Phenotype Profiling of Single Gene Deletion Mutants of E.coli Using Biolog Technology

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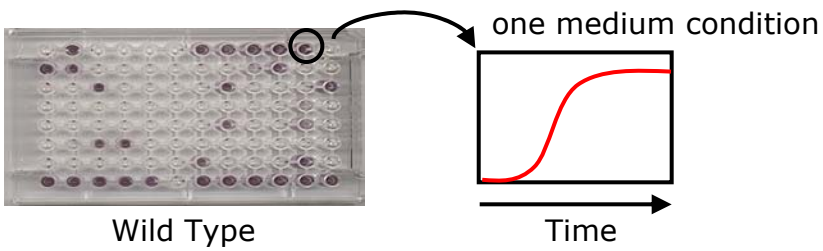
Phenotype MicroArray

- Phenotype MicroArray (PM) technology is designed to test a large number of cellular phenotypes simultaneously (Bochner et al., 2001)
- The system allows monitoring of cellular respiration during cell growth on 96-well microtiter plates under a maximum of 1920 different medium conditions by colorimetrically detection of generation of purple colored Formazane from Tetrazolium dye corresponding to the intracellular reducing state by NADH simultaneously.



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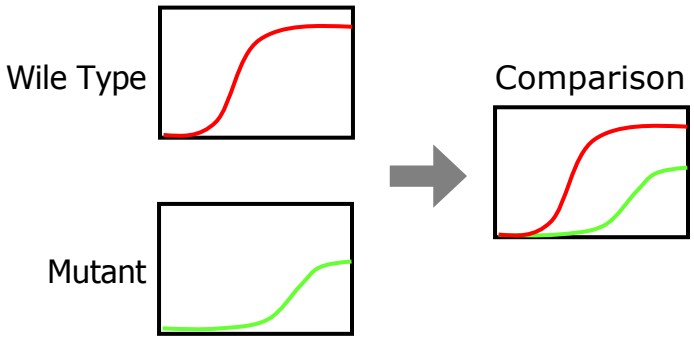
Wile Type V.S. Mutant

- Compare Single Gene Deletion Mutant to Wild Type to determine gene function



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What is clarified by PM?

- Genotype → Phenotype → Metabolic Pathway
- Knock out a gene → Which phenotypes change ?

- In this study, analysis using PM data was performed to discover new alternative pathways and identify functions of genes for which the functions have yet to be determined.

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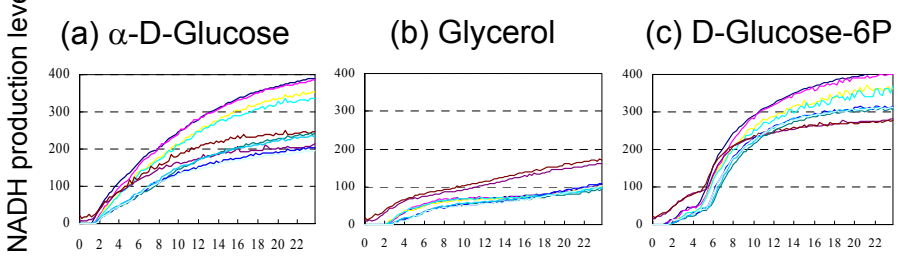
Our Approach and Method

Materials

- *Escherichia coli* K-12
  - 1 host strain (BW25113)
  - 204 mutants (Keio collection library (Baba et al., 2006))
- Growth conditions : 1920 （ex. Suger, Nitrogen , Drug etc）
- Measuring time： 15-minute intervals over 24-hour period.
  - Host strain ：10 times, Mutants ： 2 times



Web application for PM

- PM data consist of enormous amount observation points.
- It made both Excel and me overflow frequently!
- So, we are constructing web applications for PM data to search and analysis.
  - Acknowledgement
    - Yusaku Mazaki
    - Tomohiro Fujita

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Our Approach

- We report the results obtained by applying the proposed method to PM data from wild-type and 45 single gene deletion strains.
  - The strains related to central metabolism.
  - Selected 288 medium condition of carbon and nitrogen sources.
- Our methods
  - Vectorization of raw data
  - Hierarchical Clustering

