# Gut mycobiome of primary sclerosing cholangitis patients is characterised by an increase of *Trichocladium griseum* and *Candida* species

## LETTER TO THE EDITOR

We read with interest the recent Gut article by Lemoinne *et al* describing a dysbiosis of the fungal gut community in faeces of patients suffering from primary sclerosing cholangitis (PSC).[1] Though several reports, including our own previous data, support a functional and potentially pathogenic link between the intestinal bacteriota and liver inflammation in PSC,[2 3] the aetiology of the disease remains largely unknown.

We here report on the fungal mycobiome results of our cohort from Northern Germany approved by the local ethics committees (A148/14 and MC-111/15) comprising stool samples of 66 healthy control (HC) subjects, 65 patients with well-characterised PSC (including a subgroup with concomitant colitis (PSC-IBD), n=32) and 38 subjects with UC.[3] PCR and sequencing of the fungus-specific internal transcribed spacer 2 genomic region was performed as previously described[4] using the primer pair 5.8S-Fun and ITS4-Fun on an Illumina MiSeq machine. Sequencing data were subjected to quality control by using the open source package DADA2 (V.1.10)[5] in R (V.3.5.1; https://github.com/mruehlemann/ikmb_amplicon_processing). Amplicon sequence variants were taxonomically annotated using the UNITE ITS database (V.7.2).[6]

In disagreement with the findings in the French cohort,[1] overall fungal alpha diversity in the German cohort was neither altered in PSC nor in UC versus HC as calculated by Shannon species equivalent (figure 1A). None of the disease groups significantly deviated in community composition from healthy individuals (all $p_{adonis}$ >0.05; figure 1B). Fungal composition on phylum level was found to be mainly dominated by Ascomycota (figure 1C), particularly by the genera *Saccharomyces*, *Candida* and *Dipodascus* (figure 1D) in relatively higher abundance of reads when compared with the findings of Lemoinne *et al*.[1] Though our results generally validate the previously described overall fungal composition in stool, we were not able to detect the genus *Exophiala*, which was found in five PSC patients from France exclusively. Whether this is due to methodological differences (choice of primer sets, data analysis tools and sampling depth) or presence of this fungus in only a subset of PSC patients not sampled in the German cohort needs to be determined.

Disease-associated differential abundance of fungal taxa was investigated by applying Student's t-test to the arcsin-squareroot-transformed relative abundances of all genera with mean abundance >1% and present in at least 10 individuals. This analysis revealed increased levels of the genera *Candida* and *Humicola* (species level annotation suggests *H. grisea*) in PSC patients with and without concomitant colitis compared with HC (all $q_{BH}$ <0.05; figure 1E and F) and UC (all $q_{BH}$ <0.1; figure 1E) individuals. *H. grisea*, recently reclassified as *Trichocladium griseum*,[7] belongs to the fungal class Sordariomycetes, thus our results reproduce the significant increase of this class in PSC patients, as previously described by Lemoinne and colleagues, but at increased taxonomic resolution. Previous research on *T. griseum* showed that it is most frequently isolated from soil and plants but also occasionally found in patients suffering from peritonitis.[8] In addition, the validated increase of *Candida* species in PSC patients argues for an immunogenic role of these fungi, particularly with respect to earlier findings that demonstrated their high potential to induce Th17 response in T cells.[9] Increased Th17 numbers have previously been reported in PSC patients and recently been shown to be involved in PSC pathogenesis.[10]

In summary, both the significant increase of the fungal class Sordariomycetes, likely *T. griseum*, as well as of *Candida* species in stool samples of PSC patients, now found in two independent and geographically distinct PSC patient panels that were analysed with divergent methodological approaches, strongly demands for additional analyses on these fungi and their role in PSC.

