Studying the dynamics of the gut microbiota using metabolically stable isotopic labeling and metaproteomics.

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Abstract

Background: The gut microbiome and its metabolic processes are dynamic systems. Surprisingly, our understanding of gut microbiome dynamics is limited. Here we report a metaproteomic workflow that involves protein stable isotope probing (protein-SIP) and identification and quantification of partially labeled peptides. We also developed a package, which we call MetaProfiler, that corrects for false identifications and performs phylogenetic and time series analysis for the study of gut microbiome functional dynamics.

Results: From the stool sample of five mice that were fed with ^{15}N hydrolysate from *Ralstonia eutropha*, we identified 15,297 non-redundant unlabeled peptides of which 10,839 of their heavy counterparts were quantified. These peptides revealed incorporation profiles over time that were different between and within taxa, as well as between and within clusters of orthologous groups (COGs).

Conclusions: Our study helps unravel the complex dynamics of protein synthesis and bacterial dynamics in the mouse microbiome. Availability: MetaProfiler and the bioinformatic pipeline are available at <https://github.com/psmyth94/MetaProfiler.git>.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

However, the manuscript can be downloaded and accessed as a PDF.

Figures

Figure 1

Figure 1 Overview of Experimental Workflow Five 11-12 weeks old male mice were fed with a ^{15}N labeled mouse diet, where hydrolysate of the chemolithoautotrophic bacteria, *Ralstonia eutropha*, was the source of heavy nitrogen. Stool samples were collected at 10 different time points, were spiked with day 0 microbiome, and were processed by mass spectrometry. The mass spectrometric data was analyzed by OpenMS, MetaLab, and MetaProFiler to identify and quantify peptides and proteins and to establish profiles of ^{15}N incorporations over time.

A

B

Figure 3

Labeling Ratio of Proteins in Mouse Microbiome on Day 43. Phylogenetic tree where size and color of the nodes relates to the LR of the taxon; color of the cells corresponds to the phylum lineage; and the transparency denotes the rank. For nodes with annotations, the key follows the convention: [rst letter of phylogenetic rank][initials][unique identifier]. Nodes are reported when the number of distinct peptides is greater than 3 and the adjusted p-value from a one-sample t-test is below 0.05.

Annotation Legend:

pA: Actinobacteria	oD: Deferribacterales	fR: Ruminococcaceae	sAm: Akkermansia muciniphila
pB: Bacteroidetes	oE: Eggerthellales	gA: Akkermansia	sAs: Anaerotruncus sp. G3(2012)
pC: Chordata	oL: Lactobacillales	gA1: Anaerotruncus	sBv: Bacteroides vulgatus
pD: Deferribacteres	oR: Rodentia	gB: Bacteroides	sCs: Clostridium sp. ASF502
pF: Firmicutes	oV: Verrucomicrobiales	gB1: Butyrivibrio	sDs: Dorea sp. 5-2
pP: Proteobacteria	fA: Akkermansiaceae	gC: Clostridium	sEp: Eubacterium plexicaudatum
pV: Verrucomicrobia	fB: Bacteroidaceae	gD: Dorea	sFb: Firmicutes bacterium ASF500
cB: Bacteroidia	fC: Clostridiaceae	gE: Eubacterium	sLb: Lachnospiraceae bacterium A4
cB1: Bacilli	fD: Deferribacteraceae	gL: Lactobacillus	sLb1: Lachnospiraceae bacterium COE1
cC: Clostridia	fE: Eubacteriaceae	gL1: Lachnoclostridium	sLb2: Lachnospiraceae bacterium 28-4
cC1: Coriobacteriia	fE1: Eggerthellaceae	gM: Mus	sLb3: Lachnospiraceae bacterium A2
cD: Deferribacteres	fL: Lachnospiraceae	gM1: Mucispirillum	sLb4: Lachnospiraceae bacterium 10-1
cM: Mammalia	fL1: Lactobacillaceae	gO: Oscillibacter	sMm: Mus musculus
cV: Verrucomicrobiae	fM: Muridae	gP: Parabacteroides	sMs: Mucispirillum schaedleri
oB: Bacteroidales	fO: Oscillospiraceae	gR: Roseburia	sOs: Oscillibacter sp. 1-3
oC: Clostridiales	fP: Porphyromonadaceae	gR1: Ruminococcus	sPg: Parabacteroides goldsteinii

Figure 4

Functional Distribution of Heavy Labeled Proteins in Mouse Gut Microbiome on Day 43. The tree describes the functional distribution of each taxon. The size and color of the nodes relates to the LR of the taxon; color of the cells corresponds to the functional COG category; and the transparency denotes the rank. Nodes were annotated and reported in a similar fashion to figure 3.

A

B

Figure 5

Isotope Incorporation Profile. A) The top graph shows the distribution of RIA at each time point. Dark blue denotes a high density and light blue denotes a low density. At the bottom is a heatmap where the rows are peptides and the columns are the days at which the RIA was recorded. B) Similar to A except that it denotes labeling ratio over time. Both heatmaps were clustered using Ward's minimum variance, as implemented in [40], and Euclidean distance.

Figure 6

Taxon-Specific RIA and LR Proles Over Time. (A) phylum level RIA and LR proles over time; (B)

Average COG LR proles over time of the 12 most abundant genera.

Supplementary Files

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