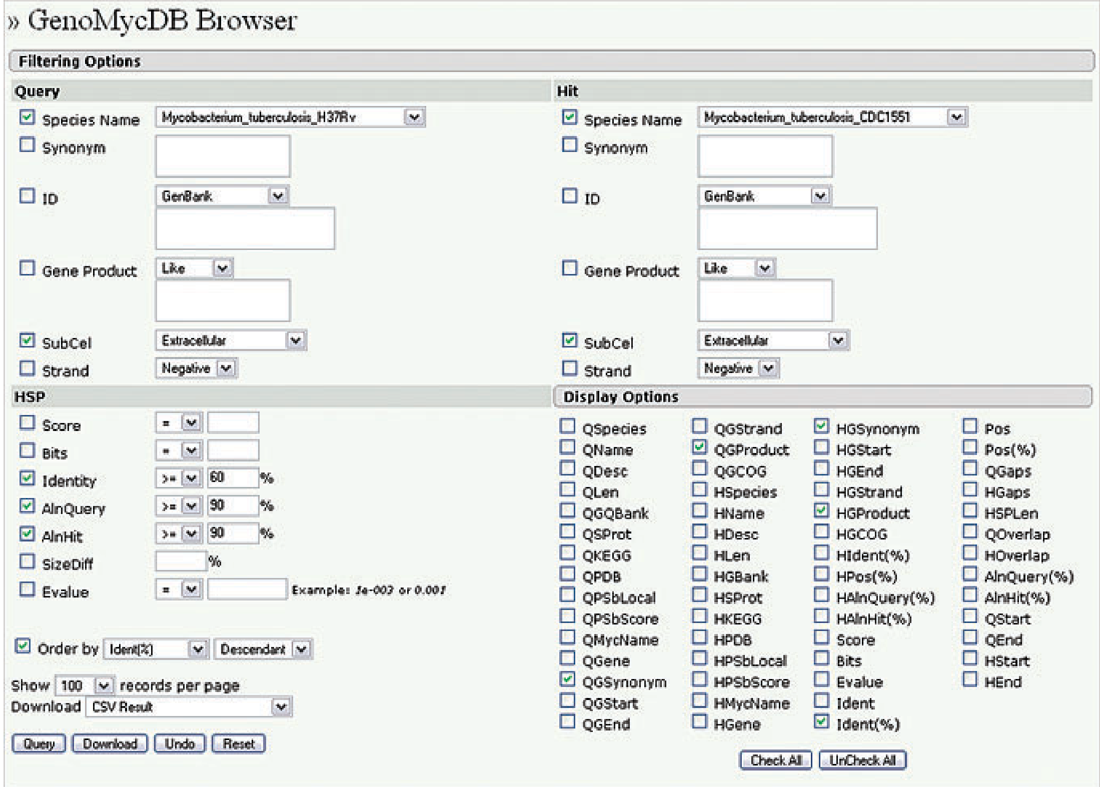
In the case of pathogenic microorganisms, especially mycobacteria, numerous potential applications of comparative genome analysis have been reported, aimed particularly at the prevention, treatment, and diagnosis of tuberculosis and other mycobacterial diseases, including i) metabolic reconstruction and identification of unique genes and virulence factors (Gordon et al., 2002), ii) characterization of pathogens and identification of new diagnostic and therapeutic targets (Fitzgerald and Musser, 2001), iii) investigation of the molecular basis of differences in pathogenesis, host range and phenotypes between clinical isolates and natural populations of pathogens (Behr et al., 1999; Brosch et al., 2001; Kato-Maeda et al., 2001; Cole, 2002), and iv) investigation of the genetic basis of virulence and drug resistance in tuberculosis-causing bacteria (Randhawa and Bishai, 2002).

With the aim of providing an on-line resource for the functional classification of mycobacterial proteins as well as for the analysis of the genome structure, organization and evolution in such species, we developed GenoMycDB, a relational database for large-scale comparative analyses of completely sequenced mycobacterial genomes based on their predicted protein content. This system presents many important advantages over similar databases, such as flexibility, scalability and cross-referencing.