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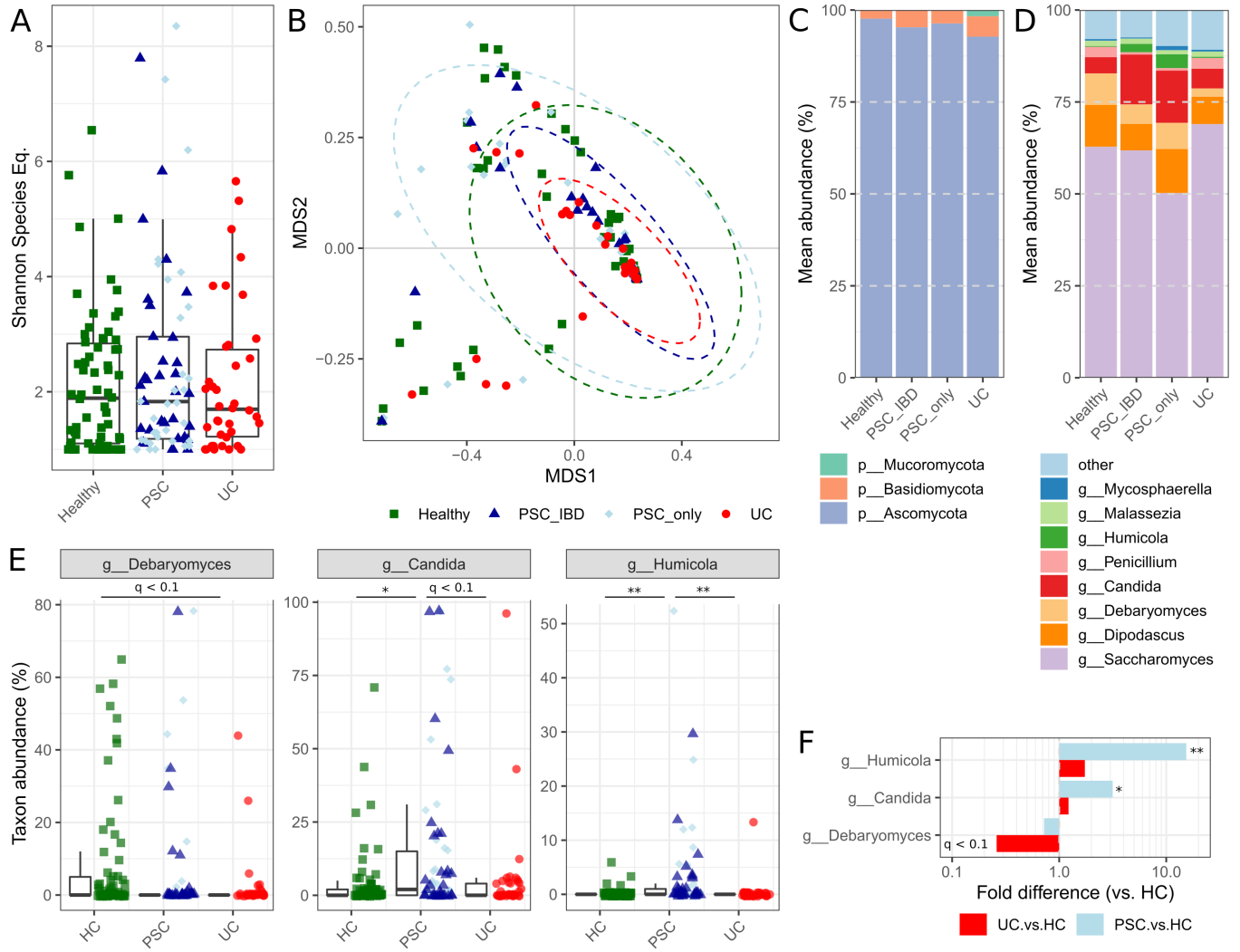


**Figure 1** Mycobiome of individuals with primary sclerosing cholangitis (PSC) and UC as well as healthy controls (HC) (all of northern German origin). Rarefaction curves for Shannon diversity of sequence variants reached plateau between 50 and 100 sequences per samples, thus samples were normalised to 100 random reads per sample. (A) Alpha diversity as presented by Shannon species equivalents (all p>0.05). (B) Beta diversity ordination of the Bray-Curtis dissimilarity based on genus-level fungal abundances (all $p_{adonis}$ >0.05). (C) Phylum-level and (D) genus-level mean abundances of all taxa with >1% mean abundance and present in at least 10 samples. (E) Group-wise box-and-whisker plots for significant genus level annotations tested for differential abundances with individual values represented as data points. (F) Differences in group-mean abundances of patients with PSC and UC, as compared with HC. *q<0.05, **q<0.01.

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**Data availability statement** Sequencing and clinical data of the patient samples used in this study can be applied for via the Popgen Biobank (Institute of Epidemiology, Christian-Albrechts-University of Kiel, Germany).

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