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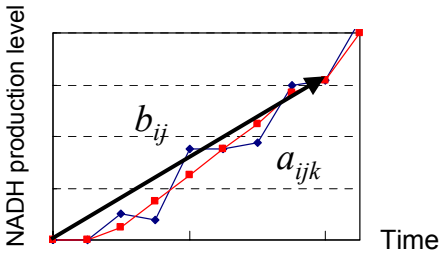
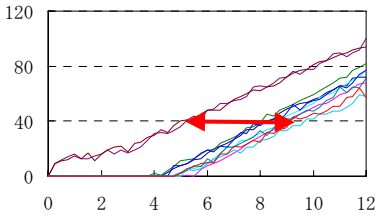
Vectorization (1)

- The original raw data less than a threshold were substituted with zero (zero-substitution).
- The data were smoothed by taking an average of consecutive nine observation points (two hour). $a_{ijk} = \frac{1}{9}\sum_{k'=k}^{k+8} x_{ijk'}$
- Each well is expressed with its maximum slope b_{ij}.
- PM data for each strain can be considered as 288-dimension vector data.

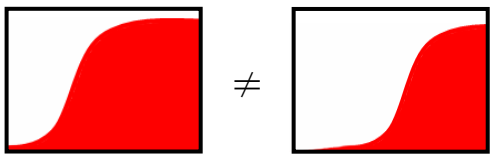


Why did we select maximum slopes ?

- Growth time shift.



  - Maximum values ?
  - Areas ?



  - Maximum slopes !

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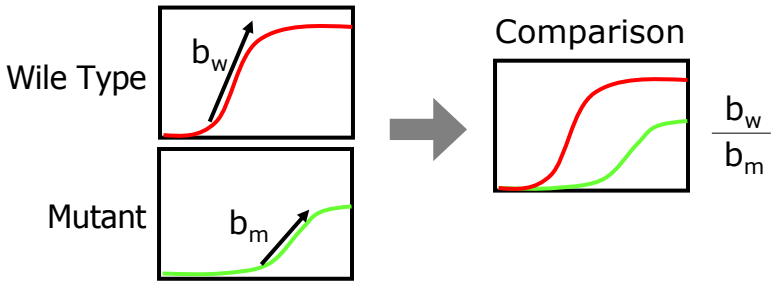
Vectorization (2)

- Calculated the ratio between vector data of mutant and wild.



- The ratio data are converted to a data of 0s, -1s and 1s by setting thresholds for the vector ratios 1.2 and 0.8.

$$v_k = (v_{k1}, v_{k2}, \ldots, v_{k288})$$

  - +1 indicates that the gene deletion activate the respiratory activity.
  - -1 indicates that the gene deletion repress the respiratory activity.

No.11

Clustering Methods

- Manhattan distance $d(v_1, v_2) = \sum_{k=1}^{n} |v_{1k} - v_{2k}|$
  - Distance between Knockout strain and other one
    - The degree of similarity using the distance tends to become larger for pairs of vector data that are less similar.
- Ward's hierarchical method
    - Less susceptible to noise and outliers as compared to other hierarchical clustering methods.

No.12

Assignment of condition and P-values to clusters

- Calculated a P-value for each experimental condition (Tavazoie et al., 1999).

$$P_{\pm 1} = 1 - \sum_{i=0}^{k-1} \frac{\binom{C}{i}\binom{G-C}{n-i}}{\binom{G}{n}}$$

  - *G* is the number of all strain data.
  - *C* is the number of the selected group of strains.
  - *n* is the number of strains with a value of +1 (or -1).
  - *k* is the number of strains with a value of +1 (or -1) within the selected strain group.

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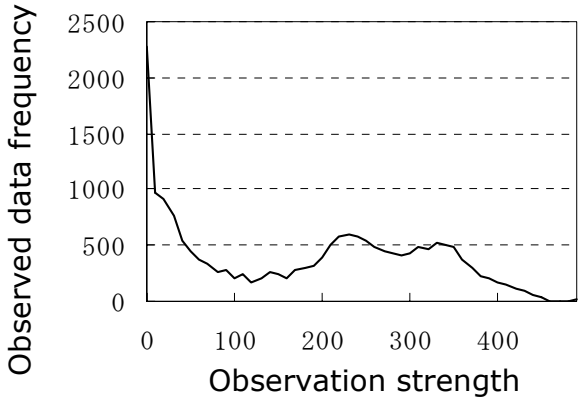
Results !