MATERIAL AND METHODS

Currently, GenoMycDB comprises the result obtained with pair-wise sequence alignments among all predicted proteins coded by the genomes of five pathogenic mycobacteria and one opportunist, respectively: *Mycobacterium tuberculosis* (strains H37Rv and CDC1551) - the causative agent of human tuberculosis; *M. bovis* (strain AF2122/97) - the etiological agent of tuberculosis in cattle and many other mammals, including humans; *M. avium subsp. paratuberculosis* (strain K10) - the etiological agent of paratuberculosis in ruminant animals, also implicated as the etiological agent of Crohn’s disease in humans; *M. leprae* (strain TN) - the causative agent of leprosy, and *M. smegmatis* (strain MC2 155) - a saprophyte, usually non-pathogenic. The database stores the computed similarity parameters of every aligned pair, providing for each protein sequence the predicted subcellular localization, the assigned COG(s) (cluster of orthologous groups), the description of the corresponding gene, and links to several important databases: GenBank (Benson et al., 2005), SwissProt/TrEMBL (Boeckmann et al., 2003), PDB (Berman et al., 2000), KEGG (Kanehisa, 1997; Kanehisa and Goto, 2000), and 2D-PAGE at the Max Planck Institute for Infection Biology (Pleissner et al., 2004).

GenoMycDB was implemented in MySQL, version 4.0.24 (http://www.mysql.com/), a high-performance but relatively simple database management system, freely available for most in-house uses (Dubois, 2000), and its graphical CGI interface, GenoMycDB Browser, was programmed in Perl, version 5.8.4 (http://www.perl.org/; Figure 1).


» GenoMycDB Browser

Filtering Options

Query

☑ Species Name: Mycobacterium_tuberculosis_H37Rv
☐ Synonym
☐ ID: GenBank
☐ Gene Product: Like
☑ SubCel: Extracellular
☐ Strand: Negative

Hit

☑ Species Name: Mycobacterium_tuberculosis_CDC1551
☐ Synonym
☐ ID: GenBank
☐ Gene Product: Like
☑ SubCel: Extracellular
☐ Strand: Negative

HSP

☐ Score =
☐ Bits =
☑ Identity >= 60 %
☑ AlnQuery >= 90 %
☑ AlnHit >= 90 %
☐ SizeDiff %
☐ Evalue = Example: 1e-003 or 0.001

☑ Order by Ident(%) Descendant

Show 100 records per page
Download CSV Result

Display Options

☐ QSpecies
☐ QName
☐ QDesc
☐ QLen
☐ QGQBank
☐ QSProt
☐ QKEGG
☐ QPDB
☐ QPSbLocal
☐ QPSbScore
☐ QMycName
☐ QGene
☑ QGSynonym
☐ QGStart
☐ QGEnd
☐ QGStrand
☑ QGProduct
☐ QGCOG
☐ HSpecies
☐ HName
☐ HDesc
☐ HLen
☐ HGBank
☐ HSProt
☐ HKEGG
☐ HPDB
☐ HPSbLocal
☐ HPSbScore
☐ HMycName
☐ HGene
☑ HGSynonym
☐ HGStart
☐ HGEnd
☐ HGStrand
☑ HGProduct
☐ HGCOG
☐ HIdent(%)
☐ HPos(%)
☐ HAlnQuery(%)
☐ HAlnHit(%)
☐ Score
☐ Bits
☐ Evalue
☐ Ident
☑ Ident(%)
☐ Pos
☐ Pos(%)
☐ QGaps
☐ HGaps
☐ HSPLen
☐ QOverlap
☐ HOverlap
☐ AlnQuery(%)
☐ AlnHit(%)
☐ QStart
☐ QEnd
☐ HStart
☐ HEnd

Figure 1. Overview of the GenoMycDB CGI interface, showing the available options for searching and displaying.

The predicted protein sequences coded by the genomes of the aforementioned mycobacteria and the features of their corresponding genes were obtained from the Reference Sequence (RefSeq) database (http://www.ncbi.nlm.nih.gov/RefSeq/) (Pruitt et al., 2000, 2005; Pruitt and Maglott, 2001) at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and, exclusively for *M. smegmatis* MC2 155, from the Comprehensive Microbial Resource database (http://cmr.tigr.org/tigr-scripts/CMR/CmrHomePage.cgi) (Peterson et al., 2001) at the Institute for Genomic Research (http://www.tigr.org/).

The compiled protein data set (24,835 sequences) was submitted to three different analyses, providing most of the GenoMycDB data source (Figure 2): i) an all against all sequence comparison using the FASTA similarity search program (Pearson and Lipman, 1988; Pearson, 1990) version 3.4t21 (ftp://ftp.virginia.edu/pub/fasta/), with the program default parameters (ktup = 2, optimized score = 16, gap opening penalty = -10, gap extension penalty = -2, matrix = BLOSUM50, filter = 0, e-value cutoff = 10); ii) the computational prediction of the subcellular localization of the proteins using the PSORTb program (Gardy et al., 2003, 2005), version 2.0.2 (http://www.psort.org/downloads/index.html), employing the model built for Gram-positive bacteria, and iii) the assignment of the proteins to COG(s) using the COGNITOR program (Tatusov et al., 2000) (xugnitor.c - ftp://ftp.ncbi.nih.gov/pub/COG/old/util/), making use of a previously described method for the classification of new sequences in pre-existing COG(s) (Tatusov et al., 1997, 2000).

