Enhancing Lyme Disease Research through LC-MS-based Metabolomics

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Introduction

Ticks are vectors for a variety of pathogens, including Borrelia burgdorferi, the causative agent of Lyme disease, and Staphylococcus aureus, a common bacterial pathogen. The role of recombinant *I. scapularis* with a Reeler domain (PIXR) is a recombinant protein with potential antibacterial properties, and its effects on various bacterial strains and tick gut microbiota are of interest. This study aims to To evaluate the impact of rPIXR on bacterial viability, biofilm formation, and gut microbiota composition, employing advanced molecular biology techniques and **metabolomic analyses** facilitated by Creative Proteomics.

Methods and Instruments

Sample Preparation

- Sample Collection: Gut samples from ticks fed on either rPIXR-immunized mice or Ovalbumin-immunized control mice were meticulously collected to ensure consistency and reliability.
- Storage: Samples were stored at −80°C to preserve metabolic integrity and prevent degradation.
- Metabolite Extraction: Metabolites were extracted using a 75% methanol solution to efficiently solubilize a wide range of metabolites. An internal standard, 2-Chloro-L-phenylalanine, was included to normalize the data and account for variability in sample processing.

Analytical Techniques

- UHPLC-QTOF-MS:

Process: Samples were analyzed using a high-resolution UHPLC-QTOF-MS system, which provides detailed chromatographic and mass spectrometric data.
Advantages: High sensitivity and resolution, allowing for the detection of low-abundance metabolites and accurate mass measurements.

- ACQUITY UHPLC-QTOF:

Process: Utilized for detailed analysis, including the identification and quantification of specific metabolites of interest.
Advantages: Improved data quality and reproducibility, critical for reliable biomarker discovery and metabolic pathway analysis.

Data Processing

- XCMS: Peak identification and alignment.
- SIMCA: Multivariate analysis to identify significant metabolic differences.
- OPLS-DA: Discriminant analysis to reveal metabolic changes associated with rPIXR treatment.

Results

➢ Metabolite Identification by UPLC-MS

Dissected guts from nymphal ticks fed on PIXR-immunized or Ovalbumin-immunized mice and processed gut supernatants for UPLC-MS-based identification of gut metabolites. A total of 59 metabolites were identified on Positive mode (73 hits in total), and 64 metabolites were identified on Negative mode (85 hits in total) and the integrated list of the **123 metabolites** is shown in **Table 1**.

ESI-Positive Mode	
1-Stearoyl-sn-glycerol	L-Methionine
2'-Deoxyadenosine 5'-monophosphate(dAMP)	L-Phenylalanine
3'-O-methylguanosine	L-Pipecolic acid
4-Guanidinobutyric acid	L-Pyroglutamic acid
5-L-Glutamyl-L-alanine	L-Saccharopine
Acetylcarnitine	L-Tryptophan
Adenine	Maltotriose
Adenosine	N-(omega)-Hydroxyarginine
alpha-D-Glucose 1-phosphate	N,N'-Diacetylchitobiose
Arginiosuccinic acid	N6,N6,N6-Trimethyl-L-lysine
Betaine	N6-Acetyl-L-lysine
Biliverdin	N-Acetyl-D-Glucosamine 6-Phosphate
Creatine	N-Benzyloxycarbonylglycine
Cytidine	NG,NG-dimethyl-L-arginine(ADMA)
Cytidine 5'-diphosphocholine(CDP-choline)	Nicotinamide
D-Alanyl-D-alanine (D-Ala-D-Ala)	Nicotinate
Dimethylglycine	Oxypurinol
Dopamine	PC(16:0/16:0)
Glycerophosphocholine	Pristanic acid
Guanosine	Protoporphyrin IX
Hypoxanthine	Pyridoxal(Vitamin B6)
Indole-3-pyruvic acid	S-Adenosyl-L-homocysteine
Inosine	S-Adenosylmethionine
L-Arginine	S-Methyl-5'-thioadenosine
L-Asparagine	sn-Glycerol 3-phosphoethanolamine
L-Citrulline	Spermidine
L-Histidine	Taurine
L-Kynurenine	UDP-N-acetylglucosamine
	Uridine 5'-diphosphate (UDP)
	Uridine 5'-monophosphate (UMP)
	Xanthine

