

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 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		ESI-Negative Mode	
1-Stearoyl-sn-glycerol	L-Methionine	2,3-Dihydroxybenzoic acid	Glycolate
2'-Deoxyadenosine 5'-monophosphate(dAMP)	L-Phenylalanine	2'-Deoxyadenosine 5'-monophosphate(dAMP)	Glyoxylate
3'-O-methylguanosine	L-Pyrogutamic acid	2'-Deoxyguanosine 5'-monophosphate (dGMP)	Guanosine 5'-monophosphate (GMP)
4-Guanidinobutyric acid	L-Pyrogutamic acid	2'-O-methyladenosine	Guanosine diphosphate mannose
5-L-Glutamyl-L-alanine	L-Saccharopine	2-Oxoadipic acid	Indole
Acetylserine	L-Tryptophan	3-Hydroxy-L-tryptophan	Inosine
Adenosine	Maltonose	3-Hydroxy-L-tryptophan	Inosine 5'-monophosphate (IMP)
Adenosine	N,N'-Diacetylchitosamine	3-Hydroxy-L-tryptophan	L-Arginine
alpha-D-Glucose 1-phosphate	N6,N6,N6-Trimethyl-L-lysine	5-Hydroxyindoleacetate	L-Glutamate
Argininosuccinic acid	N6-Acetyl-L-lysine	6-Phospho-D-glucanate	L-Histidine
Betaine	N-Acetyl-D-glucosamine 6-Phosphate	Acetyl phosphate	Linoleic acid
Biliverdin	N-Benzoyloxycarbonylglycine	Acetyl phosphate	L-Methionine
Creatine	NG,NG-dimethyl-L-arginine(ADMA)	Adenosine	L-Phenylalanine
Cytidine	Nicotinamide	Adenosine	L-Pyrogutamic acid
Cytidine	Nicotinamide	Adenosine	L-Tryptophan
Cytidine 5'-diphosphocholine(CDP-choline)	Nicotinamide	Adenosine	Maltonose
D-Alanyl-D-alanine (D-Ala-D-Ala)	Oxyphenol	Adenosine	Methylmalonic acid
Dimethylglycine	PC(16:0/16:0)	Adenosine	N-Acetyl-D-glucosamine 6-Phosphate
Dopamine	Pristanic acid	Adenosine	N-Acetylglucosamine 1-phosphate
Glycerophosphocholine	Protoporphyrin IX	Adenosine	N-Acetylserine
Guanosine	Pyridoxal(Vitamin B6)	Adenosine	N-Acetylserine
Hypoxanthine	S-Adenosyl-L-homocysteine	Adenosine	N-Acetylserine
Indole-3-pyruvic acid	S-Adenosyl-L-homocysteine	Adenosine	N-Acetylserine
Inosine	S-Methyl-5'-thioadenosine	Adenosine	N-Acetylserine
L-Arginine	sn-Glycerol 3-phosphoethanolamine	Adenosine	N-Acetylserine
L-Asparagine	Taurine	Adenosine	N-Acetylserine
L-Citrulline	UDP-N-acetylglucosamine	Adenosine	N-Acetylserine
L-Histidine	Uridine 5'-diphosphate (UDP)	Adenosine	N-Acetylserine
L-Kynurenine	Uridine 5'-monophosphate (UMP)	Adenosine	N-Acetylserine
	Xanthine	Adenosine	N-Acetylserine

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.

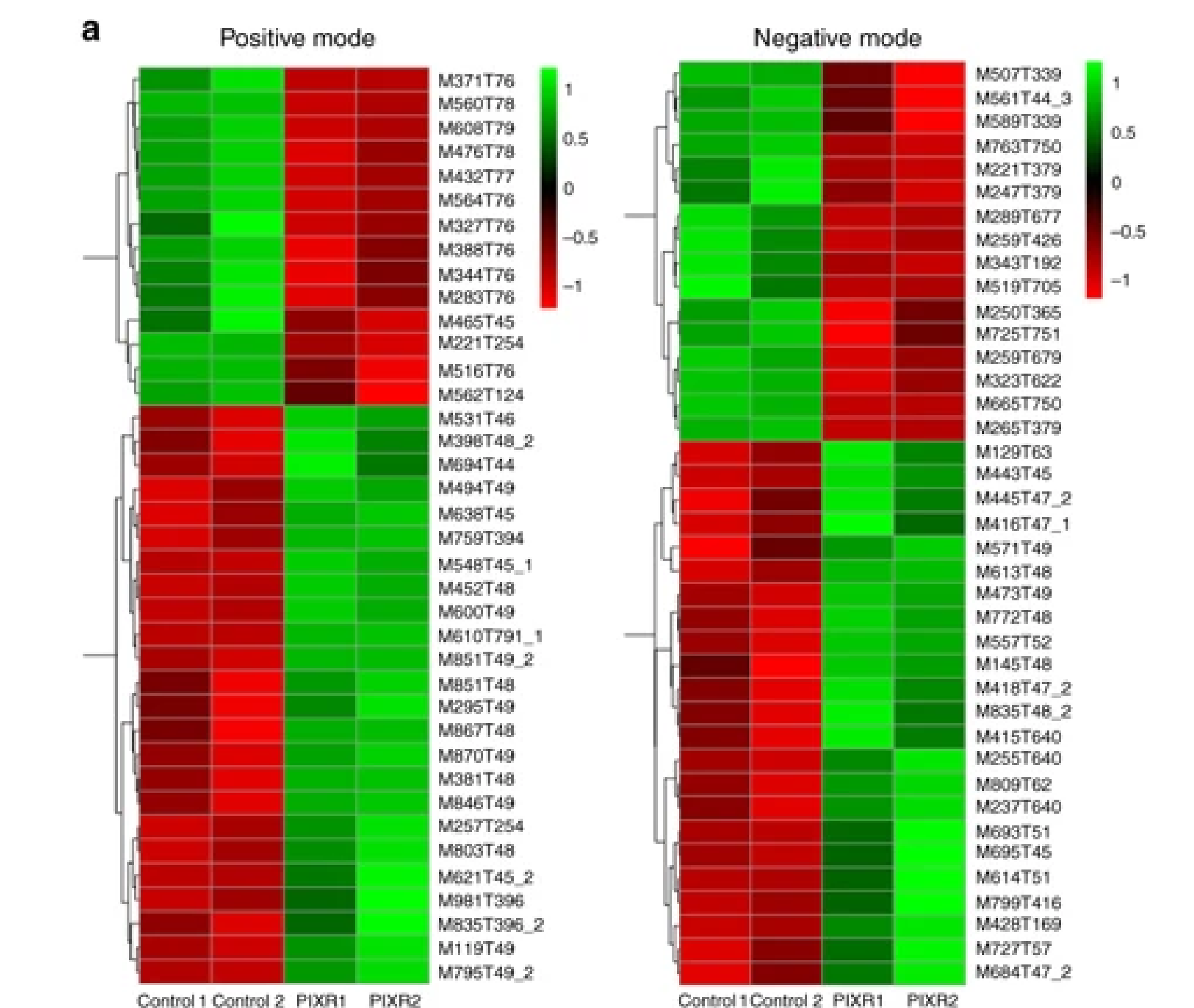


Figure 1. The heatmap of the differences in metabolite profiles between the two groups (PIXR-immunized vs. Ovalbumin-immunized)

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.

