Genomic analysis of the respiration the microbiota of human intestine

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Due to the features of anatomy and physiology, the human intestine creates the oxygen gradient, which influences the intestinal microbiota [1]. Because of this gradient, human gut provides a variety of ecological niches for both aerobic and anaerobic microorganisms [2]. The intestinal microbiota has been intensively studied during last years; however, respiratory capacities of the gut microbiota have been investigated for only a limited number of model organisms [3, 4]. Here, we present a systematic analysis of respiration genes encoded by the genomes of human gut habitants. Our study included an analysis of genes for ATP synthases, respiratory reductases for electron acceptors, and quinone biosynthesis.

We applied our genomic analysis to 254 microorganisms commonly found in the human gut. The investigated genomes belonged mostly to Firmicutes, Bacteroides, Proteobacteria, Actinobacteria, and Fusobacteria phyla of Bacteria. We found ATP synthases of F- and/or V-type in all analyzed genomes. Of the investigated genome, 111 had both F- and V-type ATP synthases, while 132 had only F-type ATP synthases, and 9 had only V-type ATP synthases. Additionally, the reference genomes demonstrated perceptible variations in the distribution of respiratory reductases. The analysis of studied genomes revealed aerobic and anaerobic reductases for tetrathionate, thiosulfate, polysulfide, sulfite, adenylyl sulfate, heterodisulfides, fumarate, trimethylamine N-oxide, dimethyl sulfoxide, nitrate, nitrate, nitrogen oxide, nitrous oxide, selenate, and arsenate. We did not find any genes for the respiration of chlorate, perchlorate, or metals. In addition to previously known terminal reductases, the two novel reductases were predicted. One of these reductases was a microaerobic extracellular one, dependent on flavins and thiols. This enzyme was found in 14 studied genomes. The second predicted reductase was an anaerobic one, reducing thiosulfate.

Among the analyzed genomes, this enzyme was only found in the one genome.

Various nitrogen oxides can be used as electron acceptors under anaerobic conditions (Figure 3A), including nitrate, nitrite, nitric oxide, and nitrous oxide [5, 6]. During the analysis of anaerobic respiration in the human gut reference genomes, we found that certain studied genomes did not contain the full set of respiratory enzymes for the reduction of nitrate to ammonia or molecular nitrogen. For incomplete genomes, the lack of certain steps could be explained by the presence of corresponding genes in non-sequenced region, by the involvement of assimilatory reductases for certain nitrogen oxides, or by inter-microbial exchange of byproducts. To test the latter hypothesis, we analyzed the studied genomes for the presence of genes encoding the corresponding respiratory and assimilatory reductases and transport proteins. Only 29 genomes contained all enzymes required for nitrate reduction to ammonia or molecular nitrogen, whereas 44 genomes had incomplete pathways. For the latter genomes, we predicted a set of exchange interactions. For example, *Lactobacillus* spp. can reduce nitrate to nitrite, which in turn could be reduced to ammonia by the other bacteria, such as *Bacteroides* spp. and *Parabacteroides* spp.

For all analyzed genomes, we also reconstructed pathways for biosynthesis of the respiratory quinones, one pathway for synthesis of ubiquinone and two alternative pathways for the synthesis of menaquinone. This reconstruction resulted in prediction of four non-orthologous replacements for previously known enzymes involved in the quinone biosynthesis. The final stages of the menaquinone biosynthetic pathway through futalosine have been unknown [7]. Using comparative genomic techniques, we predicted enzymes for these stages. The distribution of the patterns of synthesized quinones in HGM genomes was compared with that of respiratory reductases. The 225 (88.6%) out of 254 studied genomes demonstrated good agreement between the distribution of reductases and quinones; the 121 (47.6%) genomes had both reductases and quinones, whereas the remaining 104 (40.9%) genomes had neither reductases nor quinones.

Taken together, this work substantially expands our knowledge on both respiratory pathways in bacteria and physiology of the human gut microbiome. This research is a part of the large project aimed to model metabolic interactions for individual microbes and microbial

communities in human intestine as well as to model human-microbial interactions. Thus, reconstruction of complete respiratory chains, including all ATP synthases, dehydrogenases of electron donors, quinone biosynthetic pathways, and reductases of electron acceptors, is an immediate future direction. The final reconstruction of respiratory chains and their inclusion into metabolic models will further improve our knowledge and understanding of the metabolism of human gut bacteria, their interactions, and the interaction with the human host.

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