Identification of Essential Genes for Metabolic Reactions in *Mycobacterium tuberculosis* through Flux Balance Analysis

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ABSTRACT

Mycobacterium tuberculosis, the causal organism for Tuberculosis, remains one of the largest infectious killers despite availability of several drugs. The quest for novel drug targets continues with novel approaches to amalgamate *in vivo* studies with *in silico* analysis. Flux Balance Analysis (FBA) is one such computational approach utilized in organismal simulation for metabolic networks. In this study we have applied the FBA method to identify potential genes which are essential for mycobacterial metabolome. Utilizing FBA on dependence of Biomass Reaction, 75 genes were identified to be metabolically essential in *M. tuberculosis*. Three network clusters were found within the identified set of genes involving purine, phthiocerol and diaminopimelate biosynthesis pathways reiterating the importance of these networks in mycobacterial survival and pathogenicity.

INTRODUCTION

Over 10 million individuals all over the world annually succumb to tuberculosis and India alone contributes over 30% of the total mortality [1]. Despite the presence of a large research community worldwide, efficient drug discovery for *M. tuberculosis* often faces hindrances. The intelligent mycobacterium appears to have reinforcement for every pathway that it has preserved in the reductionist evolution while at the same time sustained a steady mix of regulatory and virulence factor to adapt and attack in different micro and macro-niche[2]. The presence of multiple and extensively drug resistant form of *M. tuberculosis* (MDR and XDR TB) renders designing an appropriate drug for the pathogen more difficult. To make the situation more complex, the procedure from selecting a suitable drug from *in vitro* analysis through clinical trials is lengthy and incurs exorbitant cost. Several *in silico* techniques of systems biology are currently practiced to narrow down the drug target specification.

Flux Balance Analysis (FBA) is one such computational method which carries out a metabolic analysis of a biological system within the stoichiometric and capacity constraints to identify the flow rate of involved metabolites. The advantage of having a reservoir of genome-scale metabolic network has resulted in identification of optimum behavior of any organismal metabolome which can further be utilized to identify potential drug targets in an

organism[3]. The genome-scale metabolic network reconstructions contain all of the known metabolic reactions in an organism including the genes and metabolites enzyme along with the biophysical constrains. The understanding of metabolic network coupled with survival mechanism of an organism can help determine the set of genes or reactions that are essential and optimal for its survival. The biomass composition of an organism and the effort to maximize it can be studied through FBA method and in this study we have used *M. tuberculosis* H37Rv BIGG Model iNJ661 as an input for the same [4].

MATERIALS AND METHOD

The present study used BiGG model iNJ661 which contains 661 *M. tuberculosis* H37Rv genes, 825 metabolites and 1025 reactions[4]. The primary assumption for the FBA method used here is that the maximization of biomass production requires an optimal cell performance[5]. We have analyzed the dependence of biomass reaction on each gene recursively. We deleted the genes one by one and analyzed the change in flux on biomass reaction. We declared any gene to be essential if deletion of the same gene resulted in reduction of the reaction rate by a factor of 'θ' (θ as described below).

Suppose, the reaction rate of biomass reaction in wild type *M. tuberculosis* is 'α' and later after deleting the gene from the system it becomes 'β', then, the gene is considered to be an essential gene if,

$$\frac{\beta}{\propto} < \theta$$

where,

α= 0.052549801148350436

θ=0.01[6]

We have also analyzed the system in the absolute absence of flux through the biomass reaction, i.e., θ = 0. This will result in a stringent list of metabolically essential genes.

We further utilized protein-protein interaction network (STRING) for the identified genes and visualization was performed through Cytoscape.

RESULTS AND DISCUSSION

188 genes out of 661 total genes were found to satisfy the equation with θ as 0.01. Further, strictly considering the absence of flux through the biomass reaction where θ is 0, 75 genes were identified. These genes can be termed as metabolically essential for the survival and adaptation of *M. tuberculosis*. Many of the genes identified in the present study were also termed essential gene in previous studies on *M. tuberculosis* through transposon mutagenesis, pathway enrichment techniques and metabolomics analysis[7-8]. Additionally, we have found three distinct clusters among these metabolically essential genes which are involved in purine, phthiocerol and diaminopimelate biosynthesis pathways. Phthiocerol dimycocerosate is an important surface lipid of *M. tuberculosis* which is also known to play a

significant role in patho-physiology of the mycobacteria. Our analysis supports previous studies highlighting the importance of these networks in mycobacterial metabolome.

Table I: List of metabolically essential genes of *M. tuberculosis* where θ = 0

Gene Id	Gene Name	Gene Id	Gene Name
Rv0334	rmlA	Rv1449c	Tkt
Rv0357c	purA	Rv1484	inhA
Rv0470c	pcaA	Rv1599	hisD
Rv0482	murB	Rv1601	hisB
Rv0500	proC	Rv1652	argC
Rv0618	galTa	Rv1656	argF
Rv0619	galTb	Rv1699	pyrG
Rv0772	purD	Rv1878	glnA3
Rv0777	purB	Rv2006	otsB1
Rv0780	purC	Rv2121c	hisG
Rv0788	purQ	Rv2136c	--
Rv0803	purl	Rv2158c	murE
Rv0809	purM	Rv2201	asnB
Rv0855	far	Rv2210c	ilvE
Rv0858c	dapC	Rv2220	glnA1
Rv0884c	serC	Rv2222c	glnA2
Rv1018c	glmU	Rv2245	kasA
Rv1086	--	Rv2246	kasB
Rv1201c	dapD	Rv2465c	rpiB
Rv1202	dapE	Rv2524c	Fas
Rv1285	cysD	Rv2538c	aroB
Rv1286	cysN	Rv2539c	aroK
Rv1294	thrA	Rv2540c	aroF
Rv1315	murA	Rv2860c	glnA4
Rv1323	fadA4	Rv2928	tesA