ESI-Negative Mode	
2,3-Dihydroxybenzoic acid	Glycolate
2'-Deoxyadenosine 5'-monophosphate(dAMP)	Glyoxylate
2'-Deoxyguanosine 5'-monophosphate (dGMP)	Guanosine 5'-monophosphate (GMP)
2'-O-methyladenosine	Guanosine diphosphate mannose
2-Oxoadipic acid	Indole
3-Hydroxy-L-kynurenine	Inosine
3-Hydroxypropionic acid (beta-lactic acid)	Inosine 5'-monophosphate (IMP)
5-Hydroxyindoleacetate	L-Arginine
5-L-Glutamyl-L-alanine	L-Glutamate
6-Phospho-D-gluconate	L-Histidine
Acetyl phosphate	Linoleic acid
Acetylglycine	L-Methionine
Adenine	L-Phenylalanine
Adenosine	L-Pyroglutamic acid
Arachidonic Acid (peroxide free)	L-Tryptophan
cis-9-Palmitoleic acid	Maltotriose
cis-Aconitate	Methylmalonic acid
Citrate	N-Acetyl-D-Glucosamine 6-Phosphate
Cytidine 5'-diphosphocholine(CDP-choline)	N-Acetylglucosamine 1-phosphate
Cytidine 5'-monophosphate (CMP)	N-Acetylneuraminic acid
D-Glucosamine 1-phosphate(Glucosamine-1P)	Palmitic acid
Dihydrouracil	Phosphoenolpyruvate
D-Maltose	Pyridoxamine 5'-phosphate
D-Ribose	Quinolinate
Eicosapentaenoic acid	S-Adenosyl-L-homocysteine
gamma-L-Glutamyl-L-valine	sn-Glycerol 3-phosphoethanolamine
Glutathione disulfide	Taurine
Glyceraldehyde 3-phosphate	Thymine
Glycerol 3-phosphate	UDP-N-acetylglucosamine
Glycerophosphocholine	Uracil
	Uridine 5'-monophosphate (UMP)
	Uridine diphosphate glucose(UDP-D-Glucose)
	Urocanic acid
	Xanthosine

Table 1. List of metabolites identified by Ultra performance liquid chromatography–tandem mass spectroscopic analysis of tick guts. ESI, Electrospray ionization mass spectrometry.

➢ Differential Representation

Heat map showing alterations in the profile of differentially represented gut metabolites between ticks fed on PIXR-immunized (PIXR 1 and 2-each sample representing a pool of 10 tick guts) or ovalbumin-immunized mice (controls 1 and 2 each-sample representing a pool of 10 tick guts) in the positive and negative mode (**Figure 1**). This visualization helps in identifying patterns of metabolic changes and clustering metabolites based on their differential abundance.

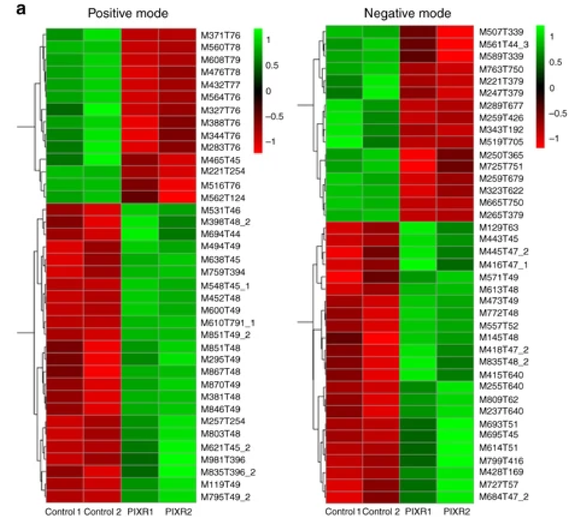
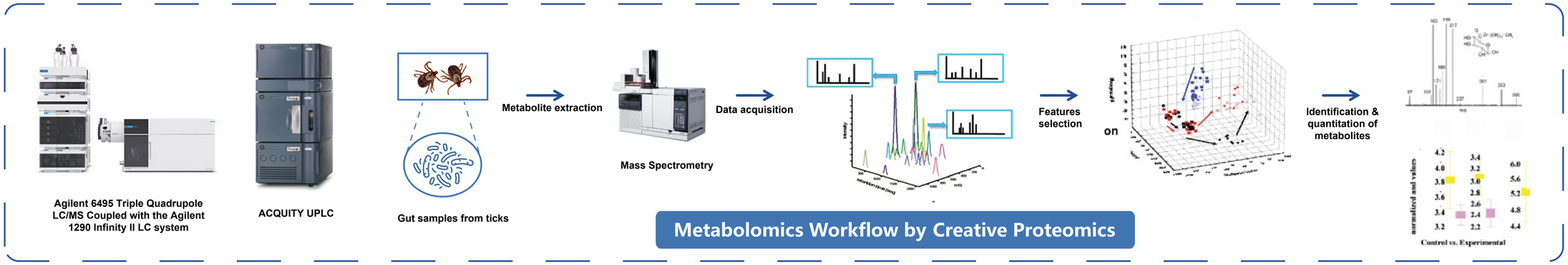


Figure 1. The heatmap of the differences in metabolite profiles between the two groups (PIXR-immunized vs. Ovalbumin-immunized)

Conclusion

- **Metabolic Identification:** The comprehensive identification of 123 metabolites revealed specific disruptions in key metabolic pathways, such as amino acid metabolism, lipid metabolism, and carbohydrate metabolism.
- **Biomarker Discovery:** Several metabolites showed significant changes between rPIXR-treated and control samples, suggesting their potential as biomarkers for rPIXR efficacy and metabolic impact.
- **Understanding Metabolic Responses:** The broad spectrum of identified metabolites provides a detailed view of the metabolic changes induced by rPIXR, facilitating a deeper understanding of its biological effects and underlying mechanisms.



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