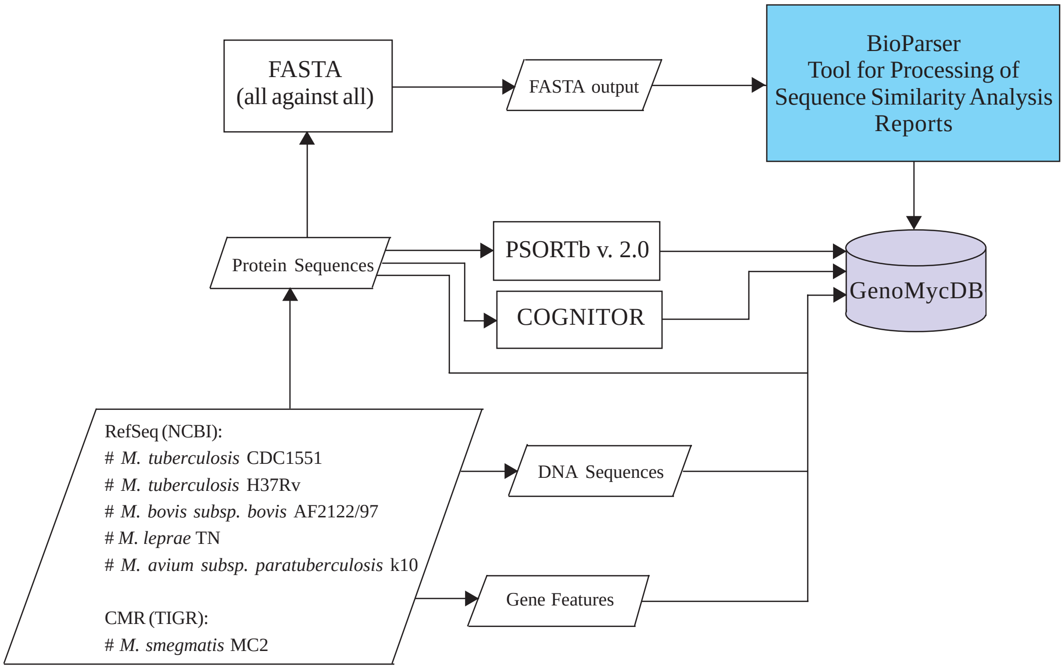


Figure 2. Flow diagram depicting the steps involved in the development of GenoMycDB.

FASTA was chosen to perform the sequence comparison because it is faster than implementations of the Smith-Waterman algorithm (Smith and Waterman, 1981), thus guaranteeing the finding of a mathematically optimal (highest scoring) solution, exhibiting almost the same sensitivity by default. The number of alignments achieved with such a comparison was exactly 1,452,022, excluding self-comparisons.

The results of the PSORTb and COGNITOR analyses are summarized in Table 1. Overall, 13,514 proteins of our dataset were assigned to pre-existing COGs. For each genome, approximately 64-74% of the predicted proteome could be assigned to COGs, except for *M. smegmatis*, for which only 13.1% of the total predicted proteins could be attributed to COGs. Since the genome annotation of this opportunist is still in progress (http://www.tigr.org/tdb/mdb/mdbinprogress.html), it is possible that the low fraction of proteins assigned to COGs is due to open reading frame prediction errors (such as frame shift) in the annotation process. The subcellular localization prediction also showed variations among these species. The most significant variations occurred in the fraction of proteins predicted to be extracellular. *M. avium* and *M. leprae* exhibited the lowest fractions (1.56 and 1.93%, respectively), followed by *M. smegmatis* (2.5%), and by *M. tuberculosis* H37Rv and *M. bovis* (approximately 3.5% for both). The *M. tuberculosis* CDC1551 strain gave the highest fraction of predicted extracellular proteins (5.06%), approximately 1.5% more than in the *M. tuberculosis* H37Rv strain genome.

Table 1. Summary of the COG (cluster of orthologous groups) assignment and subcellular localization prediction of the 24,835 proteins comprising the GenoMycDB data source.

