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Ross D. King, Andreas Karwath, Amanda Clare, Luc Dephaspe

Institutions: Aberystwyth University, Katholieke Universiteit Leuven

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Genome Scale Prediction of Protein Functional Class from Sequence using Data Mining

Ross D. King[1,2], Andreas Karwath[1], Amanda Clare[1], Luc Dehaspe[3]

(1) Department of Computer Science, University of Wales, Aberystwyth, Penglais, Aberystwyth, Ceredigion, SY23 3DB, Wales, U.K.

(3) Department of Computer Science, Katholieke Universiteit Leuven, B-3001, Belgium

(2) Author to whom correspondence should be sent.

Abstract

The ability to predict protein function from amino acid sequence is a central research goal of molecular biology. Such a capability would greatly aid the biological interpretation of the genomic data and accelerate its medical exploitation. For the existing sequenced genomes function can be assigned to typically only between 40-60% of the genes[1-4]. The new science of functional genomics is dedicated to discovering the function of these genes, and to further detailing gene function[5-8]. Here we present a novel data-mining[9-10] approach to predicting protein functional class from sequence. We demonstrate the effectiveness of this approach on the tubercle bacillus[2] genome. Biologically interpretable rules are identified that can predict protein function even in the absence of identifiable sequence homology. These rules predict 65% of the genes with no assigned function in tubercle bacillus with an estimated accuracy of 60-80% (depending on the level of functional assignment). The rules give insight into the evolutionary history of the tubercle bacillus.

1. Introduction

The formation of a theory to explain a set of observations is central to science. Computer based methods to assist in this process are becoming increasingly important[11]. Such methods are especially needed in molecular biology, where there is an overwhelming flood of new data. Here we demonstrate the effectiveness of automatic scientific discovery on an important scientific problem. We successfully apply a novel data mining approach to the problem of predicting protein functional class from sequence.

To predict the biological functional class of proteins directly from amino acid sequence, what is abstractly required is a *discrimination function*[10] that maps sequence to biological function. The existing sequence homology recognition methods can be viewed as examples of such functions: methods based on direct sequence similarity[12-13] can be considered as nearest neighbour type functions[14] (in sequence space), and the more complicated homology recognition methods based on motifs/profiles[15] resemble case-based learning functions[16]. The creation of annotated databases of protein function has now opened up the possibility of automatically identifying more general forms of discrimination function using data mining[17].

2. Data

For analysis, we selected the tubercle bacillus *(Mycobacterium tuberculosis)* genome, probably the prokaryote genome of greatest medical importance. According to the World Health Organization (WHO), tuberculosis kills 2 million people each year. Their concern about the growing epidemic has led the WHO to declare tuberculosis a global emergency[18]. We used 3,924 genes[* 2] (over 4 million base pairs) with functional class assignments from the Sanger Centre[19]. (Note that there are errors in annotation of function[20], and this adds "noise" to the data mining process[10]). The assignments of function are organised in a strict hierarchy (tree), where each higher level in the tree is more general than the level below it, and the leaf nodes are the individual functions of proteins. A subsection of the function hierarchy is shown in figure 1.

[*] For readability reasons we used "gene" throughout the paper, knowing that "potential gene" or "open reading frame" (ORF) should be used.

