Metabolic re-adjustment acclimated to biotrophic elicitation in *Chlamydomonas reinhardtii*

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Abstract

Salicylic acid (SA) is a phenolic phytohormone, playing an important role in cellular growth and development in higher plant. SA induces defense mechanism through perplexed signal transduction in which NPR1 and WRK Y70 transmits biotrophic pathogenic cues to a wide range of SA-responsive genes. The complexity of the signaling is greatly incremented by the association with other signaling modules such as jasmonic acid and abscisic acid.

Accordingly, in the current study, we explored biochemical and physiological regulation in response to SA-induced biotrophic elicitation in a photosynthetic model organism, *Chlamydomonas reinhardtii*. First, blast homology searches proposed genetic proof of the functionality that all genes synthesize SA, and genes related to signaling cascades are present in C. reinhardtii.

Subsequently, we investigated SA-driven biochemical regulation at different growth phases in dose-responsive manner using mass spectrometry-based metabolite profiling. The metabolite profiling of multi-dimensional experiment revealed highly co-regulated central carbon-nitrogen metabolism, and further proposed key control points, which can be directly linked to secondary metabolism. Particularly, TCA cycle, lipid metabolism, and carbohydrate presented dose-dependent up-regulation, which pinpoint direct functional points by SA signal transduction, and may be differentiated from other signaling cascades such as jasmonic acid and abscisic acid..

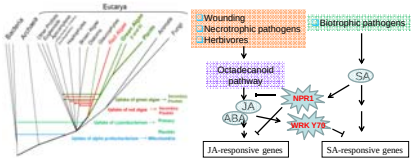
Experiment Designs

Chlamydomonas reinhardtii CC125

- Single-cell green algae lives in soil and fresh water
- Grow in defined media when supplied with illumination
- Cell cycle can be synchronized by alternating periods of light and dark
- Research in the biopharmaceuticals and the biofuel fields

Treatment I: Salicylic Acid (SA)

- Phenolic phytohormone and is found in plants
- It plays a roles in plant : growth and development
- Endogenous signaling in plant defense against pathogens



Objective

Elucidate biochemical functionality of the putative SA signaling pathways in time course and dose dependent analyses using non-targeted metabolomic approach.

Methods

Sample extraction and derivatization

Sample (1ml) were injected into 1mL of -20℃ methanol. The pellet was lyophilized in freeze dryer. Cells were disrupted and added extraction solvent, methanol : isopropanol : water (3:3:2; v/v/v). The supernatant were collected and concentrated to dryness in a vaccum concentrator. The dried pellets, 5 μL of a solution of 40 mg/mL of 98% pure methoxyamine hydrochloride in pyridine (silylation grade; Pierce) were added and shaken at 30°C for 90 min, to protect aldehyde and ketone groups. Then, 45 μL of MSTFA and a mixture of internal retention index (RI) markers, fatty acid methyl esters (FAME), were added and shaken at 37°C for 30 min, for trimethylsilylation.

Mass-spectrometry analysis

The derivatized metabolite samples were analyzed by GC/TOF MS using an Agilent 7890B GC (Agilent Technologies, Wilmington, DE) coupled with a Pegasus HT TOF MS (LECO, St. Joseph, MI).

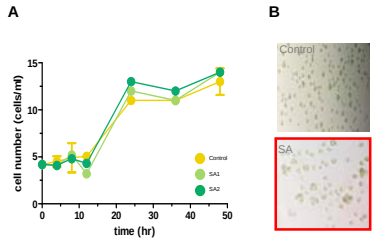
Data Processing

GC/TOF MS data were pre-processed using the LECO Chroma TOF software (ver. 3.34; St. Joseph, MI) to detect peaks and to deconvolute the mass spectra. The preprocessed data were processed using BinBase, an in-house programmed database built for metabolite identification.

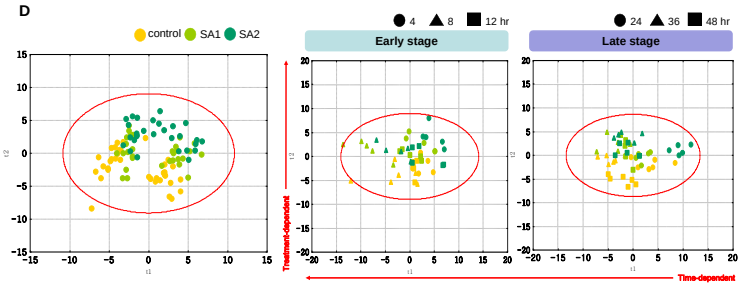
Statistical Analysis

Statistica (ver. 7.1; StatSoft, Tulsa, OK) was used for partial least square analysis (PLS) and univariate analysis. Hierarchical clustering analysis (HCA) and relevance network (RN) were performed using MultiExperiment Viewer (MeV, ver.4.8.1) to visualize and organize metabolite profiles. Pathway enrichment analysis and pathway topological analysis was performed by software R (ver. 2.8 on the linux).

