Introduction

- Research on the gut microbiome has focused predominantly on bacteria, even though fungi have been reported from the human faeces since the early 20th century (1).
- Fungi are estimated to account for only 0.01 – 0.1% of the faecal metagenome (2,3), typically dominated by Candida and Saccharomyces (4,5). Despite their alleged low abundance, a fungal cell is typically 100-fold larger than a bacterial cell, representing a greater overall biomass than that implied by the fungi’s DNA content (6,7).
- Fifteen studies spanning from 1917 – 2018 (including a study on ossified faeces) have reported 315 different fungal species detected with culture-independent and culture-dependent methods from healthy human faeces.
- Although ~87% of the fungi detected from the human gut have been reported only from single studies, some species are more commonly encountered than others (Figure 1), suggesting the existence of a resident human gut mycobiome.
- But few studies have attempted to determine which fungi are likely to be active in the human gut, and which are transient. Many fungi are unable to grow at physiological [37°C] temperatures.
- Different gut mycobiome profiles have been found in people from different countries (8), and climate is known to affect the colonisation ability of fungi (2). Fungi are ubiquitous in the environment (9); with diet appearing to have the strongest correlation with gut mycobiota composition (10-14).
- The link between fungal taxa and obesity has been explored solely in a small cohort by Rodriguez et al., suggesting that a decrease in fungal biodiversity, but not overall abundance, may help to differentiate between obese and healthy individuals, and discriminate those with poor glucose and lipid metabolism (7).

Aims

The diversity of the healthy human gut mycobiome in New Zealand population has not been previously explored. Our aim was to culture fungi from faeces of healthy individuals under conditions analogous to the human gut.

Methods

Eligibility criteria for recruitment:
(1) Overall good health status;
(2) Body Mass Index (BMI) >25 kg/m²;
(3) Aged 18 – 75 years;
(4) No use of long-term medications, probiotics, antifungal or antibiotic preparations, over-the-counter supplements, or non-steroidal anti-inflammatory or any other preparations known to have an effect on the gut.
(5) No gastrointestinal illness in the previous two weeks;
(6) No history of severe gastrointestinal disease.

Facial sample collection and culturing:

- Self-collected fresh faecal sample processed within 12h.
- Spread-plating of faecal aliquots on Sabouraud dextrose agar (SDA).
- Incubation for 14 days at 37°C in darkness, under aerobic and anaerobic conditions (achieved with BD GasPak™ Gas Generating Container System).
- Plates assessed visually every 4 – 7 days.

Isolation of pure fungal colonies:

- Colonies assessed visually, based on their gross morphology, and with lactophenol blue staining and light microscopy to determine whether they were mould, yeast or bacteria.
- Fungal colonies sub-cultivated onto fresh SDA plates to obtain pure colonies.

Molecular identification of fungal isolates:

- Extraction and amplification of 1.5 b-ITS2 region of the ribosomal DNA of the isolated pure fungal colonies with the REPOXtract™ N-Amyl Plant PCR Kit (Sigma-Aldrich).
- Sequencing of the amplified 1.5 b-ITS2 region (Sanger sequencing by capillary electrophoresis).
- Analysis of the sequenced fungal DNA with Genemapper programme; query sequences compared with known DNA sequences in a non-redundant database with Megablast programme.

Results

- The study recruited 21 participants that matched inclusion criteria (14 females, 7 males).
- In total, 88 fungal isolates from 21 study participants were cultured. The majority of isolates (93%) were sub-cultured from SDA plates supplemented with chloramphenicol and gentamicin, mainly under aerobic or near-anaerobic rather than totally anaerobic conditions.
- Pure cultures were not able to be isolated for 3 fungal species in the study, (one mould and two yeasts). Negative control plates showed no sign of contamination.

Conclusions

- By cultivating fungi at body temperature in anaerobic or near-anaerobic darkness, our results reflect fungi that are more likely to be active in the gut, and hence be regarded as autochthonous (resident).
- Comparison with previous studies shows consistency with the molecular identification results.
- Candida albicans was the most prevalent species cultured from 52% of the healthy human faeces in our study, which is consistent with this taxon being the most commonly reported worldwide, and with the predicted Candida albicans carriage rates of 30-60 % in the healthy human gut (4).
- Six species previously unreported from the healthy human gut by other researchers were identified in this study. Candida brocaciensis, Coniochaeta hoffmannii, Hanseniaspora pseudugilliermondii, Aspergillus fumigatus, Aspergillus tubingensis, and Paecilomyces dactylobrushus were all cultured from single individuals in our study.
- Aspergillus dermatitidis was cultured from three participants in our study, but has been reported only by Nash et al. (2) using culture-independent methods, suggesting the black yeast may be more prevalent in the healthy human gut of urban New Zealand residents, although the sample size is too small to be certain.

References