The effect of implementing objective functions in analyzing the changes of enzyme activity profiles

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To maximize the target metabolic fluxes, the use of an objective function in determining the Elementary Mode (EM) Coefficients (EMCs) somehow gives some impacts. This paper describes the effect of applying the objective function, i.e., Maximum Entropy Principle (MEP), on estimating the EMCs. MEP is applied to the Gene Modification of Flux (GMF) [1] [2] – a method that uses the elementary modes of a metabolic network map to predict its associated flux distribution under different genetic conditions.

1. Introduction

The 'communication' of regulatory network systems in the level of gene expression, protein-protein interaction and metabolism involves thousands of genes, enzymes and metabolites. This 'communication' changes due to several constrained conditions; such as environmental and genetic conditions. This 'communication' can be predicted to derive the complete pictures of a metabolic network system.

Flux Balance Analysis (FBA) is one of the examples of pathway analysis tools that work based on stoichiometric and objective functions; where these will be used to identify a solution boundary. An objective function acts as an optimization factor that maximizes the targeted cell growth, energy or metabolite synthesis [1]. On the other hand, complex metabolic pathways can be analyzed based on elementary modes (EMs). EMs are a minimal enzyme set that able to operate at steady state, through all irreversible reactions used in an appropriate way [3].

This paper investigates the effect of implementing an objective function - Maximum Entropy Principle (MEP) on estimating EMCs. In addition, a number of enzyme activity data profiles of *Escherichia coli* (*E. coli*) [4] [5] [6] are collected and analyzed by the Genetic Modification of Flux (GMF) algorithm with MEP.

2. Method

2.1 Genetic Modification Flux (GMF)

Genetic Modification of Flux (GMF) [1] [2] is a combination of two algorithms; modified CEF (mCEF) and Enzyme Control Flux (ECF). The main purpose of this algorithm is to predict the flux distribution of gene knockout mutants by utilizing the EM analysis in connecting various network levels.

One of main problems in EM-based analysis is how to estimate the Elementary Mode Coefficients (EMCs). This problem is due to the complexity calculation of EMCs, yet the coefficient values are important in deriving a metabolic model. To overcome this problem, we used MEP to perform GMF. Figure 1 summarizes the implementation process of this application.

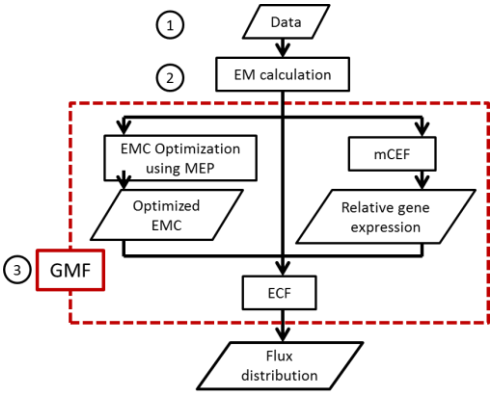

Figure 1: GMF implementation

This algorithm is as follows:

- A data file that contains reactions and metabolic data model is generated
- The EM calculation is performed using CellNetAnalyzer (CNA) [7]
- GMF is applied by using the generated EMCs that will be:
  1. further optimized by MEP to produce a set of optimized EMCs
  2. used by the mCEF algorithm to produce relative gene expression
- A flux distribution is predicted through the optimized EMCs and relative gene expression

2.2 Data

To investigate the feasibility of the proposed algorithms, a number of *E. coli* metabolic network publications are gathered and analyzed. Some respective publications are listed in Table 1.

TABLE 1. Data model publications

Knockout Mutant	Condition	Reference
gnd	Dilution rate: 0.2 h^{-1}	[4]
pykF	Sampling time (batch culture): 4 hours	[5]
sucA	Dilution rate: 0.2 h^{-1}	[6]

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3. Result and Discussion

We compared the flux predictions for wild type and genetic mutants. Figure 2 shows the performance of the predicted (P) and experimental (E) metabolic fluxes at several key branch points with respect to an independent experiment for wild-type (WT) cells. Figure 2 demonstrates that the use of MEP optimizes effectively their WT flux values.

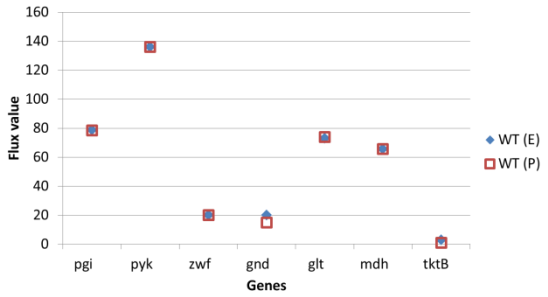

Figure 2: Comparison of experimental (E) and predicted (P) metabolic fluxes at several key branch points from an independent experiment

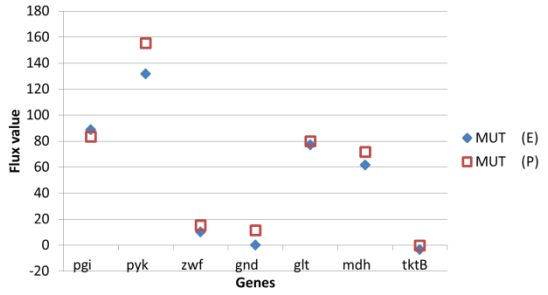

Figure 3: Comparison of experimental (E) and predicted (P) metabolic fluxes at several key branch points in the central metabolic network for a *gnd* knockout mutant (MUT).

As shown in Figure 3, the predicted flux distribution of a *gnd* knockout mutant (P) by GMF with MEP is relatively consistent with experimental data (E).

The use of MEP is effective in estimating the EMCs. However, it is important to note that further investigation should be made on the data preparation [8].

4. Conclusion

A number of original metabolite data publications are collected and tested using the GMF algorithm with MEP. The proposed algorithm predicted the flux values of WT accurately, yet the results in some genetic mutants remain to be improved. Further investigation should be made [1] [2] [8].

References

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