A Method for Species Comparison of Metabolic Networks Using Reaction Profile

YUKAKO TOHSATO†

Comparative analyses of the metabolic networks among different species provide important information regarding the evolution of organisms as well as pharmacological targets. In this paper, a method is proposed for comparing metabolic networks based on enzymatic reactions within different species. Specifically, metabolic networks are handled as sets of enzymatic reactions. Based on the presence or absence of metabolic reactions, the metabolic network of an organism is represented by a bit string comprised of the digits “1” and “0,” called the “reaction profile.” Then, the degree of similarity between bit strings is defined, followed by clustering of metabolic networks by different species. By applying our method to the metabolic networks of 33 representative organisms selected from bacteria, archaea, and eukaryotes in the MetaCyc database, a phylogenetic tree was reconstructed that represents the similarity of metabolic network based on metabolic phenotypes.

1. Introduction

To obtain the energy necessary for cellular activities, cells within organisms take up many kinds of material in the form of food, etc. The cells break down and synthesize materials required for self-maintenance and growth via an enormous number of chemical reactions. These chemical reactions occurring in an organism are known collectively as “metabolism,” which consists of enzymatic reactions that result in the conversion of certain compounds (substrates) into other compounds (products) by the action of enzymes (proteins). As the product of a reaction is used as the substrate of other reactions, a large-scale and complex metabolic network is formed. These reactions are now stored in several public databases, including KEGG, and MetaCyc, which are available on the World Wide Web. For example, KEGG is a collection of manually drawn metabolic maps.

Metabolisms are important targets for understanding cell processes. Comparative analysis of the metabolic networks among different species provides essential information on the evolution of organisms and on pharmacological targets, and there has been a great deal of research in this area in recent years. Some examples of the application of computer analysis to metabolic networks include (1) metabolic pathway clustering based on genomic information, where metabolic pathways are compared by assigning genes on the genomes to each of the enzymes that constitute a specific pathway and (2) pathway alignment based on functional similarity among enzymes where metabolic pathways are clustered by assigning enzymes based on an enzymatic hierarchy. On the other hand, phylogenetic classification based on single genes, such as rRNA, does not provide a complete and accurate picture of evolution because it does not take into account evolutionary leaps due to gene transfer, duplication, deletion, and functional replacement. Thus, importance is placed on comparison and investigation of phylogenetic trees created from a variety of standpoints. Here, I focus on comparison of phylogenetic trees between metabolic phenotypes and genomic sequences, and a variety of related proposals have been made.

In this study, a method was developed for comparing different species based on metabolic expression profiles. The method consists of comparing different species to acquire new knowledge regarding interspecies phylogenies. This is done by considering the metabolic network as a set of metabolic reactions, and the whole network is expressed as a bit string encoding the presence (1) and absence (0) of reactions that comprise the network.

Section 2 discusses related research. The proposed method is described in Section 3, and Section 4 presents results obtained using the proposed method in an actual comparison of metabolic networks. Finally, Section 5 summa-
rizes problems and future work.

2. Related Work

There have been previous studies related to the purpose of this research, as reported by Hong et al.\(^9\). They proposed a method for species comparison of metabolic networks based on the combination of metabolites that comprised the enzymatic reaction, and analyzed the metabolic pathways of 42 microorganisms. The method proposed by Hong et al. classifies the overall metabolic networks into 64 individual sub-networks on the basis of metabolic map classifications. The numbers of reactions involved in each sub-network were counted and used for estimation of the reaction content \( p_{ij} \),

\[
p_{ij} = 100 \times \frac{r_{ij}}{R_j}
\]

where \( r_{ij} \) is the number of reactions in the \( j \)th sub-network in organism \( i \), and \( R_j \) is the number of non-duplicate reactions involved in the \( j \)th sub-network. In this case, the reaction content becomes synonymous with a sub-network. The Pearson correlation coefficient was used to assess the degree of similarity \( D \) between the reaction content \( p_{i1}, p_{i2}, \ldots, p_{iN} \) of organism \( i \) with the reaction content \( p_{j1}, p_{j2}, \ldots, p_{jN} \), which is defined as:

