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Impact of Metabolomics in Symbiosis Research

Alba Chavez-Dozal and Michele K. Nishiguchi

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<http://dx.doi.org/10.5772/66631>

Abstract

In symbiotic associations, there is a constant molecular complexity that allows establishment and maintenance of the relationship. Metabolomic profiles have enabled researchers to explain symbiotic associations in terms of their underlying molecules and interactions between the symbiotic partners. In this review, we have selected studies on symbioses as examples that have helped to explain the metabolic integration of bacterial symbionts and their hosts in an effort to understand the molecular fingerprint of animal-microbial symbioses.

Keywords: symbiosis, mutualism, metabolomics, co-clustering analysis

1. Introduction

The intimate association between two organisms is a very complex biological phenomenon; nevertheless, it is a very common way of life for every living organism on Earth. Symbiotic associations with one or many phylogenetically different organisms provide a fascinating view into how symbionts adapt and co-evolve. As Chaston and Douglas beautifully described in their comprehensive review [1], the omics revolution has transformed our ability to understand symbiotic associations at the molecular level. Researchers have adopted multiple techniques with great fervor in an effort to decipher the basis and complexity of symbiotic associations. Until recent years, the molecular pathways of symbiotic associations could only be studied in the context of genetic changes (transcriptomic studies) and protein profiles (proteomics); however, it is very likely that the establishment of a mutualistic association involved multiple evolutionary changes in the biochemistry and metabolic network of all the partners involved in the symbiosis [1]. Omics biology brings challenges and opportunities; one of the recent advances is the ability to construct a molecular metabolic catalog of an organism within a symbiotic association.

Metabolomics refers to the analytical approach used to study different cell products (“chemical fingerprints”) that help to understand the physiological state of an organism [2].

In this section, we provide a comprehensive description of four experiments where the approach of metabolomics was selected in a particular type of animal-microbial symbiosis, in order to answer specific questions in symbiosis research.

2. Exemplars of metabolomic approaches in symbiosis research

2.1. Inferring metabolic interactions in arbuscular mycorrhizal symbiosis

Our exemplar of metabolomics studies of microbe-plant interactions is a set of observations by Schweiger et al. [3] that describe species-specific leaf metabolic responses to arbuscular mycorrhiza (AM) [4]. Arbuscular mycorrhiza is a unique symbiotic association between root arbuscular mycorrhizal fungi (AMF) and plants [4]. This is an ancient and widespread association where the fungus improves water uptake to the host plant, and in return the fungus receives plant carbohydrates. The fungus is restricted to the roots of the plant; however, the biochemical pathways and the involvement of exchanged substances are reflected on systemic root tissues affecting the chemical composition of plant tissues (defined as “phyto-metabolome”) [4].

Comparative studies conducted on five different plant-AMF associations demonstrate that foliar metabolome is highly plant-species-specific, with low degrees of conservation across species. The experimental design was crucial to the success of this analysis, with the metabolome analysis performed on leaves of five plant species exposed to the worldwide distributed AMF *Rhizophagus irregularis*. Furthermore, the study took into account the implications of metabolite fluctuation at different leaf developmental stages and plant-reproductive status. Additionally, mycorrhizal plants were compared with control plants that received a sterilized inoculum. The results from this study indicate the high specificity of plant metabolome responses to the same AMF colonization; among the most striking findings indicate that metabolomics responses related to phosphate uptake, citric acid cycle, and amino acids were species-specific [3]. **Figure 1** summarizes the most important findings of this interesting study.

2.2. Metabolomic profile of the ryegrass-endophyte symbiosis

Along the lines of microbe-plant interactions, there is an interesting study conducted by Cao et al. [5] that is of particular relevance for symbiosis research. The metabolomics profile of perennial ryegrass (*Lolium perenne*) infected with endophytic fungus (*Neotyphodium lolii*) provided understanding of regulatory biochemical mechanisms for the production of beneficial alkaloids.

