Enhanced untargeted metabolomics workflow using LC-QTOF and Metaboscape for analysis of gut microbial metabolism

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Introduction

Untargeted metabolomics data analysis strategies must handle large data files and produce biologically and statistically relevant data. There is an unmet need for an efficient single bioinformatic solution for handling these data sets.

Metaboscape, Bruker's integrated untargeted metabolomics software solution, was used to analyze a model gut microbe system exposed to the synthetic hormones ethinyl estradiol and levonorgestrel found in common oral contraceptives. A comparison was made with the open source strategies XCMS and GNPS.

Results indicate significant down regulation of amino acids in Bacteroides fragilis, up regulation in Lactobacillus rhamnosus, and a mediating effect in a co-culture. This may indicate that supplementation of a probiotic may ameliorate the effects of synthetic hormones on gut microbial metabolism.

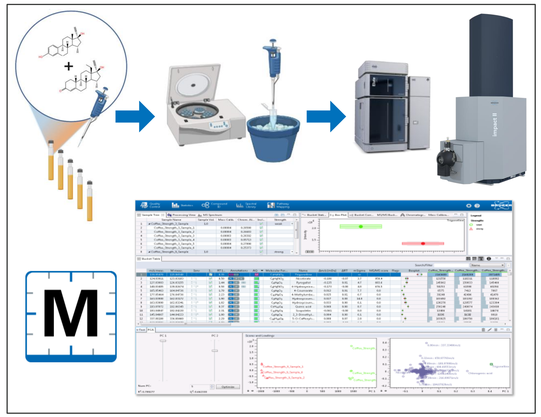

Fig. 1 Experimental design of gut microbe study. Anaerobic cultures are grown in BHI media until OD600 ≈ 1, washed in PBS followed by metabolite extraction. LCMS acquisition is performed in reverse phase with data dependent acquisition in positive mode. Data was analyzed using Metaboscape 2021b and an open source workflow.

Methods

Bacteroides fragilis and Lactobacillus rhamnosus were grown in anaerobic conditions and exposed to either ethinyl estradiol (E = 0.7 µM) and/or levonorgestrel (L1 = 3 µM, L5 = 50 µM, LE = 0.7 µM E + 3 µM L) versus control. Hormone/bacterial conditions were grown until late log phase. Cell culture pellets were washed in minimal media, followed by addition of 2:2:1 acetonitrile:methanol:water. Extracts were dried down and reconstituted in 1:1 acetonitrile:water proportional to protein concentration measured with a BCA assay.

Untargeted metabolomics data was acquired using a 20-min gradient on an Imtakt Scherzo-SM column coupled with a Bruker Impact II QTOF using autoMSMS in positive mode. XCMS was used to perform feature detection, retention time alignment and statistical analysis of metabolite features between hormone/bacterial conditions. GNPS was used to perform MSMS fragmentation pattern matching. Metaboscape 2021b was employed as a comparison for improved feature annotation and statistical analysis.

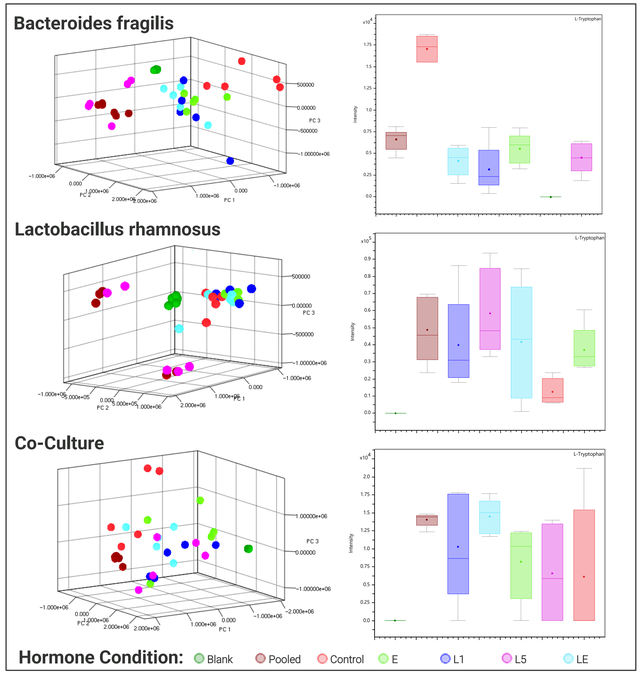

Fig 2. Statistical analysis of three different bacterial conditions and five hormone conditions. PCA results and t-test results (tryptophan example above) were assessed.

