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Approaches to the analysis of fungal communities using the example of the human mycobiome patient with COVID-19

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Motivation and Aim: The human microflora is a complex composite system that includes various microorganisms. To an extent, the methods of modern biology are based on the study of the major component of the human microflora, represented by the bacterial part. However, there is a growing interest in the study of fungal microflora in the scientific community. Interest in this topic has grown against the COVID-19 pandemic. Thus, in people with COVID-19, an increased presence and change in the spectrum of fungal microorganisms was described [1]. At the same time, complications from fungal infections have become the cause of severe disease [2, 3]. At the same time, there is no understanding of the patterns of formation of a complicated picture of the course of COVID-19 through the addition of nosocomial bacterial or fungal flora.

Today, there are a number of studies showing that COVID-19 affects various human organs, including the intestines and its microflora. A significant amount of information has already accumulated in the literature on the study of changes in bacterial flora. However, much less research has focused on the human fungal microflora (i. e., mycobiome).

The aim of the work was to assess changes in the human mycobiome against the background of COVID-19, as well as to identify factors associated with changes in the composition of the fungal community. In our study, we carried out a taxonomic annotation of the mycobiome of patients with COVID-19 at the time of hospitalization and compared it with the state of the fungal microflora formed during treatment for coronavirus infection. The work also demonstrates differences between groups of patients at the first and second time points, which allows us to obtain a basic understanding of the trend in the direction of changes in the mycobiome during COVID-19 therapy.

The fungal part of the human microflora represents a minor component, thereby limiting the range of methods for studying it. Currently, the most common technology for studying fungal communities is amplicon sequencing of the internal transcribed spacer (ITS). However, in practice, only ITS1 or ITS2 regions from the full ITS cluster are used. In turn, they make it possible to reflect the taxonomy of different fungi with varying efficiency [4]. In turn, the full-length region ITS1-5.8S-ITS2 has a much stronger

phylogenetic signal and will better describe the taxonomy and composition of the fungal community.

Another rather important difference between the study of fungal ITS and bacterial 16S is the relatively high variability in the lengths of ITS amplicon sequences. Polymerase chain reaction, which is used in the preparation of libraries for sequencing, in turn, can have a certain bias in the assessment of fungal composition. Based on this, one of the tasks of this work is to find an optimal procedure for normalizing ITS to the length of the sequence itself.

Methods and Algorithms: The study included fecal samples (n = 117) obtained from patients during the first day of hospitalization (first time point) and closer to recovery (second time point). The control group consisted of 8 fecal samples taken from healthy donors. With this study, we would also like to expand the geographical understanding of the fungal microflora of the intestine. For the assembled collection of samples, a dataset of sequencing full-length sections of the ITS1-5.8-ITS2 regions was obtained on the Illumina MySeq platform with a library size of 300 + 300.

In the resulting data set, some of the sequences, as expected, do not cover the middle region of 5.8S rRNA and for this reason, it is not possible to use standard approaches for processing amplicon data using dada2. In our work, we applied sequence mapping to the fungal ITS UNITE database using bwa. Then the counts were calculated for each covered sequence in the database. We considered a successful hit to be those records that cover more than 95 % of the maximum possible length that we could cover with the available library size, in our case 600 bp.

We decided to further normalize the resulting tables with record counts in UNITE to the lengths of aligned records by calculating the TPM metric, which is actively used in transcriptomics. In addition, we also analyzed the clr transformed shares of the relative representations of the counts obtained on the raw matrix.

Agglomeration of entries in the table was carried out using the tip_glom function on a phylogenetic tree built using mafft + fasttree for sequences from the UNITE reference database. Clustering of samples by enterotype was assessed using DirichletMultinomial. DESeq2 was used to identify differentially represented fungi. Reduced dimensionality imaging was performed using PCoA. To identify factors associated with mycobiome diversity, we used the nonparametric PERMANOVA test.

Results: The major part in the analyzed array is represented by fungi of the genus *Candida*, in particular *Candida albicans*, which was found in all samples. However, the minor components of the mycobiome may vary. Thus, in a number of samples, the presence of representatives of the non-albigans *Candida sp.* group is noted: *Candida parapsilosis, Candida sake, Candida dubliniensis, Candida tropicalis* and *Candida mesenterica*. A significant part of the samples also includes representatives of the *Dipodascacea* family - *Yarrowia lipolytica* and *Geotrichum candidum*. The analysis also revealed that *Geotrichum candidum* is the main driver of differences between time points; its completion is characteristic of the first time point. *Yarrowia lipolytica* and *Saccharomyces cerevisiae* tend to be overestimated at the first point and underestimated at the second.

There were no obvious major differences in the taxonomic profile of fungal compositions between the different groups of people studied. However, as a result of the study, it was noted that the fungal alpha diversity of the group of sick patients was generally higher than that of healthy patients (p-value < 0.05), but no statistically significant differences were found between the time points themselves. However, it is

worth noting that alpha diversity in patients at the first time point is slightly higher than at the second, which indirectly hints at a trend towards the return of fungal microflora to a healthy state.

In the analysis of factors of individual characteristics of patients and characteristics of the treatment procedure using PERMANOVA 2, it was determined to be statistically significant. These are the presence of inflammatory bowel disease (p-value < 0.05) and the presence of diabetes (p-value < 0.05). The literature also shows an association of fungal microflora with inflammatory bowel disease and diabetes [5, 6]. At the same time, it was noted that the total percentage of explained variance in beta diversity exceeds 60 %, which suggests that the diversity of human fungal microflora is largely explained by individual characteristics of the person and the treatment procedure.

For a more accurate representation and association of the mycobiome with various factors, further research is needed that will expand the array of available information

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