N. lolii is a naturally occurring fungus whose complete cycle occurs within perennial ryegrass. The fungus grows between the cells of the host plant drawing nutrients from it, and in return, the endophyte produces chemical compounds that provide resistance to drought, pests, and protection from overgrazing. Therefore, the aim of this study was to gather metabolomics information and combine it with microarray data in order to obtain a better understanding of

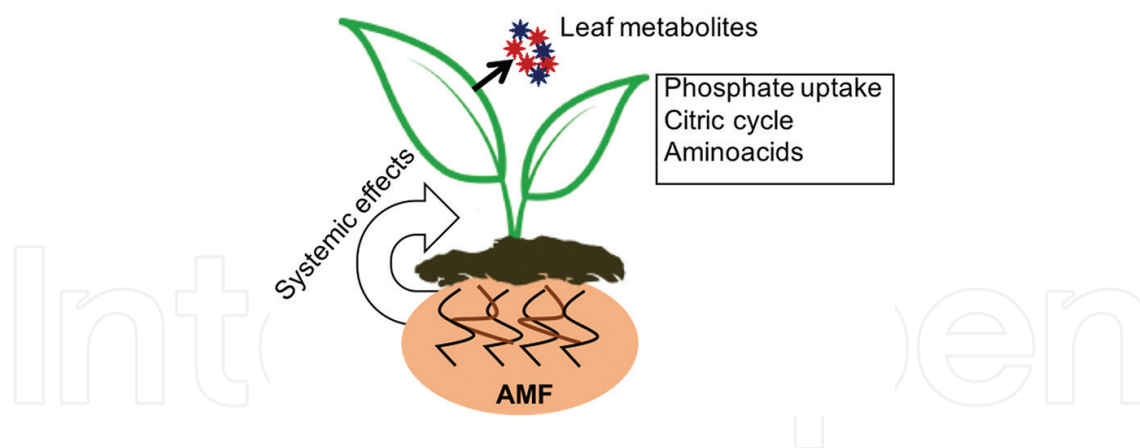


Figure 1. Summary of findings on the leaf phytometabolome when plants are exposed to the same arbuscular mycorrhizal fungi (AMF). Leaf metabolites detected included carbohydrates, organic acids, amino acids and derivatives, cyclic polyols, and sugar alcohols. Metabolites are differentially regulated primordially affecting the phosphate and citric acid cycles.

the biochemical mechanisms involved in the cross talk between partners, with the eventual purpose of achieving genetic manipulation of beneficial metabolite production (in particular manipulation of alkaloids).

Twenty-four perennial ryegrass samples comprising three tissue types (immature leaves, blades, and mature leaves) were examined of both endophyte-infected plants and endophyte free as a control. Targeted metabolomics analysis was used as the quantitative approach that provided identities of 70 metabolites based on the available databases of reference compounds. The use of targeted metabolomics in combination with microarray data provided better identification and classification Accuracy of compounds, as well as greater insights into the dynamics and fluxes of the newly identified metabolites. Results of this comprehensive study included the identification of accumulated alkaloids in the mature tissues of endophyte-infected ryegrass, and the co-clustering analysis of microarray data-identified genes with distinctive expression patterns which coincide with the pattern of alkaloid accumulation [5]. **Figure 2** summarizes the findings of this study. Results of this study indicate that co-clustering analysis is not a straightforward task no matter what kind of algorithm is used, and that the integration of transcriptomics and metabolomics can generate noisy data. However, this study demonstrated that co-cluster analysis could be a comprehensive choice to gain a more complete understanding of a complex biological system involving two entirely different taxa that are intertwined in their metabolic capabilities.

2.3. Metabolomic profile of symbiotic protection against pathogens

It is believed that specific strains from the gut microbiota can influence host immunity and protect from infection by pathogenic bacteria. One example is the early and prevalent gut colonizer *Bifidobacterium*, which is considered part of the healthy normal gut flora. It is believed that different strains of *Bifidobacterium* protect against enteropathogenic *Escherichia coli* O157:H7 infection in mice; however, the potential molecular and cellular mechanisms underpinning this protective effect are still under investigation [6].

