

Galactomyces Geotrichum MK017: In Silico Model of Succinic Acid Metabolic Pathway

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INTRODUCTION

- Succinic acid or C4-dicarboxylic acid produced by *G. Geotrichum* MK017 is precursor used for preparation of pharmaceuticals, antibiotics, vitamins, surfactants, detergents and food (Fig. 2).
- Metabolic pathways are constructed by using strategy to predict metabolic pathway from a reference database (Table 1) of known pathways (metabolites, enzymes, reactions) by using known genome sequence to obtain key informations about metabolism and physiology of *G. Geotrichum* to develop static model.
- Metabolic computational tools were used to gain insight into the intracellular metabolic conditions of *G. Geotrichum* MK017, including metabolic flux analysis (MFA), probabilistic models of network structure and network accuracy.
- The concept of network based Statistical Inference were used to construct probabilistic Models of Network Structure (the Inferrelator, Probabilistic Boolean network, Dynamic Bayesian networks), evaluate network accuracy (predicted and true network) were used in modelling of *G. Geotrichum* Succinic acid Metabolic pathway (Fig. 1).

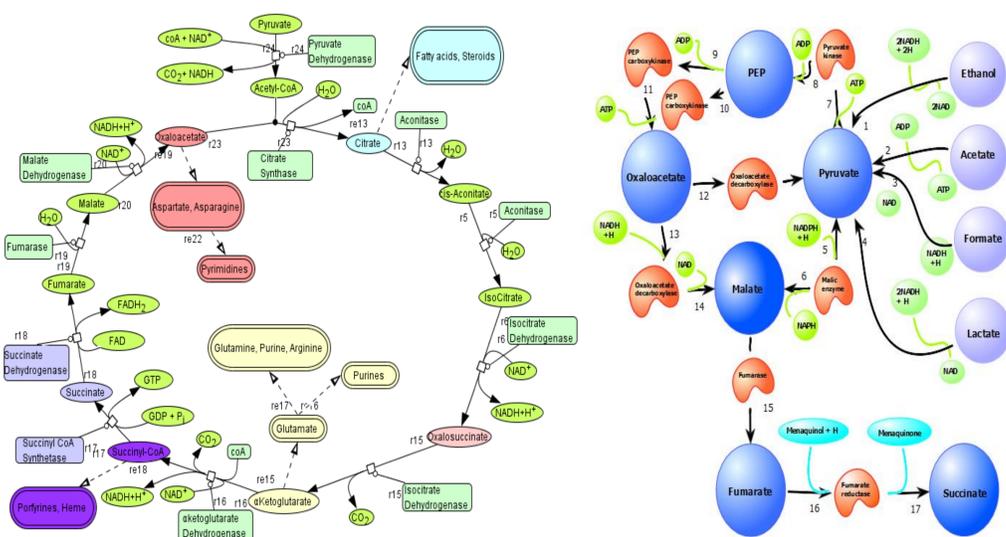


Fig. 1 Citric acid cycle and succinic acid metabolic pathway

Succinate is important medicinal product of citric acid cycle. At each turn of the cycle, three NADH, one FAD₂, one GTP (ATP), two CO₂ are released in oxidative decarboxylation reactions. Acetate reacts in the form of acetyl-coenzyme A. The effect of one turn of the cycle is: 2 acetate → succinate + 2H

Table 1. Classification of databases used in silico modeling of pathways

	MetaCyc/BioCyc	KEGG PATHWAYS	BioCarta	BioModels
Curation	Manual and automated	Automated	Manual	Manual
Size	~621+ pathways	~289 pathways	~355 pathways	~126 models
Nomenclature	EC, GO	EC, KO	None	GO
Organism coverage	~500 species	Various	Primarily human and mouse	~475 species
Visuals	Species-specific custom	Reference and species-specific	Animated, cartoonish	Non-standardized
Primary usage	PGDB, comput. biology	PGDB, pathway comparisons	Human pathways, disease	Simulations, modeling