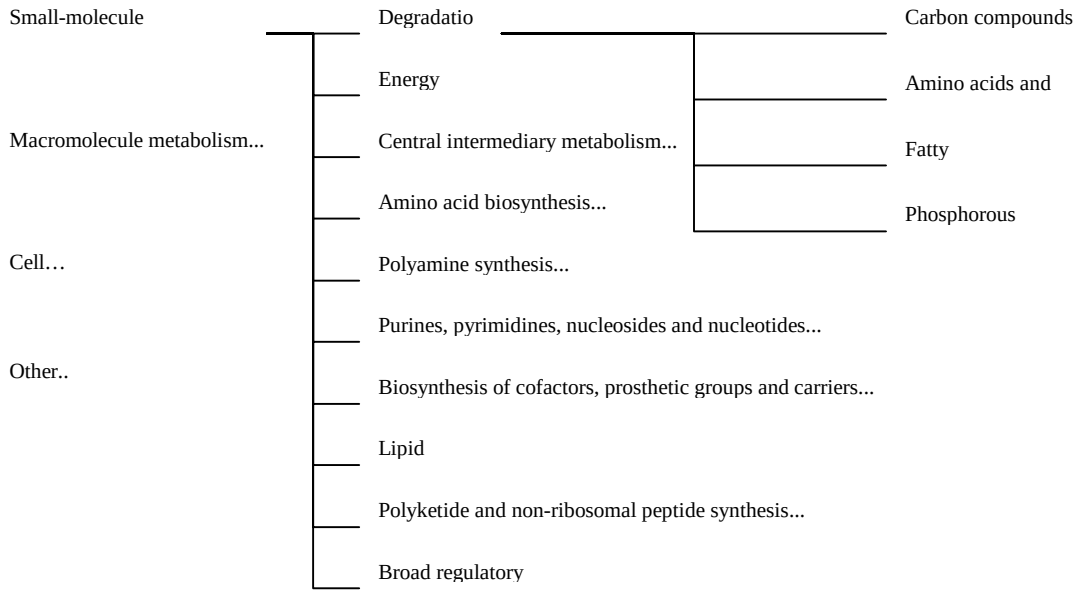

Figure 1 A subset of the genes functional hierarchy.

For example, a typical protein in the tubercle bacillus is L-fuculose phosphate aldolase (Rv0727c fucA), its top-level class assignment is “Small-molecule metabolism”, its second-level class is “Degradation”, and its third-level class is “Carbon compounds”. We attempted to learn discriminatory functions for every level of the functional hierarchy. Success on these different levels would demonstrate the generality of the approach.

To generate the database to mine we formed a single deductive database of genes and their known functional assignments. We then processed this data to form sequence descriptions of the genes. Therefore, these descriptions are solely based on features that can be computed from sequence alone. The most commonly used technique to gain information about a sequence is to run a sequence similarity search, and this was used as the starting point in forming descriptions. The basic data structure in the deductive database is the result of a PSI-BLAST search[13] (we used the parameters: e = 10, h = 0.0005, j = 20, NRProt 16/11/98). NRProt is a large protein sequence database collecting together protein sequences a variety of multi-genome protein databases. Using each gene, and each protein identified as having sequence similarity to it, we formed an expressive description based on: the frequency of

singlets and pairs of residues in the gene; the phylogeny of the organism from which each protein was obtained - from SWISS-PROT[21]; SWISS-PROT protein keywords (membrane, transmembrane, inner_membrane, outer_membrane, repeat, plasmid, and alternative_splicing); and the length and molecular weight of the gene. This description resembles a "phylogenic profile"[7], but is more general and expressive. In total 5,895,649 facts were generated. Table 1 shows the available types of data and their descriptions.

Type of data	Description
hom(A)	refers to a homologous protein (*A*) found by PSI-BLAST.
keyword(A, Word)	refers to a SwissProt keyword found in *A*.
classification(A, Class)	refers to the phylogenic classification of the organism *A* came from, taken from SwissProt.
species(A, Species)	refers to the species of *A*, taken from SwissProt.
mol_wt_rule(A, Weight)	refers to the molecular weight of *A*: 1 very low, 2 low, 3 medium, 4 high, and 5 very high.
amino_acid_ratio_rule(Residue, Weight)	refers to the percentage composition of the residue in the sequence.
e_val_rule(A, Weight)	refers to the PSI-Blast sequence similarity measure (note that a low value means a high sequence similarity).
e_val_gt e_val_lteq	refers to the PSI-Blast sequence similarity measure,greater than or less than/equal to a certain value
mol_wt_lteq(A, Weight) mol_wt_gt(A, Weight)	refers to the molecular weight of *A* being greater than or less than/equal to some value
amino_acid_pairs_wg	and others similar, refers to the number of pairs of these two amino acids, in this case tryptophan and glycine