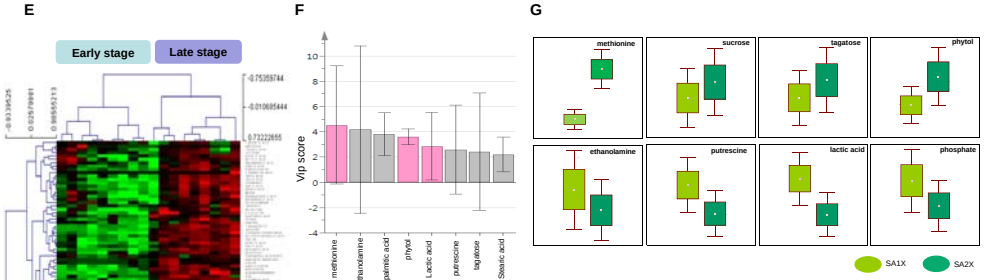
Results



(A) Cell Growth control and experiments Cell growth measured by cell number. SA1 were treated with salicylic acid 0.5mM. SA2 were two times of concentration of SA1. **(B) Morphological changes of *C. reinhardtii* by treatments** Cells were imaged under mid-exponential phage by microscope (400X). After adding SA cells were continuously divided after one cell cycle. **(C) Number of significantly changed metabolites** The number of significant metabolic alterations (p<0.05) in SA-treated cultures over 48hr. Red bars indicate increase and blue bars represent decrease in the abundances. Up-regulation of wide range of metabolites was observed in SA1 group while SA2 group showed suppression of the majority of metabolites.



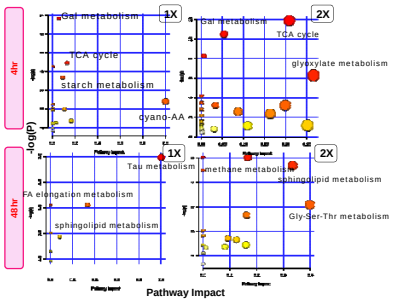
(D) Supervised multivariate statistics using partial least square (PLS) analysis of GC-TOF MS data Differential metabolic phenotypes were captured by SA treatment in dose-responsive manner. In addition, the score plot demonstrated time-dependent separation along with all sample groups according to vector 1 (t1). When the dataset was divided into early and late stages, the control group was clearly separated from the treated while the dose-dependent metabo-phenotype was partially recognized along with different culture time.

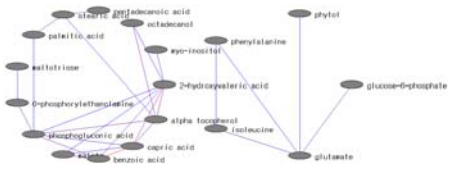


Compounds	VIP score
Methionine	4.53955
Ethanolamine	4.17185
Palmitic acid	3.81054
Phytol	3.60794
Lactic acid	2.85956
Putrescine	2.58006
Tagatose	2.41357
Stearic acid	2.20311
Sucrose	1.60478
Phosphate	1.15248

(E) Hierarchical clustering analysis (HCA) was performed to link the expression-based correlation with chemical/biochemical relevance. The statistics also confirmed relative linkage distance within same group and across different samples. **(F) The graphical readout of variable importance scores (VIP)** were performed for screening treatment-specific metabolite alteration indicates the most unique features among different treatment and time points (Vip score > 1). The detailed list is displayed in the table.

(G) Relative abundances of statistically different metabolites by VIP. Some of exemplary metabolites of VIP were depicted by box-whisker plot to present relative abundance and dynamic changes. Methionine and sucrose display dose-dependent up-regulation while ethanolamine shows opposite pattern.





(H) Pathway over-representation and topological analysis were conducted using Hypergeometry method and Relative-betweeness centrality. The analysis pinpoints dose-dependent down-regulation in TCA cycle, lipid metabolism, and carbohydrate metabolism, which can imply the direct functional points by SA signal transduction.

(I) Relevance network was constructed based on the coregulatory distance matrix in late stage Arbitrary threshold 0.4~1.0 was applied to build the network where oval presents metabolite and edge shows the correlation linkage using relevance network algorithm. Different edge color indicate the intensity of correlation.

Conclusions

1. Proof of biochemical activity of SA signaling pathway by MS- based metabolomic approach
2. Precise capture of time- and dose-dependent dynamics of metabolic responses to SA
3. Distinct metabolomic phenotype between the early and late stage
4. Discovery of signal transduction-specific metabolic pathway
5. Link to future study toward to secondary metabolism investigation

References

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