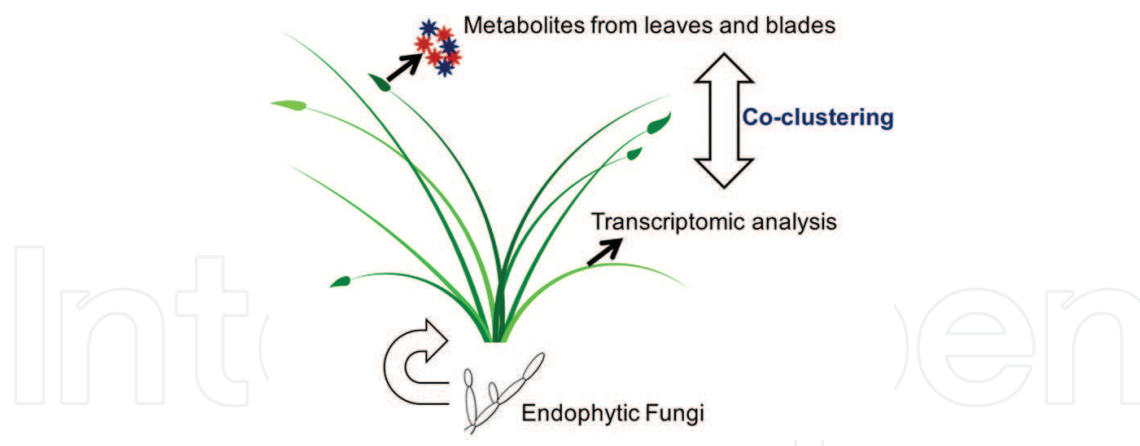


Figure 2. Summary of the study conducted by Cao et al. Co-clustering analysis of microarray and metabolomics data on endophytic-infected ryegrass indicate a set of genes and metabolites that are important for alkaloid production.

One study conducted by Fukuda et al. [7] used a combined “omics” strategy in an effort to gain a better understanding of the protective effect of *Bifidobacterium* over its mice host. Experiments designed comprised mice infected with different species of the symbiotic bacterium *Bifidobacterium* (including *B. longum* and *B. adolescentis*) and the pathogen *E. coli* O157:H7. The life span of co-infected mice was observed and transcriptomic and metabolomic profiles were conducted. **Figure 3** diagrams the experimental design of this study. This sophisticated analysis included a combination of sequencing, the platform used for metabolite detection was HPLC-MS (high-performance liquid chromatography-mass spectrometry) and for the analysis of products, the dataset was subjected to a multivariate analysis method named PLS (partial least squares) projection to latent structures. Typical data-processing flow included detection of signal peaks and normalization of dataset to generate a matrix of the products detected. For their statistical analysis, the method selected was PCA (principal component analysis) and CL (cluster analysis).

Results from this study indicate that mice bearing the strain *B. longum* survived, whereas those infected with *B. adolescentis* died. Metabolomic profiles between the two treatments revealed that the concentration of fatty acids (acetic acid in particular) was significantly elevated in those mice that survived *E. coli* infection. Furthermore, mice that survived showed an increased expression of genes involved in ATP-binding-cassette carbohydrate transporters [7]. Observations from the study suggest that the elevated production of acetic acid improved intestinal defense, thereby enhancing the barrier function of colon epithelial cells inhibiting the transport of *E. coli* toxins.

2.4. Metabolomics of a beneficial marine bacterium

The marine luminescent bacterium *Vibrio fischeri* establishes a symbiotic association with numerous sepiolid squids and monocentric fishes. *V. fischeri* infects a specialized light organ in the mantle (body) cavity of host squids and produces bioluminescence that is used by its host to avoid predation in a behavior known as counterillumination. In return, the squid host provides an enriched habitat for *Vibrio* to reproduce and to form bacterial communities of

monospecies biofilms. The ability of *V. fischeri* to form a biofilm in the light organ of its squid host plays a central role in establishment and maintenance of the symbiotic association. This interesting symbiotic association has been the center of attention of many researchers, and has been investigated for more than 25 years; however, as indicated for other examples of mutualistic associations, the molecular basis of the squid-*Vibrio* symbiosis is still obscure.

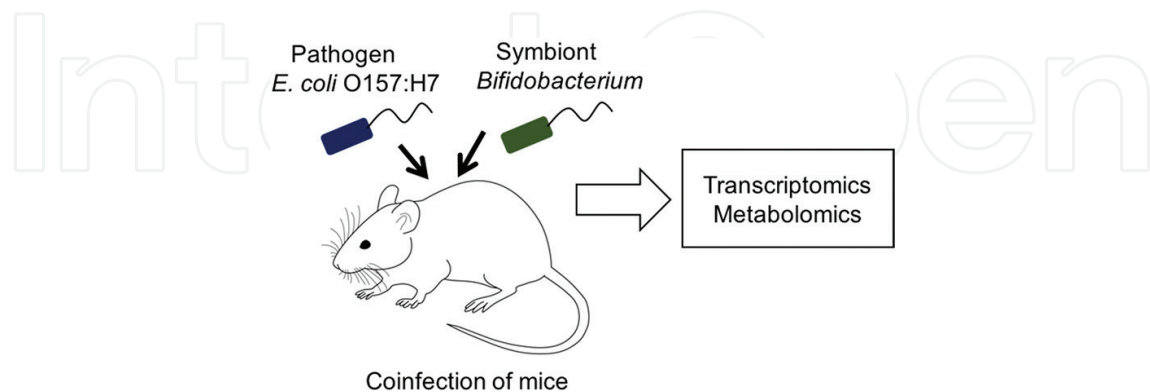


Figure 3. Summary of the experiment conducted by Fukuda et al. Mice were coinfectd with beneficial strains of *Bifidobacterium* and the pathogenic strain of *Escherichia coli* O157:H7. Combined transcriptomic and metabolomic profiles revealed an increase in acetate and fructose transporters in those mice that survived lethal infection.