Selection of threshold for zero-substitution

- The value of 100 was set as the threshold for zero-substation.
  - Low observation strength may lead to unstable experimental measurement.



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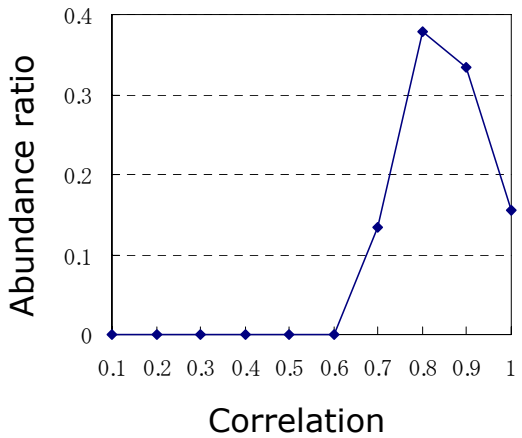
Repeatability of the PM data

- Calculated Pearson correlation coefficients between PM data for 10 trials in the wild-type.
  - 0.67 ～ 0.96 (Ave. 0.81)



High repeatability

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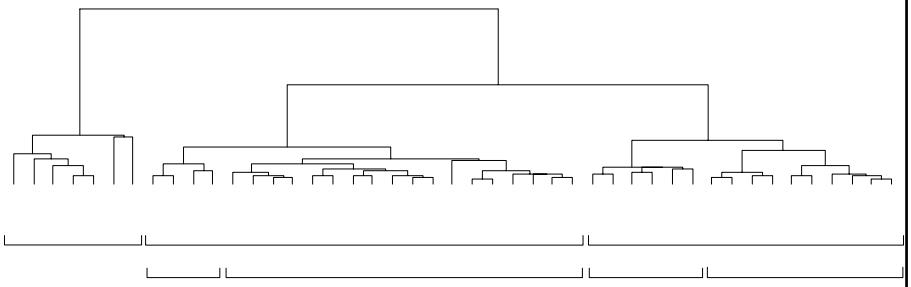
Clustering Result

- 45 single-gene-knockout mutants in central metabolism under 288 condition.
- Three major clusters C_1 to C_3 were obtained.



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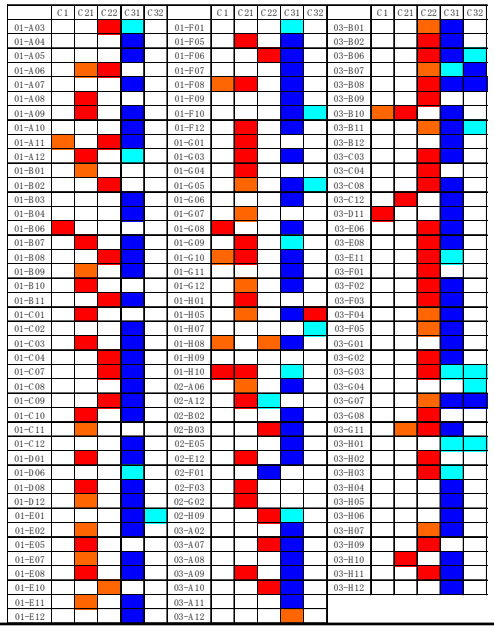
Characteristic growth condition



Phenotype profiles of these five clusters.

- P_{+1} <= 0.05
- P_{+1} <= 0.1
- P_{-1} <= 0.05
- P_{-1} <= 0.1

C_2 group activated cellular respiratory activity.

C_3 group repressed cellular respiratory activity.