Species	Number of proteins	Assigned COGs	Cellular wall	Cytoplasm	Cytoplasm membrane	Extracellular	Unknown
M. avium subsp. paratuberculosis K10	4350	3230 (74.2%)	11 (0.25%)	2399 (55.15%)	663 (15.24%)	68 (1.56%)	1209 (27.79%)
M. bovis AF2122/97	3920	2738 (69.8%)	8 (0.20%)	2065 (52.68%)	593 (15.13%)	136 (3.47%)	1118 (28.52%)
M. leprae TN	1605	1186 (73.9%)	-	853 (53.15%)	250 (15.58%)	31 (1.93%)	471 (29.35%)
M. smegmatis MC2 155	6844	899 (13.1%)	20 (0.29%)	3842 (56.14%)	1096 (16.01%)	171 (2.50%)	1715 (25.06%)
M. tuberculosis CDC1551	4189	2687 (64.1%)	9 (0.21%)	2093 (49.96%)	587 (14.01%)	212 (5.06%)	1288 (30.75%)
M. tuberculosis H37Rv	3927	2774 (70.6%)	8 (0.20%)	2086 (53.12%)	598 (15.23%)	135 (3.43%)	1100 (28.01%)

The FASTA output file was analyzed with the BioParser program (Catanho et al., 2006) (http://www.dbbm.fiocruz.br/BioParser.html), a tool designed for the processing of sequence similarity analysis reports; the results were parsed and automatically stored in a local MySQL database, comprising the central structure of the GenoMycDB: tables bp_query, bp_hit and bp_hsp (Figures 2 and 3).

The proposed structure is simple and intuitive; for each aligned pair present in the sequence similarity report, the attributes related to the query and hit sequences are stored (without redundancy) in the bp_query and bp_hit tables, respectively. The attributes that characterize