\[
D = \frac{1}{N} \sum_{k=1}^{N} \left( \frac{p_{ik} - \bar{p}_i}{\sigma_i} \right) \left( \frac{p_{jk} - \bar{p}_j}{\sigma_j} \right)
\]

where \( \bar{p}_i \) and \( \bar{p}_j \) are the averages of values in \( p_{i1}, p_{i2}, \ldots, p_{iN} \) and \( p_{j1}, p_{j2}, \ldots, p_{jN} \), respectively. \( \sigma_i \) and \( \sigma_j \) are the standard deviations of these values. Then, clustering was performed using the furthest neighbor method\(^8\).

Nevertheless, with this definition of Hong’s method, if the numbers of reactions in the metabolic map are identical, these will not be distinguished, even where the types of reaction are different. Consider the case where, within organisms \( S_1, S_2, \) and \( S_3 \), the presence or absence of enzymatic reactions \( r_1, r_2, r_3, \) and \( r_4 \) have relationships shown in Fig. 1. The reaction contents of two organisms \( S_2 \) and \( S_3 \) become the score value 75; even where the existing enzyme reactions are of different types, they thus become the same score. In addition, the clustering results from the method of Hong et al. are impacted by the metabolic map classifications.

Taking the points described above into consideration, a method is proposed as described in the following section.

3. Method

3.1 Metabolic Network and Reaction Profile

The metabolic network of an organism is treated as a set of the enzymatic reactions. Consider two different organisms, \( S \) and \( S' \), with metabolic networks \( N \) and \( N' \), respectively. The set \( R \) of all reactions included within the metabolic networks of organisms \( S \) and \( S' \) is taken as \( R = \{r_1, r_2, \ldots, r_n\} \). Here, multiple isozymes catalyzing the same reaction were counted only once, and multifunctional enzymes were counted as many times as they catalyze different reactions. Enzymatic reactions are distinguished by the combinations of metabolites — called “reaction types.” Duplication of reaction types is not allowed within a set \( R \). For \( R \), the reaction profile of organism \( X \) is represented by a bit string \( P_X = b_{x1}b_{x2} \cdots b_{xn} \) (a sequence of digits “0” and “1”). When a bit \( b_{xi} \) is set to 1 in a bit string, it means the corresponding reaction \( r_i \) \((1 \leq i \leq n\) is present for organism \( X \), while 0 means the reaction is absent.

3.2 Similarity Measure between Reaction Profiles

For defining the degree of similarity, a variety of numerical methods, such as the Pearson correlation coefficient, have been proposed. However, in this study, the Tanimoto coefficient\(^4,11\) was used. The Tanimoto coefficient is an index that strongly shows the relative correlation between two elements\(^4\).

The degree of similarity \( T(X,Y) \) of the reaction profile \( P_X = b_{x1}b_{x2} \cdots b_{xn} \) of organism \( X \) and the reaction profile \( P_Y = b_{y1}b_{y2} \cdots b_{yn} \) of organism \( Y \) are defined in accordance with the Tanimoto (Jaccard) coefficient as follows.

\[
T(X,Y) = \frac{N_z}{N_x + N_y - N_z}
\]

\( N_x \) and \( N_y \) are the numbers of 1 bits in the reaction profiles \( P_X \) and \( P_Y \), respectively, and \( N_z \) is the number of common reactions in both reaction profiles \( P_X \) and \( P_Y \). By definition, \( T(X,Y) \) is the number in the range 0 to 1; the closer to
1, the higher the degree of similarity between the two reaction profiles, while the closer to 0, the lower the degree of similarity between the two reaction profiles.