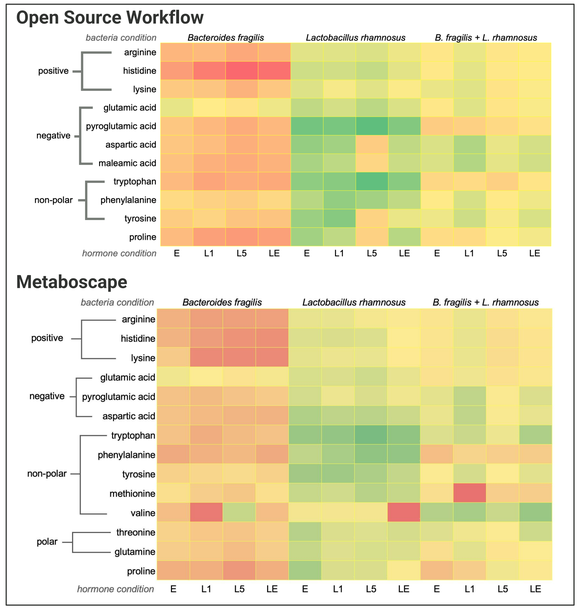

Fig 3. Heat map of annotated amino acids. General trend shows down regulation in *B. fragilis*, up regulation in *L. rhamnosus* and a mediating effect in *co-culture*.

Results

This study highlights the differences observed in metabolites in gut microbial cultures when exposed to different hormone conditions. An open source workflow using XCMS Online for feature analysis and GNPS for metabolite identification was compared with Metaboscape 2021b.

Multivariate PCA statistical analysis was performed with both XCMS and Metaboscape to highlight changes between hormone conditions. Univariate statistics showed up/down regulation was significantly altered in amino acids in both workflows.

Metaboscape was able to annotate 14 amino acids while XCMS/GNPS was only able to identify 11. Valine peak detection in Metaboscape was not complete for B. fragilis and LGG. However, this is likely a result of QTOF parameters and chromatography. A total of 431 unique features were identified using Metaboscape while this assessment could not be made in the open source workflow.

Summary

Data analysis using Metaboscape provides a rapid and facile workflow for analyzing untargeted metabolomics data (Fig 2).

Amino acids were identified as a major class of compounds that are dysregulated in gut microbes in the presence of ethinyl estradiol and/or levonorgestrel (Fig 2, 3).

These trends (not all statistically significant) show down regulation in amino acids in *B. fragilis*, up regulation in *L. rhamnosus* and a mediating effect in *co-culture* (Fig 3).

Trends were consistent between both the manual open source workflow using XCMS/GNPS and Metaboscape 2021b (Fig 3).

Amino acids are involved in neurotransmission and may impact downstream effects involving mood and behavior.

Metaboscape provides a more simplistic way to draw comparisons between sample classes in a user-friendly platform without the need for prior knowledge in programming.

More unique features are annotated with Metaboscape versus manual annotation with the open source workflow. In addition, isotopes and adducts are automatically bucketed into single features.

Metaboscape analysis was rapid, taking approximately one day to analyze compared to ~4 weeks using XCMS/GNPS.

References

- Gowda, H., et al., *Analytical Chemistry*, **2014**, 86(14), 6931-6939
- Wang, M., et al., *Nature Biotechnology*, **2016**, 34(8), 828-837

Conclusion

- Untargeted metabolomics was performed on gut microbial cultures using Bruker Impact II QTOF
- Metaboscape provides rapid annotation and statistical analysis compared to open source workflow
- Gut microbes have different regulatory effects on amino acid production in the presence of synthetic hormones.

Untargeted Microbiome Metabolomics