Abstract

Gut microbiota is considered an important factor in the development of various diseases including inflammatory bowel disease (IBD, n = 127), Ulcerative colitis, Crohn´s disease, and colorectal cancer (CRC, n = 64). A part of this thtesis is to prepare clinical material of different sorts (stool, biopsy) for sequencing on Illumina Miseq platform. This is achieved trough DNA isolation, amplification of 16S and internal transcribed spacer (ITS), normalization and ligation of sequencing adaptors. The aim of this project is to describe the differences between microbiota in healthy and diseased subjects in case of IBD or unimpaired and tumorous tissue for CRC patients. This research is also being based on cultivation, where a fresh stool samples (n = 3) are cultivated in a broad range of conditions, which enables us to obtain ecophysiological and species diversity of these samples by traditional and molecular methods. The cultivable fungi are also assigned reliable taxonomy by amplification of relevant genes (ITS1, β tubulin, second largest subunit of RNA polymerase II, RPB2) followed by both-sided Sanger sequencing. Selected species of fungi are processed into lysates, which are used for stimulation of mice macrofage cell line (RAW). Therefore the impact on immunity response is studied *in vitro* and analyzed by immunological methods such as fluorescence-activated cell sorting (FACS) and colorimetric assays, enzyme-linked imunosorbent assay and Griess reaction.

This thesis is not limited on a sole describtion of microbiome changes, but provides complex preview of the intestinal microbiome and its links to intestinal disease trough variety of emploeyed methods.

Key words: mycobiome, microbiota, Illumina, ITS rDNA, diversity, inflammatory bowel disease, colorectal cancer, *in vitro* tests, imunoassays