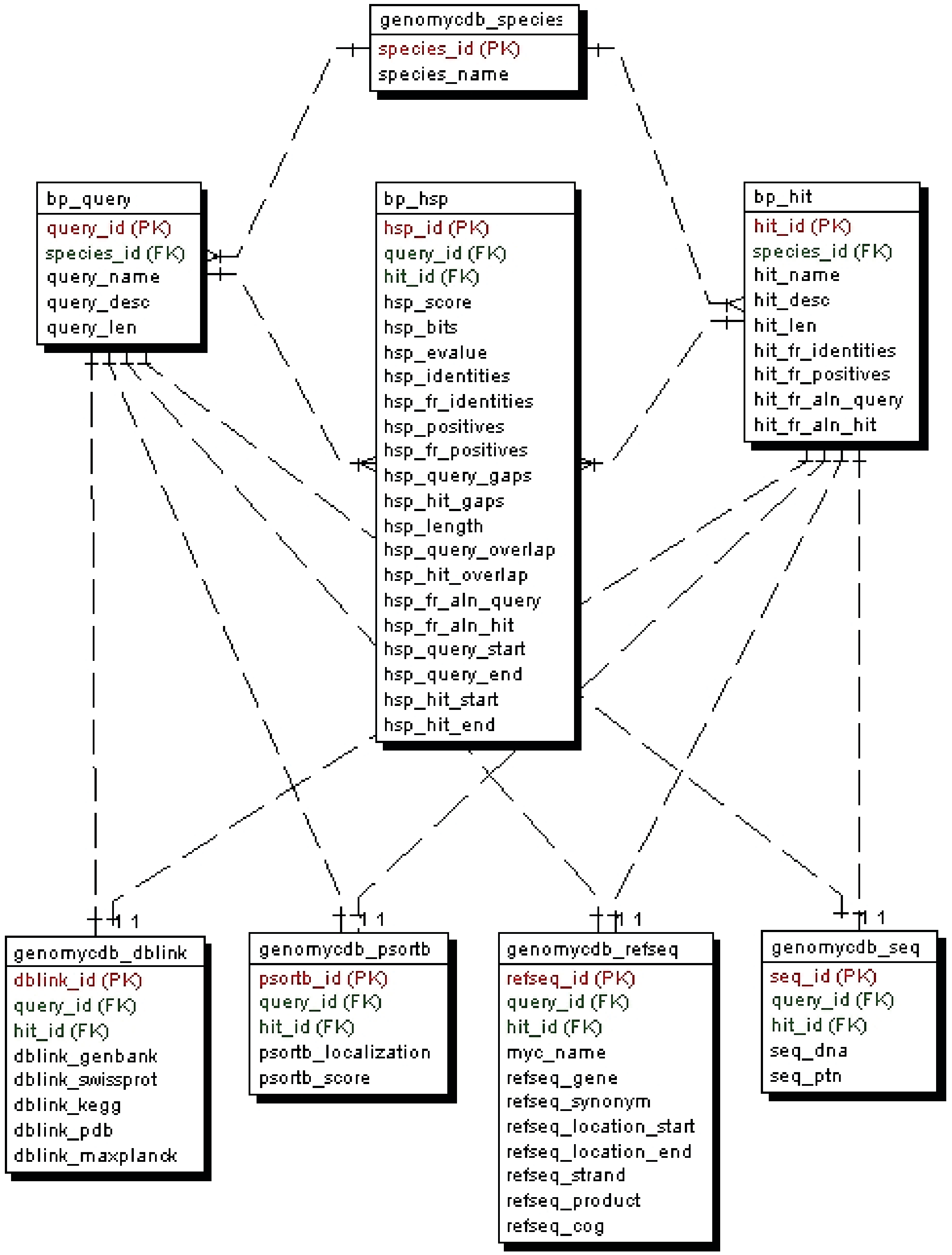


Figure 3. Entity-relationship diagram showing the relational structure of GenoMycDB. The entities and their relationships are described in the text. PK = primary key; FK = foreign key.

each alignment, otherwise known as HSP (high scoring pair), are stored in the bp_hsp table, which is linked to the query and hit tables by two foreign keys: *query_id* and *hit_id*, respectively (Catanho et al., 2006).

Five additional tables were included in GenoMycDB (Figures 2 and 3), containing the following data/information:

- genomycdb_species - comprises the scientific name of each species/strain represented in GenoMycDB (*species_name*);
- genomycdb_dblink - includes the identifying numbers of each mycobacterial protein sequence in the following databases: GenBank (*dblink_genbank*), SwissProt/TrEMBL (*dblink_swissprot*), PDB (*dblink_pdb*), KEGG (*dblink_kegg*), and 2D-PAGE at the Max Planck Institute for Infection Biology (*dblink_maxplanck*);
- genomycdb_psortb - consists of the predicted subcellular localization of each protein (*psortb_localization*) and the score obtained in the prediction analysis (*psortb_score*);
- genomycdb_refseq - provides for each mycobacterial protein: the GenoMycDB derivative name (the species name followed by a sequential number representing the relative position in the genome of the corresponding gene from the origin of replication) (*myc_name*), the name of the corresponding gene (*refseq_gene*), the synonym of the gene (*refseq_synonym*), the localization of the gene in the genome (*refseq_location_start*, *refseq_location_end*, and *refseq_strand*), the protein description (*refseq_product*), and the assigned COG(s) (*refseq_cog*);
- genomycdb_seq - provides the protein (*seq_ptn*), and DNA (*seq_dna*) sequence of each mycobacterial protein.

All these five tables are linked to the bp_query and bp_hit tables by the *query_id* and *hit_id* foreign keys, respectively. The bp_query and bp_hit tables are linked to the genomycdb_species table by the *species_id* foreign key (Figure 3).

RESULTS

GenoMycDB was designed for large-scale comparative analysis, offering a variety of searching/retrieving methods (Figures 1 and 3). The selection of aligned pairs with specific attributes can be done i) based on one or multiple alignment parameters (section *Filtering Options*, sub-section *HSP*) - raw score (*Score*); bit score (*Bits*); fraction of identical positions for a given HSP (*Identity%*); fraction of the query and/or hit sequence that has been aligned within a given HSP (*AlnQuery%* and *AlnHit%*, respectively); difference in length, expressed as a fraction, between the query and hit sequences (*SizeDiff*), and number of alignments expected by chance (*Evalue*) - and/or ii) based on one or multiple features characterizing one or both sequences of the aligned pair (section *Filtering Options*, sub-sections *Query* and *Hit*) - species name (*Species Name*); synonym of the corresponding gene(s) (*Synonym*); identifying number(s) of the protein(s) in the GenBank, KEGG, PDB or SwissProt/TrEMBL database (*Id*); presence or absence of a given key word in the protein description (*Gene Product*); predicted subcellular localization of the protein (*SubCel*), and DNA strand where the corresponding gene is located (*Strand*). Users can conveniently choose one field or a combination of fields to formulate the search, taking into account that a logical *AND* connects all these fields to each other. The *Display Options* section exhibits all available attributes that can be selected to compose the result (Table 2).

Table 2. Summary of the attributes available for displaying (as appears in the *Display Options* section of GenoMycDB Browser), with their corresponding description.

