**Examining the source of fungi in the stool of mice**

Just as it is possible that all healthy human gastrointestinal fungi come from diet and saliva, the fungi detected in the GI tracts of other animals, including mice, may be derived from other sources. To briefly examine this, we amplified and sequenced the ITS2 from DNA isolated from mouth swabs, fur swabs and samples, and feces from two different cages of 9-week-old male C57BL/6J mice. In addition, cage bedding, pillows, and food that had not yet been exposed to mice were examined for fungi.

There were 21 ITS2 OTUs detected in stool samples, with no observed cage effect (Table S8). Fungi were largely undetectable from mouth and fur samples, however, 12 of the stool OTUs were present in fresh food, and one was found in the bedding. Together, these accounted for 77% of the stool reads. All remaining OTUs, except for *Aspergillus fumigatus* which was detected in two different stool samples, were only detected in individual mice. *A. fumigatus* is common throughout the environment, including in indoor air (Shelton et al., 2002). Therefore, the only fungi present in the stools of multiple mice were also present in their food, bedding, or possibly air, suggesting these lab mice did not possess fungi unique to their GI tracts.

**Methods**

Using the MO BIO PowerSoil kit, DNA was extracted from mouth swabs, fur swabs, fur samples, and feces of four 9-week-old male C57BL/6J mice from two different cages. In addition, DNA was extracted from cage bedding, pillows, and food never exposed to mice. The ITS2 region was amplified from the DNA samples, sequenced, and analyzed as described above. Animal care and experimental procedures were approved by Baylor College of Medicine’s Institutional Animal Care and Use Committee in accordance with all guidelines set forth by the U.S. National Institutes of Health.

**Reference**

Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl Environ Microbiol. 2002;68(4):1743-53. Epub 2002/03/28. PubMed PMID: 11916692; PubMed Central PMCID: PMCPMC123871.