amino_acid_pair_ratio_qh	and others similar, refers to the ratio of one amino acid to another in the gene, in this case the ratio of glutamine(q) to histidine(h). This ratio is not a percentage, not out of a hundred, instead it's a ratio out of a thousand. So for example 2.8 means 0.28%.
amino_acid_ratio_g	and others similar, refers to the percentage composition of the residue in the sequence of the gene, in this case the percentage of glycine
psi_iter_gt psi_iter_lteq	refers to the number of iterations of the PSI_BLAST search (greater than or less than/equal to some number)

Table 1 Database facts and their description. These facts are generated for each of the genes.

3. Data Mining method

We then mined this database to generate rules that predict protein functional class from sequence description. This was done using a combination of clustering and rule learning (see Figure 2).

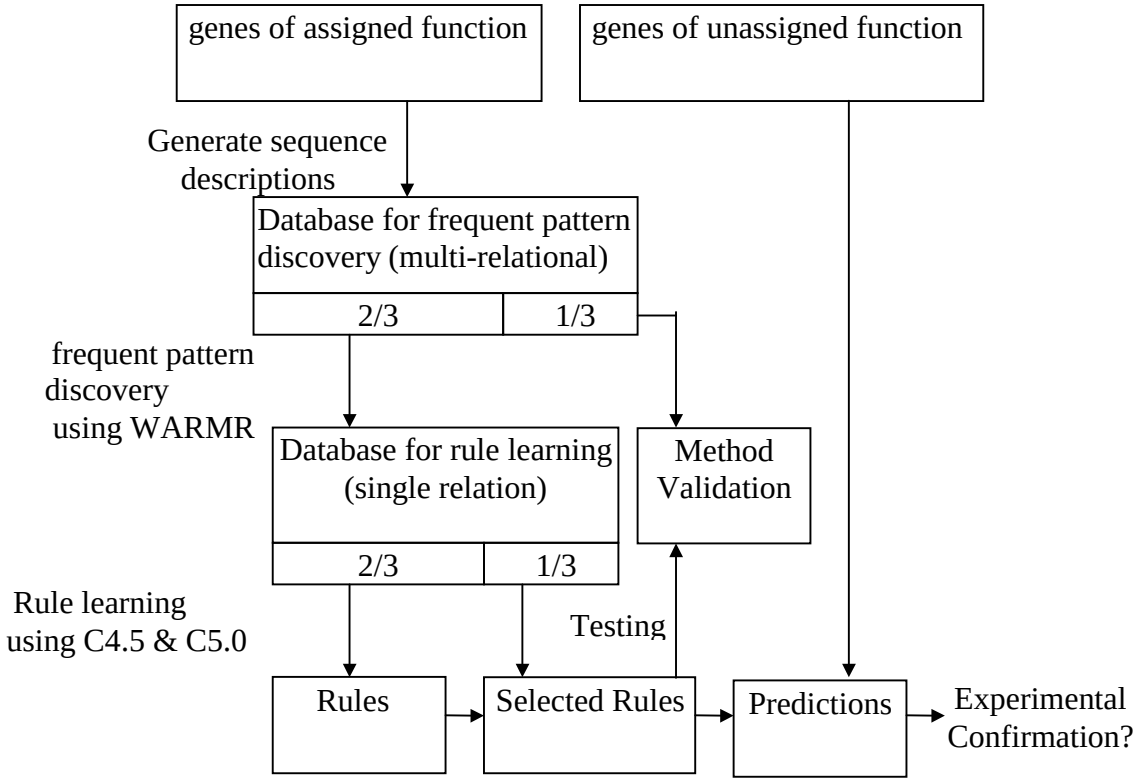

Figure 2 Flow chart of the data mining methodology. The genes of known functions were split randomly, one third to be held out for the final testing and two thirds to be used to generate prediction rules. The data used to generate the rules were in turn split randomly, two thirds to actually generate the rules and one third to be used as validation set for selecting good rules according to accuracy and coverage. After the selection of good rules, we tested their accuracy on the held-out test data, and also used them to predict biological function for the genes of currently unknown function (classified as "Unkown" or "Conserved Hypotheticals").

