Reconstruction and analysis of integrated metabolic reaction network of *Bacillus subtilis*

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1 **Introduction**

Metabolic pathway reconstruction is a process that involves collecting information about genes, proteins, reactions and metabolites that participate in major and minor metabolic activities and the relations among these entities can be represented as a network. Most often the system of interest is a single cell within which a metabolic network operates. *Bacillus subtilis* was selected for our study because it is one of the most well studied prokaryotes (second only to *E. coli*) and because knowledge about its essential genes is well available and minimal genome *B. subtilis* strains have been developed. To extract essential reactions in the metabolic networks of *B. subtilis*, we focused on centrality measures of reactions based on their degree and betweenness in the network because degree quantifies the number of other reactions with which a reaction is connected to and betweenness quantifies the number of shortest paths touching a reaction compared to the number of shortest paths between all pairs and these points are regarded as important for analyzing metabolic pathways. In this study, we determined the hub and bottleneck reactions and inferred the relation between hubs and bottlenecks, and their biological functions.

2 **Method**

2.1 **Reconstruction of integrated metabolic pathway**

Although several genome-scale network reconstructions describe *B. subtilis* metabolism, they differ in scope and content, and use different terminologies to describe the same chemical entities. We manually extended the pathway of *B. subtilis* central metabolism proposed by Goelzer *et al.* by collecting information from several databases (KEGG, MetaCyc, BRENDA) and published papers [1-3].

2.2 **Construction of reaction networks**

Metabolic pathways are sometimes represented as networks of metabolites and sometimes as networks of reactions. In the present study we followed the later approach. We constructed a reaction network by connecting every two reactions who share at least a common compound as a substrate or a product. Figure 1 show how a reaction network is constructed by placing connection between reactions (i.e enzymes), and removing all metabolites from the pathway information.

Figure 1: Construction of reaction network
3 Results and Discussion

3.1 The reconstructed metabolic network

Table 1 shows the number of genes and the number of reactions associated to the integrated network we constructed and also those for the network model used in [2]. As a result of added information from multiple sources, the number of genes associated with metabolic reactions increased from 433 to 749, and the number of metabolic reactions increased from 448 to 713.

Table 1: Comparison of reconstructed metabolic network

<table>
<thead>
<tr>
<th></th>
<th>No of genes associated with metabolic reactions</th>
<th>No of metabolic reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goelzer et al Model</td>
<td>433</td>
<td>448</td>
</tr>
<tr>
<td>Consensus reconstruction</td>
<td>749</td>
<td>713</td>
</tr>
</tbody>
</table>

3.2 Identification of hubs and bottlenecks in the reaction network

Figure 2(a) and 2(b) show the distributions of reactions with respect to degree and betweenness centrality respectively, and Figure 2(c) shows the x-y plot of degree and betweenness centralities of metabolic reactions. Spearman’s rank correlation coefficient between degree and betweenness centralities is about 0.5 and highest 5% of hubs and bottlenecks are highlighted and characterized. We identified that some compounds related to hub reactions are glutamate, pyruvate and 2-oxoglutarate which are metabolites related with regulatory protein activity of B. subtilis. And the reactions associated with essential genes tend to be not hubs but bottlenecks. Furthermore, the non-hub bottlenecks are correspond to phenylalanine, tyrosine and tryptophan biosynthesis superpathway. As whole we can say that hubs are related to protein activity regulations and bottlenecks are related to essentiality.

![Figure 2](image)

Figure 2: Distribution and X-Y plot of degree and betweenness of the reaction network, Histogram of the number of reactions for (a) degree, and (b) betweenness, and (c) the relation of degree and betweenness

References