Display Option	Description
QSpecies	Query species
QName	Query name
QDesc	Query description
QLen	Query length
QGQBank	GenBank identifying number of the query sequence
QSProt	SwissProt/TrEMBL identifying number of the query sequence
QKEGG	KEGG identifying number of the query sequence
QPDB	PDB identifying number of the query sequence
QPSbLocal	PSORTb subcellular prediction of the query sequence
QPSbScore	PSORTb subcellular prediction score of the query sequence
QMycName	GenoMycDB derivative name of the query sequence
QGene	Name of the query sequence gene
QGSynonym	Synonym of the query sequence gene
QGStart	Start position of the query sequence gene in the genome
QGEnd	End position of the query sequence gene in the genome
QGStrand	DNA strand where the query sequence gene is located
QGProduct	Description of the query protein sequence
QGCOG	Protein query sequence assigned COG(s)
HSpecies	Hit species
HName	Hit name
HDesc	Hit description
HLen	Hit length
HGBank	GenBank identifying number of the hit sequence
HSProt	SwissProt/TrEMBL identifying number of the hit sequence
HKEGG	KEGG identifying number of the hit sequence
HPDB	PDB identifying number of the hit sequence
HPSbLocal	PSORTb subcellular prediction of the hit sequence
HPSbScore	PSORTb subcellular prediction score of the hit sequence
HMycName	GenoMycDB derivative name of the hit sequence
HGene	Name of the hit sequence gene
HGSynonym	Synonym of the hit sequence gene
HGStart	Start position of the hit sequence gene in the genome
HGEnd	End position of the hit sequence gene in the genome
HGStrand	DNA strand where the hit sequence gene is located
HGProduct	Description of the hit protein sequence
HGCOG	Protein hit sequence assigned COG(s)
HIdent(%)	Overall fraction of identical positions across all HSPs (aligned regions only)
HPos(%)	Overall fraction of conserved positions across all HSPs (aligned regions only)
HAlnQuery(%)	Fraction of the query sequence which has been aligned across all HSPs (not including intervals between non-overlapping HSPs)
HAlnHit(%)	Fraction of the hit sequence which has been aligned across all HSPs (not including intervals between non-overlapping HSPs)
Score	Raw score
Bits	Bit score
E-value	Expect value for the HSP (e-value)

Continued on next page

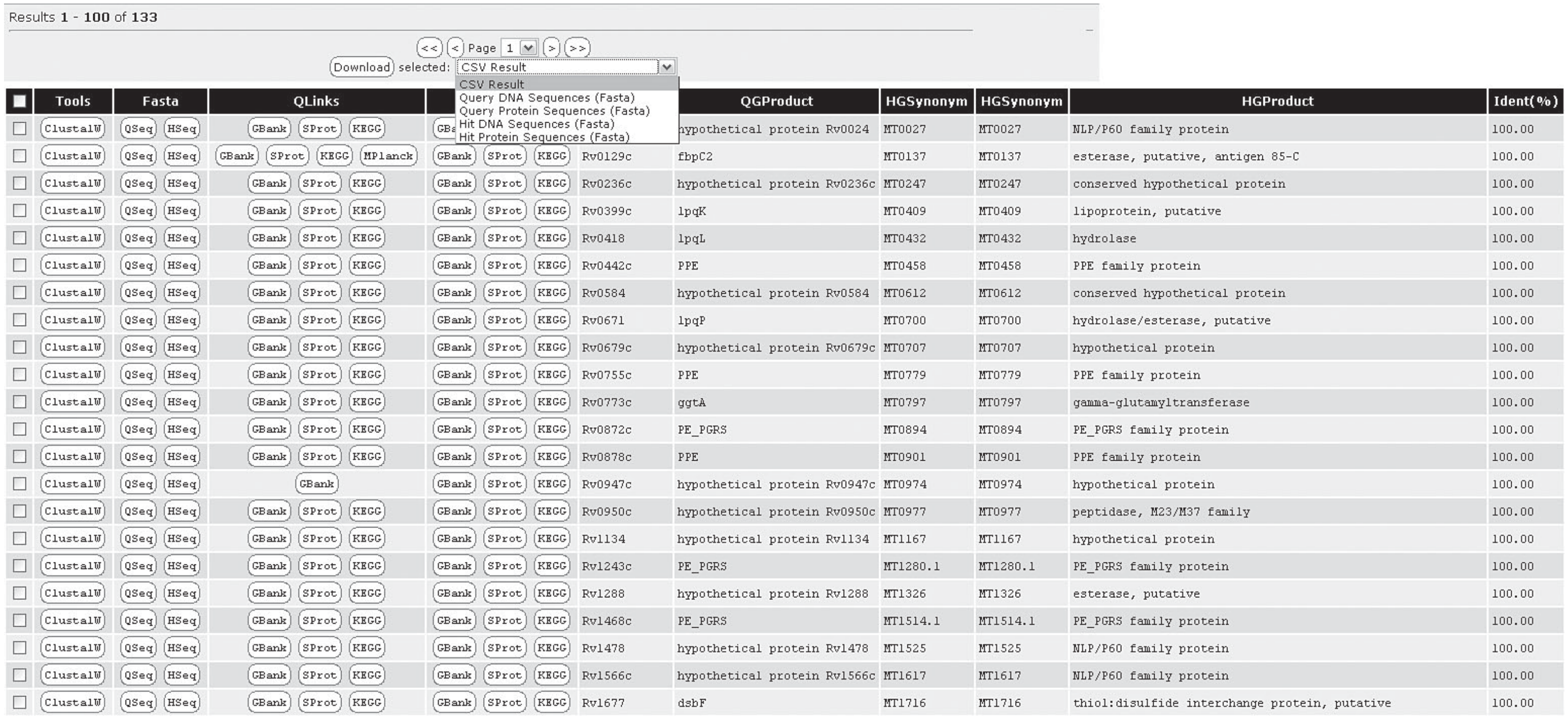
Table 2. Continued.