This hybrid approach has proved successful in the past on other scientific discovery tasks[22]. It is powerful because clustering improves the representation for learning (using the expressive power of inductive logic programming - ILP[23]), and discrimination efficiently exploits the pre-labeled examples. WARMR[22] is an ILP data mining algorithm that is used to identify frequent patterns (conjunctive queries) in the sequence descriptions. In this experiment roughly 18,000 frequent queries are discovered. These are converted into 18,000 Boolean attributes for rule learning, where an attribute gets value 1 for a specific gene if the corresponding query succeeds for that gene. Conversely, if the query fails, the corresponding attribute is assigned value 0.

The machine learning algorithms C4.5 and C5[24] were used to induce rules that predict function from the attributes. Good rules were selected on a validation set, and the unbiased accuracy of these rules estimated on a test set. Rules were selected to balance accuracy with unidentified gene coverage. In general the correct balance of accuracy and coverage for any particular application depends on the relative cost of making errors of commission and omission[25] (making incorrect predictions v missing genes). The system can be tuned to select different balances. The prediction rules were then applied to genes that have not been assigned a function.

4. Results

It was possible to find good rules that predict function from sequence at all levels of the functional hierarchies, as shown in Table 2. The number of rules found are those selected on the validation set. A rule predicts more than one homology class if there is more than one sequence similarity cluster in the correct test predictions. A rule predicts a new homology class if there is a sequence similarity cluster in the test predictions that has no members in the training data. Average test accuracy is the accuracy of the predictions on the test proteins of assigned function (if conflicts occur, the prediction with the highest *a priori* probability is chosen). Default test accuracy is the accuracy that could be achieved by always selecting the most populous class. "New functions assigned" is the number of genes of unassigned function predicted.

	Level 1	*Level 2*	*Level 3*	*Level 4*
Number of rules found	25	30	20	3
Rules predicting more than one homology class	19	18	8	1
Rules predicting a new homology class	14	15	1	0
Average test accuracy	62%	65%	62%	76%
Default test accuracy	48%	14%	6%	2%
New functions assigned	886 (58%)	507 (33%)	60 (4%)	19 (1%)

Table 2

The test accuracy of these rules is far higher than possible by chance. Of the genes originally in the "Conserved Hypothetical" or "Unknown" function classes, 985 (65%) were predicted to have a function at one or more levels of the hierarchy. The rule learning data, the rules, and the predictions, are given at: http://www.aber.ac.uk/~dcswww/Research/bio/ProteinFunction/.

We illustrate the value of the rules by describing rule TB_C50_1_26 shown in figure 3.

If the percentage composition of lysine in the gene is > 6.6%
Then its functional class is "Macromolecule metabolism"

Figure 3 Example rule found for the Macromolecule metabolism functional class.

This top-level rule is 85% (11/13) accurate on the test set (the probability of this result occurring by chance is estimated at 1.2×10^{-5} as the class Macromolecule metabolism covers ~25% of examples). The rule correctly predicts the following proteins (rpsG (S7), rpsI (S9), rpsL (S12), rpsT (S20), rplJ (L10), rplP (L16), rplS (L19), rplX (L24), rpmE (L31), rpmJ (L36), infC (IF-3)). These proteins are all involved in protein

translation. When the training data are included the rule covers 46 out of the 58 proteins known to be involved in ribosomal protein synthesis and modification. The two errors (of commission) made in the test data were groEL2 a "60 kD Chaperonin 2 gene" and Rv3583c a "putative transcriptional regulator". The rule predicts the function of five genes classed as "Conserved Hypotheticals" (Rv566, Rv854, Rv910, Rv2185, Rv2708) and ten genes classed as "Unknowns" (Rv123, Rv810, Rv909, Rv1893, Rv1955, Rv2061, Rv2517, Rv2819, Rv2822, Rv3718). The prediction rule is consistent with protein chemistry, as lysine is positively charged which is desirable for interaction with negatively charged RNA. The choice of lysine over arginine for the positively charged residue may be connected with the high GC content of the *M. tuberculosis* genome[2] - lysine is coded by the codons AAA and AAG while arg is coded by CGU, CGC, CGA, and CGG.