In a recent study conducted by Chavez-Dozal et al. [8], both proteomic and metabolomic profiles were performed in parallel in strains of *V. fischeri* in their biofilm form and compared to profiles of free-living (or planktonic) *V. fischeri* cells of the same strain. The main objective of this study was to obtain a comprehensive profile of the molecular components to provide the first meta-proteome profile of biofilms that are important for establishment of this mutualistic association. A summary of this study is illustrated in **Figure 4**.

Biofilms are a complex microbial community composed of cells encased within a self-produced exopolymeric matrix. Expression profiles of biofilm communities reveal the composition of the matrix, which include a combination of lipids, polysaccharides, proteins, and DNA [9, 10].

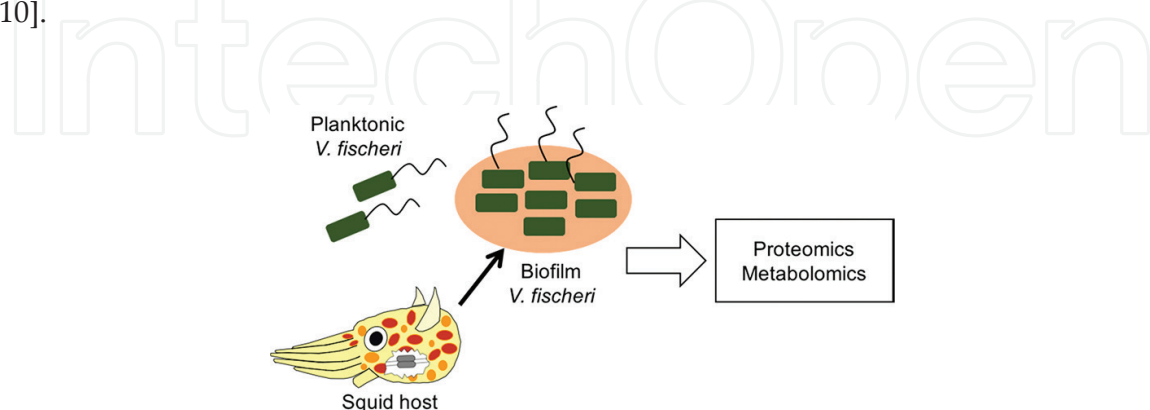


Figure 4. Summary of the experiment conducted by Chavez-Dozal et al. [5]. Proteomic and metabolomic profiles were performed in planktonic cells and biofilm communities of the same strain of *Vibrio fischeri*. Results revealed an upregulation of biofilm matrix components and molecules related to multiple stress responses.

Results of this study revealed a time-resolved picture of approximately 100 proteins and 200 metabolites present in the biofilm state of *V. fischeri*. The most important components found in this study include proteins, sugars, and molecules that form part of the exopolysaccharide matrix of biofilms; surprisingly, an increased concentration of intermediates of the glycolysis pathway was found to be prevalent during the biofilm state [8]. Results from this study suggest that molecules involved in the construction of the biofilm matrix are essential to bacterial community formation, a process that has been known to activate stress responses such as upregulation of alternative anaerobic pathways. The reported findings of this study have broad implications for *V. fischeri* ecology, since many of the symbiosis-regulated genes are not yet described. The combination of proteomics and metabolomics has therefore provided a link between protein regulation and function during different phases of the symbiosis, improving our understanding of the mechanisms that are important for successful host colonization.

3. Concluding remarks

Metabolomic approaches are increasingly selected for multiple purposes of symbiosis research. Although other “omic” approaches are needed to understand molecular function in symbiotic associations, the emerging use of metabolomics provides a new level of biochemical sophistication. The different examples provided in this mini review are only some of the pillar studies that included the use of either metabolomics or a combinational analysis of metabolomics with transcriptomics/proteomics of different mutualistic systems; however, many more studies are in progress using metabolomics profiles to define and characterize molecular and biochemical pathways that are important for establishment and persistence of symbiotic associations. The advancement of technologies that allows higher resolution of minute concentrations of proteins and their modulation will expand the area of metabolomics research and will enable a better perspective of the physiological state of organisms as single entities (otherwise known as the holobiome).

Author details

Alba Chavez-Dozal and Michele K. Nishiguchi*

*Address all correspondence to: nish@nmsu.edu

Department of Biology, New Mexico State University, Las Cruces, NM, USA

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