A simulated metagenomic analysis of the gut microbiota of Anorexia Nervosa patients using PICRUST

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AUTHORS:Farhaan Kanji^{*1}, Himanshi Khurana¹, Caitlin Sherry¹ and Evangeline Ng¹.

[where * indicates the primary author.]

INSTITUTION:

1. Department of Science, McMaster University, Hamilton, ON

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Abstract

Introduction:

Mack *et al.* (2016) studied the fecal bacteria and archaea of 55 European normal-weight participants (NW), 55 European patients with anorexia nervosa (ANT1), and 44 ANT1 patients following a body mass index increase (ANT2). Spreadsheets of identified microbes and their relative abundance per patient were uploaded to the EBI Metagenomics web server by Mack *et al.* We aimed to further study the functions of the identified microbes using the PICRUST algorithm (Langille, 2013) and see if these functions are consistent with published literature.

Methods:

Spreadsheets were downloaded from EBI Metagenomics (Project# ERP012549) in JSON Biom format and uploaded to a Galaxy cloud server hosting PICRUST. All data transformations can be viewed at <http://hutterhower.sph.harvard.edu/galaxy/u/farhaansgroup/h/anorexi-astem-2017>. Transformed datasets were downloaded, appended with a .biom file extension, converted to the SPF format using STAMP v2.1.3 (Parks, 2014), and merged into a single file using Microsoft Excel for analysis with STAMP. Differences in propionate metabolism between ANT1, ANT2, and NW samples was chosen for further study.

Results & Discussion:

The proportion of propionate metabolism genes was not significantly different between ANT1 and NW samples ($p=0.08$), but was different between ANT2 and NW samples ($p=0.01$) using a pair-wise Welch's t-test (0.95 CI) with a Storey FDR multiple test correction. In comparison, Mack *et al.* detected no differences in propionate concentration between AN and NW fecal samples using gas chromatography while Morito *et al.* (2015) found lower concentrations of propionate in Japanese AN versus NW fecal samples using liquid chromatography.

Our discrepancy with Mack *et al.* could have arisen since PICRUST cannot analyze the genes of eukaryotes, PICRUST is limited by the depth and breadth of the gene annotations in the KEGG database, and our experimental setup cannot provide data on gene expression. Moreover, 18% of V4 16S rRNA DNA sequences could not be matched to any bacteria or archaea by EBI Metagenomics. In conclusion, while in silico experiments can be useful to predict microbial functions in a sample, in this case, our PICRUST-based hypothesis that fecal samples from Mack *et al.* would have different concentrations of propionate between AN and NW samples was not borne out by Mack *et al.*'s chromatography experiments. Nonetheless, the conflicting findings between us, Mack *et al.*, and Morito *et al.* warrants further research on whether microbes mediate carbohydrate metabolism differently in patients with a history of anorexia nervosa versus controls.

Keywords

microbiome, metagenomics, anorexia nervosa, carbohydrate metabolism

A Simulated Metagenomic Analysis of the Gut Microbiota of Anorexia Nervosa Patients using PICRUST

Liquification of 200 mg of fecal sample from normal-weight (NW), anorexia nervosa patients before (ANT1) or after a weight gain (ANT2)

Isolation of DNA using contaminant-binding buffers and centrifugation

Amplification of small portions of the DNA extracts corresponding to the bacterial and archaeal V4 16S rRNA gene ("V4 DNA") using PCR

The Illumina MiSeq determines the sequence of each DNA strand by copying each DNA strand using fluorescent dyes that can be detected by a computer

Genes Involved in Propanoate Metabolism

Patient-specific microbial counts were derived by matching experimentally-generated V4 DNA sequences with classified sequences in the Greengenes database. We further analyzed the microbial counts by matching each microbe from each patient to characteristic genes catalogued in the KEGG database using PICRUST.

A similar proportion of identified genes in ANT1 and NW samples were involved in propanoate metabolism, but a higher proportion were found in ANT2 samples versus NW samples. In contrast, Mack *et al* did not measure significantly different concentrations of propanoate between fecal samples using gas chromatography.

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