For example, the reaction profiles of organisms $S_1$, $S_2$, and $S_3$ of Fig. 1 become 1111, 1110, and 0111, respectively. Here, $T(S_1, S_2) = 3/4 = 0.75$, and $T(S_2, S_3) = 2/4 = 0.5$; thus, the similarity between the reaction profiles of $S_1$ and $S_2$ is higher than that between the reaction profiles of $S_2$ and $S_3$.

3.3 Clustering

Using the degree of similarity $T$ as defined in Section 3.2, a dissimilarity score $D(A, B)$ was defined between reaction profiles.

$$D(A, B) = 1 - T(A, B)$$

Then, on the basis of the dissimilarity $D$, a distance matrix for all organisms was created. Clustering was performed on the dissimilarity matrix. Although there are various clustering methods, such as the group average method and the centroid method, in this study, the furthest neighbor method was used in addition to the method proposed by Hong, et al.

4. Experiments and Results

4.1 Experiments and Results

To evaluate the effectiveness of the proposed method, the metabolic networks were compared among different species.

Reaction profiles were constructed from the MetaCyc database update version 2004-09-27, 33 sequenced organisms (6 archaea, 26 bacteria, 1 eukaryote). A list of the organisms is shown in Table 1. Table 1 lists organism name, abbreviation, and the number of enzyme reactions found in that organism. The number of enzyme reactions within the table is the number of enzyme reactions in the metabolic networks of each species.

The MetaCyc database was provided in flat file format, and reconstructed with MySQL.

| Table 1 | List of organisms used in this analysis. |
| --- | --- |
| **Organism** | **Code** | **Number of Reactions** |
| Archaea | | |
| 1 Archaeoglobus fulgidus DSM4304 | AfD | 791 |
| 2 Methanococcus jannaschii DSM26661 | MjD | 693 |
| 3 Methanobacterium thermoautotrophicum delta H | MtD | 702 |
| 4 Pyrococcus furiosus DSM 3638 | PfD | 720 |
| 5 Thermoplasma acidophilum DSM 1728 | TaD | 502 |
| 6 Thermoplasma volcanium GSS1 | TvG | 773 |
| Bacteria | | |
| 1 Aquifex aeolicus VF5 | AaV | 687 |
| 2 Borrelia burgdorferi B31 | BbB | 473 |
| 3 Clostridium acetobutylicum ATCC824 | CaA | 896 |
| 4 Caulobacter Crescentus | Cc | 812 |
| 5 Campylobacter jejuni NCTC 11168 | CjN | 728 |
| 6 Campylobacter jejuni RM1221 | CjR | 682 |
| 7 Escherichia coli K-12 | EcK | 1041 |
| 8 Escherichia coli O157 | EcO | 855 |
| 9 Enterococcus faecalis V583 | EfV | 817 |
| 10 Haemophilus influenzae KW20 Rd | HiK | 836 |
| 11 Helicobacter pylori 26695 | Hp2 | 542 |
| 12 Helicobacter pylori J99 | HpJ | 614 |
| 13 Mycobacterium leprae TN | MtT | 745 |
| 14 Neisseria meningitidis serogroup A Z2491 | NmA | 790 |
| 15 Neisseria meningitidis MC58 | NmM | 800 |
| 16 Pseudomonas aeruginosa PA01 | PaP | 1093 |
| 17 Porphyromonas gingivalis W83 | PgW | 796 |
| 18 Streptococcus pneumoniae R6 | SpR | 848 |
| 19 Streptococcus pneumoniae T1GR4 | SpT | 717 |
| 20 Streptococcus thermophilus LMG 18311 | StL | 762 |
| 21 Streptococcus pyogenes MGAS10394 | Sy1 | 874 |
| 22 Streptococcus pyogenes MGAS3232 | Sy2 | 768 |
| 23 Streptococcus pyogenes SF370 serotype M1 | Sy3 | 868 |
| 24 Vibrio cholerae N16961 | VcN | 848 |
| 25 Yersinia pestis CO92 | YpC | 1184 |
| 26 Yersinia pestis KIM | YpK | 946 |
| Eukarya | | |
| 1 Human | Hu | 1187 |
The distance matrix is calculated using Perl. Statistical processing software R Version 2.3.0 was used for clustering and for the creation of a phylogenetic tree diagram. As a result, 33 reaction profiles consisting of 3744 bits were obtained. The phylogenetic tree obtained as is shown in Fig. 2. The abbreviations (e.g., MtD and MjD) in Fig. 2 represent the organism names, and these correspond to their formal names shown in Table 1.