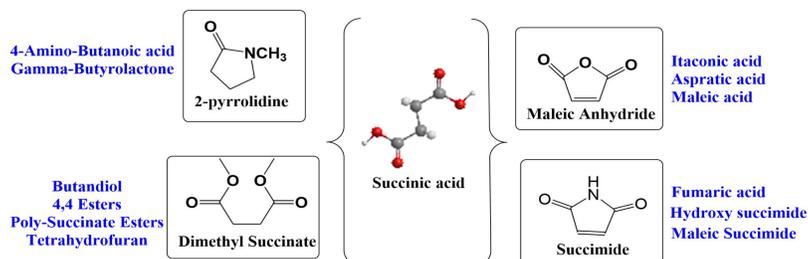


Fig. 2 Pharmaceuticals synthesized from succinic acid metabolic pathway

METHODS

- Succinic acid and other metabolites of metabolic pathway were analyzed by LC-Q-TOF-MS system (UHPLC-qTOF-MS system (Agilent Technologies, Waldbronn, Karlsruhe, Germany) that consisted of a 1260 LC system, a Jetstream electrospray ionization (ESI) source, and a 6540 UHD accurate-mass qTOF spectrometer. A Poroshell EC-C18 column (2.1 × 100 mm, 2.7 μm, Agilent) was used for compound separation. The mobile phase was composed of 0.1% acetic acid, 0.01% formic acid (Solvent A) and MeOH (Solvent B). Data analysis like normalization, T-testing, analysis of variance (ANOVA), clustering were performed in OriginPro 2016 (OriginLab, NorthHampton, USA), STATISTICA 10 (STAT, Tulsa, OK) (Fig. 3, 4).
- Databases and software used for building system models -genome annotation data (Entrez Gene, GOA, Uniprot), biochemical pathways & functional associations (KEGG, GO, the SEED, MetaCyc, BioCyc, Transport DB), Regulatory Sequences (EPD, TRED), model, model parameter repositories (DQCS, BioModels) and Computer-Aided Design (CAD) tool for synthetic biology Cytoscape_v 3.3.0 (NIGMS, Bethesda, USA), Celldesigner 4.0 (SBGN, Germany), Maven (Rabinowitz Lab, Princeton, USA), BioClipse (Upsalla, Sweden), R-Studio 3.3.1 (Boston, USA).

CONCLUSION & REFERENCES

- Antifungal transport proteins carriers, channels, pumps with pharmaceutically important properties were determined. The energetics and kinetics of carriers fumarate/succinate were characterized in *G. Geotrichum* MK017 (Table 3).
- Hong, S.H. and Lee, S.Y. Metabolic flux analysis for succinic acid production by recombinant *Escherichia coli* with amplified malic enzyme activity, *Biotechnol. Bioeng.*, 74(2):89, 95, 2001.
 - J.O. Kröwer, J.O. Nielsen, L.M. Blank, *Metabolic Flux Analysis*, Springer New York, 2014.

RESULTS & DISCUSSION

- Succinic acid pathway is formalized and analyzed by stoichiometric and constraint-based modeling techniques, including FBA, MFA (Table 2, Fig. 5). Several algorithms were provided for computational strain design/ metabolic engineering.

Table 2. Intracellular Succinic acid flux distributions

	SUCCINIC ACID FLUX
CO₂ consumption	22.8
NADH formation	31.5
Glucose consumption	86.4
Malic enzyme flux	60.1
PEP carboxyl flux	90.4

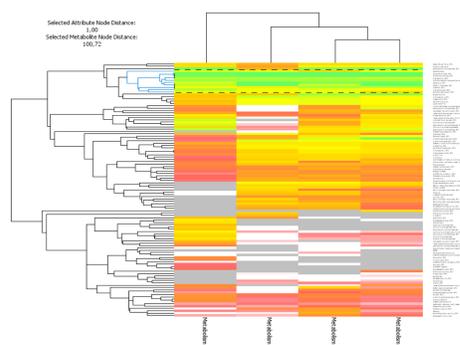


Fig. 3 Heatmap of metabolites from G.Geotrichum MK017

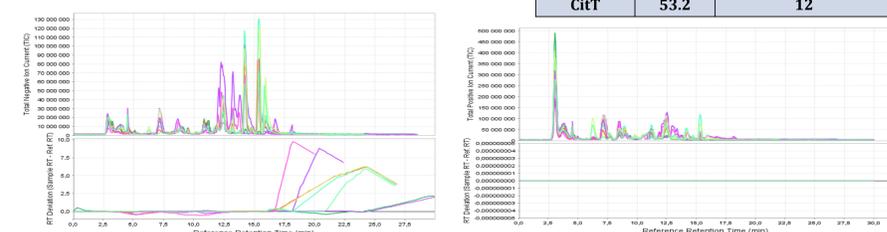


Fig. 4 POS, NEG mode LC-MS-QTOF and chromatograms of metabolites analyzed from G.Geotrichum MK017

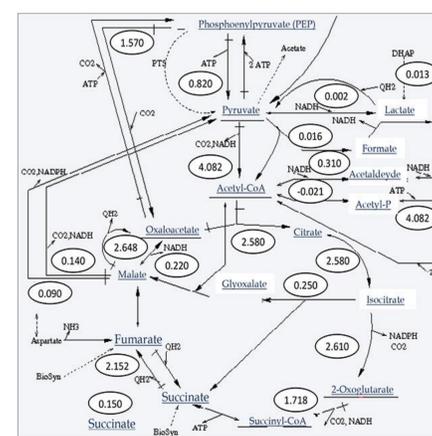


Fig. 5 Succinic acid flux analysis in G.Geotrichum MK017

Table 1. Succinic acid carriers from G. Geotrichum MK017

Carriers	Mr	Transmembrane Helices Predicted
DcuA-D	45-49	12-14
DctA	45.2	10-12
CitT	53.2	12

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