Display Option	Description
Ident	Number of identical residues
Ident(%)	Fraction of identical positions for a given HSP
Pos	Number of conserved residues
Pos(%)	Fraction of conserved positions for a given HSP
QGaps	Number of gaps in the query alignment
HGaps	Number of gaps in the hit alignment
HSPLen	Length of HSP (full length of the alignment)
QOverlap	Length of query participating in alignment minus gaps
HOverlap	Length of hit participating in alignment minus gaps
AlnQuery(%)	Fraction of the query sequence which has been aligned within a given HSP
AlnHit(%)	Fraction of the hit sequence which has been aligned within a given HSP
QStart	Query start position from the alignment
QEnd	Query end position from the alignment
HStart	Hit start position from the alignment
HEnd	Hit end position from the alignment

COG(s) = cluster of orthologous groups; HSP = high scoring pair.

The result of each search is displayed as a table, in which each line corresponds to a particular alignment, and each column represents a sequence or an alignment attribute (Figure 4). The first columns, namely, *Tools*, *Fasta*, *QLinks*, and *HLinks*, offer different means to analyze a selected sequence or pair of sequences individually; it is possible to execute a global alignment between the sequences using the CLUSTAL W program (Thompson et al., 1994) (http://www.ebi.ac.uk/clustalw/), at both levels: protein and DNA (*ClustalW*); in addition, one can visualize the sequence(s) in the FASTA format (*QSeq* and *HSeq*), or access the page(s) of the sequence(s) in other database(s) (*GBank*, *SProt*, *KEGG*, *PDB*, or *MPlanck*). There are two different ways to export the result: i) save the selected records displayed in the browser or all records returned in a table format flat file, choosing the *CVS Result* option in the *Download* drop-down button of the page containing the result (Figure 4) or in the similar button of the GenoMycDB Browser main page (Figure 1), respectively, and ii) save the sequences (DNA or protein) of the selected pairs or the whole sequence set (DNA or protein) corresponding to all records returned in a FASTA format flat file, choosing the appropriate option (*Query DNA Sequences*, *Query Protein Sequences*, *Hit DNA Sequences*, or *Hit Protein Sequences*) in the same *Download* drop-down buttons and pages.

In summary, GenoMycDB provides an on-line resource for large-scale comparative analysis of completely sequenced mycobacterial genomes based on their predicted protein content. Through the GenoMycDB Browser, users can dynamically select pairs or groups of potential homologs between selected species/strains based on different aspects of similarity between the aligned sequences and/or on particular features characterizing one or both sequences of the aligned pair. One or multiple alignment parameters can be defined to establish a reliable cutoff of similarity to infer homology. Links to several important databases are dynamically produced for each record in the customized searching result, expanding and facilitating the analysis of the data. Sequences (both protein and DNA) of individually selected records can be globally aligned,



Results **1 - 100** of **133**

<< < Page 1 > >>

Download selected: CSV Result

CSV Result
Query DNA Sequences (Fasta)
Query Protein Sequences (Fasta)
Hit DNA Sequences (Fasta)
Hit Protein Sequences (Fasta)