4.2 Discussion

Within the phylogenetic tree shown in Fig. 2, the six species of archaea — Methanobacterium thermoautotrophicum delta (MtD), Methanococcus jannaschii DSM2661 (MjD), Archaeoglobus fulgidus DSM4304 (AfD), Pyrococcus furiosus DSM 3638 (PfD), Thermoplasma volcanium GSS1 (TvG), and Thermoplasma acidophilum DSM 1728 (TaD) — were classified within the same cluster (Cluster 1). Further, the sole eukaryote, Human (Hu), was located apart from these organisms (Cluster 2). The proposed method successfully achieved distinct separation of the archaea, the bacteria, and the eukaryote. The proposed method showed a similar tendency to the clustering results for archaea and bacteria using the method of Hong, et al. 8).

The 26 species of bacteria were widely separated; they were divided into gram-positive proteobacteria, gram-negative bacteria, and other. Nine species were classified as gram-positive bacteria, and the eight species of gram-negative bacteria — Streptococcus thermophilus LMG 18311 (StL), Clostridium acetobutylicum ATCC824 (CaA), Streptococcus pneumoniae R6 (SpR), Streptococcus pneumoniae TIGR4 (SpT), Enterococ-
there may exist groups with metabolic networks similar to those of gram-positive bacteria, as well as groups with metabolic networks that are not similar to those of gram-positive bacteria. This was recently reported by Zhang, et al.\textsuperscript{16}. With regard to these organisms, further detailed investigations are required to determine where on the metabolic map their characteristics are located.

Although the proposed method closely resembles the method proposed by Yamada, et al.\textsuperscript{4}, there are several important differences, including the purposes of the respective methods and the fact that genes are used as the standard in the method of Yamada, et al.

### 5. Conclusions and Future Work

A method was proposed for comparing metabolic networks among different species based on their reaction profiles by considering metabolic networks as a set of enzymatic reactions. This method is a combination of the commonly used bit expression for data, the Tanimoto coefficient method, and clustering, and is relatively easy to implement. The method was applied for 33 actual species, and its validity was demonstrated.

It is anticipated that metabolic network data will become available in future for a variety of organisms, including eukaryote-specific metabolic pathways. It would be possible to obtain more knowledge considering the diversification of metabolic phenotypes using this method. Further knowledge will be acquired by comparison of phylogenetic trees between metabolic phenotypes and genomic sequences.

Nevertheless, the analysis of metabolic networks based on the method proposed here has the problem that it is markedly impacted by data deficiencies. Thus, it will be necessary to introduce (1) verification of influence that lack of data has on clustering results, (2) consideration of the divergent-pathways, (3) comparison of results according to a variety of clustering methods, and (4) statistical scale that will guarantee the reliability of results.

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Yukako Tohsato is an Assistant Professor of Department of Bioscience and Bioinformatics, Ritsumeikan University. She received her M.E. degree from Kyushu Institute of Technology in 1997. She worked at Mitsubishi Electric Co. from 1997 to 1999. She received her Ph.D. degree from Osaka University in 2002. From 2002 to 2003 she worked as a Research Associate at Osaka University. From 2003 to 2004, she worked as a Researcher at Osaka University. She is a member of IPSJ.