

Combined Metabolome and Transcriptome Analysis of *Bacillus subtilis* Cells Cultured on Different Carbon Sources

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1 Introduction

One of the key words characterizing the post-genome science is high-throughput and comprehensive chemical analysis such as DNA microarray chips. These chemical analyses that realize the measurement of transcriptome, proteome, and protein-protein interactions reveal the flow of genetic information from genome to phenotypes. However, such analysis was not available for metabolites that are chemical basis of phenotypes; there was no measured data for each intracellular metabolite how much amount is there in cells. Due to the lack of metabolomics data, metabolites are treated as a black box in the flow of genetic information.

Recently we have successfully developed the method of chemical analysis for metabolomics [1]. Here we apply the method to the metabolomics of *Bacillus subtilis* cells that were cultured on five different carbon sources. We routinely measured the intracellular concentration of 88 metabolites; those in the glycolysis, TCA cycle, and pentose-phosphate cycle pathways, and amino acids, nucleotide, and cofactors. Firstly, we compare the metabolite profiles among the cells grown on different carbon sources. Secondly, we link metabolite profiles to the corresponding transcriptome data. This is the first study to interpret transcriptome data by referring to metabolome data.

2 Method and Results

2.1 Metabolome Analysis

Bacillus subtilis cells were inoculated into 100 ml of S6 medium in a 500 ml Erlenmeyer flasks and incubated in a water-bath reciprocal shaker at 37°C. S6 medium contains either 25 mM glucose, 25 mM fructose, 50 mM glycerol, or 37.5 mM malate as a carbon source. Yeast extracts were not added to the medium. We carefully kept the constant doubling time of cells in each medium and harvested the cells in 10 ml culture medium on membrane filter (1.0 micrometer) at the middle of logarithmic phase (A600 = 1). Cells were washed with water and extracted with methanol. Methanol extracts were washed with chloroform to remove lipids and lyophilized after removing proteins and other macromolecules [1]. Lyophilized samples were analyzed by using capillary electrophoresis (CE) coupled to mass spectrometry (MS). Metabolites are roughly separated by CE and quantitatively measured by single ion monitoring of MS. Analytical conditions of CE/MS were described in our previous report [1].

Among the 88 metabolites that are measurable on the CE/MS system, 62 metabolites were detected. Metabolite profiles were prepared for the cell extracts. Figure 1 is an example of such profile.

2.2 Transcriptome Analysis

DNA microarrays and fluorescent-labeled cDNA were prepared and hybridized, and fluorescence intensities of 3942 genes spotted on the microarrays were measured as described previously [2].

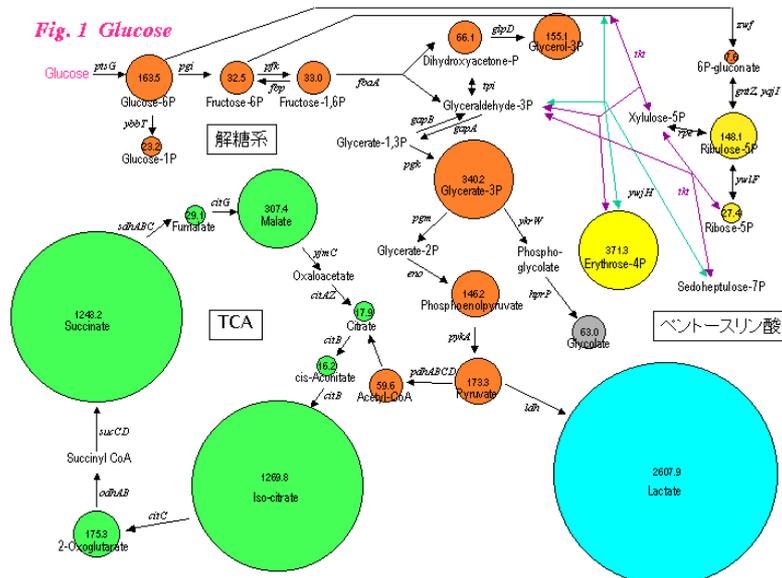


Figure 1: Metabolite profile of *Bacillus subtilis* cells grown on glucose as a carbon source. Metabolites and pathways are drawn as circles and arrows, respectively. Size of circles increases with the cellular concentration of metabolites. Metabolites indicated by not circle but letter are under the detection limit or not measurable in the present CE/MS system.

3 Discussion

Concentration of metabolites measured in the present study is the static amount of each metabolite in the *B. subtilis* cells. Metabolite profiles of the cells have two features.

Firstly, some metabolites are higher in intracellular concentration while others are lower. Metabolites of higher concentrations are not always of biological significance. Intracellular concentration of metabolite might be regulated by in-and-out flux by enzymatic reactions. However, no relationship is found between intracellular concentration of a metabolite and gene expression of the corresponding enzymes that utilize it as a substrate or product. It is necessary to develop a new bioinformatics tool to understand the biological meanings of the differences in their concentrations.

Secondarily, metabolite profiles are almost the same among the cells that were grown on different carbon sources. The present analysis showing that the *B. subtilis* cells took the same profile suggests that intracellular concentration of the individual metabolites were optimized to the maximum cell growth independent on carbon sources.

It will be interesting to compare metabolite profiles of *B. subtilis* cells grown in different growth phases and environments and to analyze whether different organisms have similar or species-specific metabolite profiles.

References

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