Not all rules are as simple as the example in figure 3, a more complex rule is shown in figure 4. This rule predicts the level two functional class "Degradation of macromolecules ". The rule is 62.5% accurate (5/8) on the test set. It predicts 3 genes which are currently classified as "Unknown" or "Conserved Hypothetical". The errors of commission are rplV (synthesis and modification of macromolecules), Rv1566 (Virulence) and ponA2 (peneillin binding protein).

If
there exists a homologous protein in SwissProt with the keyword "membrane" AND
there exists a homologous protein in Bacillus subtilis AND
there does not exist a homologous protein with very low molecular weight, a large percentage of glutamic acid, and medium sequence similarity AND
there does not exist a homologous protein in SwissProt with good sequence similarity, low percentage of cysteine, the keyword "transmembrane" and a fairly high molecular weight AND
there does not exist a firmicutes sp. protein in SwissProt with the keyword "transmembrane", with medium molecular weight, and a very high amount of low entropy sequence AND
there exists a homologous mammalian protein in SwissProt with the keyword "repeat" with very high molecular weight
Then its functional class is "Degradation of Macromolecules".

Figure 4 A more complex rule for the classification of "Degradation of Macromolecules".

For those proteins correctly predicted by each rule we carried out all-against-all PSI-BLAST searches. If all the proteins could be linked together by PSI-BLAST scores < 10 then the proteins were considered homologous. It was found that many of the predictive rules were more general than possible using sequence homology. This was shown in two ways: the rules correctly predict the function of sets of proteins that are not homologous to each other, and they correctly predict the function of proteins that are not homologous to any in the training data (Table 2). Such rules provide a way of predicting function in the absence of recognisable sequence homology. The other rules, those of equal power to sequence homology, are also valuable as they provide a novel way of detecting homology.

5. Discussion

The discovered rules are important in two ways: they make predictions that are useful in determining the functions of genes of currently unknown function, and they provide evolutionary insight. The actual function of a gene can only be determined by "wet" experiment. However, bioinformatic techniques such as sequence homology detection, and the prediction rules presented here, can make such experimental determination simpler. It is clearly more efficient to test a high probability hypothesis than to randomly test for possible functions. We look forward to the testing of our predictions by other workers, and we are designing automatic methods to test the rules ourselves.

The existence of general rules for predicting biological function raises the question of their evolutionary causation. How are such rules possible, given the notoriously complicated mappings between function and structure, and structure and

sequence? Several possibilities exist: the rules are paralogous[26] with homology so distant as to be undetectable by sequence analysis; convergent evolution has occurred, forcing proteins with similar function to resemble each other; or horizontal evolution has transferred functional related groups of protein into the organisms. Evidence in favour of a role for distant homology is that it is possible to predict function better than random based on predicted secondary structure alone, and secondary structure is better conserved over evolution than sequence[27]. Evidence against this is that we have found little evidence for common SCOP database[28] "superfamily" and "fold" classifications for proteins predicted by the same rule. Convergent evolution seems to be the dominant factor in rules such as TB_C50_1_26 (Figure 3). Evidence for horizontal transfer of genes into *M. tuberculosis* is the importance of phylogeny in many rules where a paralogous explanation seems to be ruled out.

6. Conclusion

We have demonstrated the utility of automatic knowledge discovery techniques by showing that they can discover prediction rules that are effective and of biological interest in functional genomics. The data mining approach described is extendable to analysis of other forms of bioinformatic data, such as expression profiles, pathway analysis, structural studies, etc.[5-8] Information from all these diverse approaches will be able to be combined together to produce more powerful predictions than any single one in isolation.

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