■	Tools	Fasta	QLinks			QGProduct	HGSynonym	HGSynonym	HGProduct	Ident(%)
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBa		hypothetical protein Rv0024	MT0027	MT0027	NLP/P60 family protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG MPlanck	GBank SProt KEGG	Rv0129c	fbpC2	MT0137	MT0137	esterase, putative, antigen 85-C	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0236c	hypothetical protein Rv0236c	MT0247	MT0247	conserved hypothetical protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0399c	lpqK	MT0409	MT0409	lipoprotein, putative	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0418	lpqL	MT0432	MT0432	hydrolase	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0442c	PPE	MT0458	MT0458	PPE family protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0584	hypothetical protein Rv0584	MT0612	MT0612	conserved hypothetical protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0671	lpqP	MT0700	MT0700	hydrolase/esterase, putative	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0679c	hypothetical protein Rv0679c	MT0707	MT0707	hypothetical protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0755c	PPE	MT0779	MT0779	PPE family protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0773c	ggtA	MT0797	MT0797	gamma-glutamyltransferase	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0872c	PE_PGRS	MT0894	MT0894	PE_PGRS family protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0878c	PPE	MT0901	MT0901	PPE family protein	100.00
☐	ClustalW	QSeq HSeq	GBank	GBank SProt KEGG	Rv0947c	hypothetical protein Rv0947c	MT0974	MT0974	hypothetical protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv0950c	hypothetical protein Rv0950c	MT0977	MT0977	peptidase, M23/M37 family	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv1134	hypothetical protein Rv1134	MT1167	MT1167	hypothetical protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv1243c	PE_PGRS	MT1280.1	MT1280.1	PE_PGRS family protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv1288	hypothetical protein Rv1288	MT1326	MT1326	esterase, putative	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv1468c	PE_PGRS	MT1514.1	MT1514.1	PE_PGRS family protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv1478	hypothetical protein Rv1478	MT1525	MT1525	NLP/P60 family protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv1566c	hypothetical protein Rv1566c	MT1617	MT1617	NLP/P60 family protein	100.00
☐	ClustalW	QSeq HSeq	GBank SProt KEGG	GBank SProt KEGG	Rv1677	dsbF	MT1716	MT1716	thiol:disulfide interchange protein, putative	100.00

Figure 4. Overview of a result returned by querying GenoMycDB, showing the attributes and values returned and the available exporting options (*Download selected*). In this example, the search was performed based on the following criteria: "display the records between *Mycobacterium tuberculosis* H37Rv and *M. tuberculosis* CDC1551 in which the fraction of identical positions in the HSP is equal to or greater than 60% *AND* the fraction of the query sequence that has been aligned within the HSP is equal to or greater than 90% *AND* the fraction of the hit sequence that has been aligned within the same HSP is equal to or greater than 90% *AND* the predicted subcellular localization for both sequences (query and hit) is extracellular" (see the selected fields for this search in Figure 1). Only the first 22 records of a total of 133 are shown in descending order of the fraction of identical positions in the HSP.

allowing more detailed examination of the compared pair. Different ways of exporting and visualizing the results are offered, making it easier to process and analyze the information.

DISCUSSION

The application of comparative genomic methods for the study of pathogenic microorganisms has been successfully explored, especially in mycobacteria. Several databases and computational tools have been created, aiming to organize, integrate and analyze the wealth of information generated by large-scale sequencing projects of mycobacterial genomes and other organisms (http://genolist.pasteur.fr/; http://myco.bham.ac.uk/). However, with very few exceptions (Uchiyama, 2003; Choi et al., 2005), these databases and tools do not allow massive and/or dynamic comparisons of such data. Usually, searches in these databases are genome-guided, and comparisons between genomes/genes are either pre-computed or manually accomplished, since the provided datasets are not related to each other. In addition, the parameters employed to compare the data are commonly pre-defined, giving little or no freedom to the user. Some of them have outputs that are quite difficult to interpret, and inconsistent sequence annotation is another relevant problem.

As demonstrated in Results, GenoMycDB overcomes the aforementioned problems, offering a flexible, scalable, functional, cross-referenced, and user-friendly system for the comparative genomic analyses of representatives of the genus *Mycobacterium*. Furthermore, the same structure and database interface can easily be applied to other groups of genomes, extending the potential of our system.

In our laboratory, GenoMycDB is currently being used to study the nucleotide evolutionary rates among protein-coding regions of mycobacteria, to analyze point mutations and polymorphisms among selected protein-coding regions of *M. tuberculosis* complex species, and to investigate the factors shaping codon usage in mycobacteria. In addition, the database is presently being used to annotate the genome of BCG Moreau, a vaccine strain derived from *M. bovis* used to prevent tuberculosis in the Brazilian population; this bacterium is being sequenced in our laboratory (gap closure phase). Therefore, GenoMycDB provides a valuable tool for the comparative analyses of mycobacterial genomes, making it possible to identify evolutionary, structural, and functional relationships between proteins in such genomes.

Future developments include new search fields, logical operators, sequence analysis and visualization tools, new sequenced mycobacterial genomes, and additional sequence features.

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