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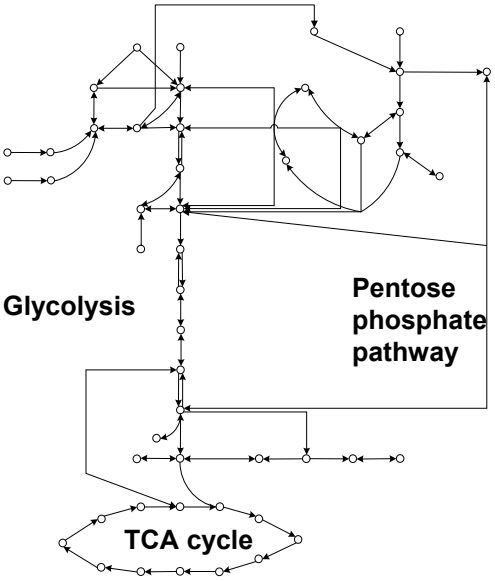
Distribution of gene-knockout affects



Cluster C_1: green
Cluster C_{21}: red
Cluster C_{22}: pink
Cluster C_{31}: blue
Cluster C_{32}: light blue

- The mutants of cluster C_1 are located at the early stage of the glycolysis.
- Four mutants in cluster C_{31} are closely related to the TCA cycle.

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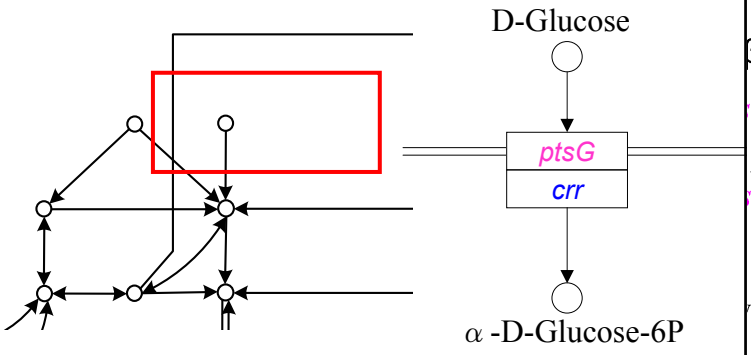
Analysis result for Phoshotransferase system

- PtsG and Crr form enzyme II complex as PTS (phosphotransferase) system .
- However, deletion of *ptsG* and *crr* genes affect opposite direction in phenotype profiles.



- Crr might function as switching for further steps after transportation of Glucose.

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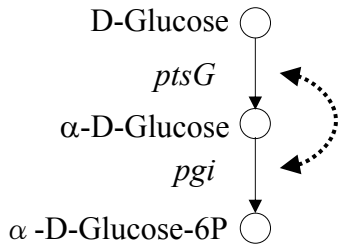
Phenotype Similarity and Pathway Distance (1)

- What kind of relationship exist between them ?
- Minimal pathway distance : The number of the compound on shortest path between given genes.



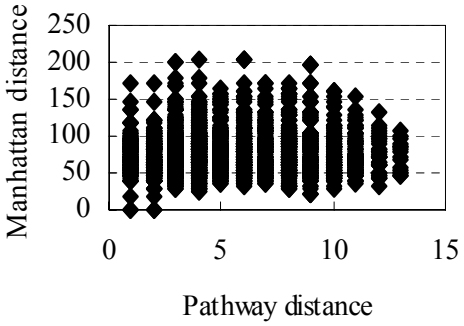
Between *ptsG* and *pgi*
Distance: 1

- Major metabolic path data is represented one adjacency matrix of a directed graph.
- The step between the two compounds in the same metabolic map can be extracted using shortest paths algorithms.

No.21

Phenotype Similarity and Pathway Distance (2)

- We calculated the minimal pathway distance for all strain pairs whose knockout genes are involved in central metabolism.
- For established pairs, phenotypic similarity were determined.



- The results showed no correlation between them!

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Conclusion

- We performed to analyze further insight into central metabolic pathway network to PM data.
  - Medium conditions that activate or repress cellular respiratory activities for the different strain groups were identifies.
  - These results suggested the possibility of metabolism steps with unknown bypass route.
- However, our proposal methods have insufficient sensitivity to continue to identify functions of genes of uncertain function of to analysis for further large-scale data.
  - Robustness
    - Alternative passes
    - Bypass route
    - Unknown passes

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Future works

- Computational method for prediction about bound strength among known reactions.
- Double gene knockout experiments.
- Combination PM and another high-throughput data.

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Thank you very much for your attention.