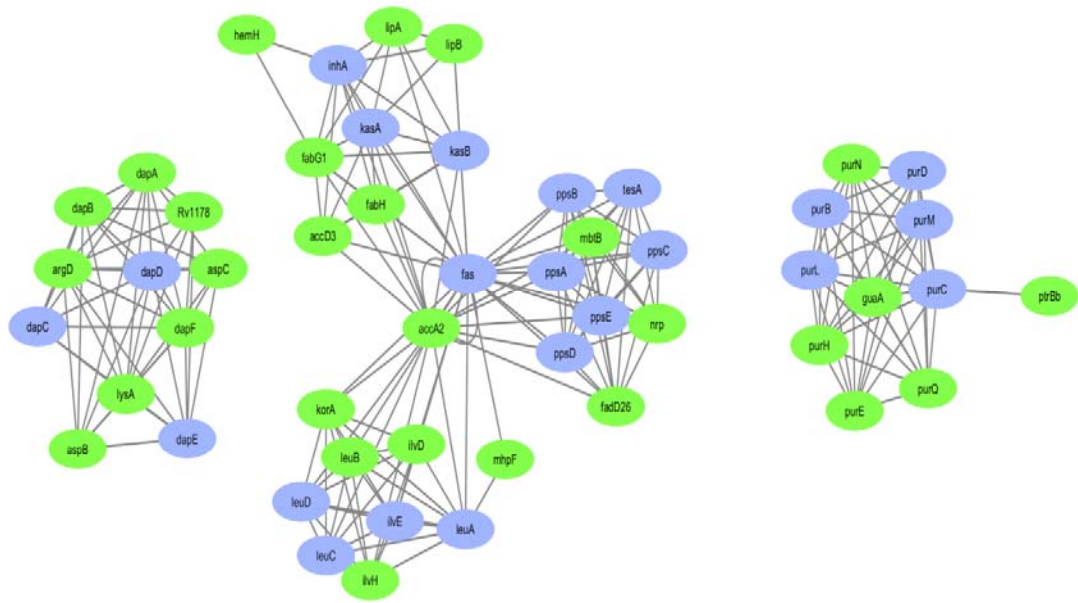|			
Rv1338	murI	Rv2931	ppsA
Rv1350	fabG2	Rv2932	ppsB
Rv1381	pyrC	Rv2933	ppsC
Rv1383	carA	Rv2934	ppsD
Rv2935	ppsE	Rv3372	otsB2
Rv2947c	pks15	Rv3465	rmlC
Rv2981c	ddlA	Rv3581c	ispF
Rv2987c	leuD	Rv3582c	ispD
Rv2988c	leuC	Rv3709c	Ask
Rv3001c	ilvC	Rv3710	leuA
Rv3248c	sahH	Rv3800c	pks13
Rv3264c	manB	Rv3801c	fadD32
Rv3340	metC		

Figure I. Interaction network of metabolically essential genes of *M. tuberculosis* reveal three prominent cluster

CONCLUSION

Flux Balance Analysis is less time consuming and relatively easy to compute thus proves to be a good method for *in silico* identification of drug targets. The structure of the metabolic network is a critical factor for the accuracy of FBA as reported in several studies and with our expanding knowledge about *M. tuberculosis* metabolic network, the input models for the FBA analysis needs to be updated regularly. The present work identifies 75 metabolically essential genes of *M. tuberculosis*, the interactome of which could be explored as target for drug combination.

REFERENCES:

1. World Health Organization (2017). *Global Tuberculosis Report*. Licence: CC BY-NCSA 3.0 IGO

2. Bhaduri, A., Misra, R., Dhamija, N. (2015). To eat or not to eat: Understanding the tussle between mycobacteria and host autophagy. *Indian Journal of Microbiology, 55*(4), 456-459. doi: 10.1007/s12088-015-0541-9.

3. Orth, J., Thiele, I., Palsson, B. (2010). What is flux balance analysis? *Nature Biotechnology, 28*(3): 245–248.

4. King, Z. A., et al. (2016). BiGG Models: A platform for integrating, standardizing, and sharing genome-scale models. *Nucleic Acids Research*, *44*(Database issue), D515-D522. doi:10.1093/nar/gkv1049

5. Raman, K., Chandra, N. (2009). Flux balance analysis of biological systems: Applications and challenges. *Briefings in Bioinformatics, 10*(4), 435-449.

6. Deutscher, D., et al. (2008). Can single knockouts accurately single out gene functions? *BMC Systems Biology 2*, 50. doi: 10.1186/1752-0509-2-50

7. Sassetti, C. M., Boyd, D. H., Rubin, E. J. (2003). Genes required for mycobacterial growth defined by high density mutagenesis. *Molecular Microbiology, 48*(1), 77-84.

8. Xu, G., et al. (2014). Screening essential genes of *Mycobacterium tuberculosis* with the pathway enrichment method. *Molecular Biology Reports 41*, 7639-7644. doi:10.1007/s11033-014-3654-z

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