DOI 10.18699/SBB-2022-284

Gene networks underlying the mechanisms of resistance to inflammatory factors in *Bifidobacterium longum* and *Lacticaseibacillus rhamnosus*

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Key words: Bifidobacterium longum; Lacticaseibacillus rhamnosus, RNA sequencing; inflammatory process; pro-inflammatory cytokines; transcription start site; transcriptome

Motivation and Aim: Bifidobacteria and lactobacillus are a key component of the commensal gut microbiota conferring considerable health benefits and supporting the normal functioning of the host organism. As permanent residents of the normal gut microbiota, bifidobacteria and lactobacillus have evolved to adapt the host's immune response whose priority is to eliminate pathogenic agents. The mechanisms that ensure the survival of commensals during inflammation and maintain the stability of the core component of the normal gut microbiota in such conditions remain poorly understood. We performed transcriptome analysis of the *B. longum* GT15 and *L.rhamnosus* K32 strains to investigate the mechanisms lying behind the response of bifidobacteria and lactobacillus to signaling molecules of the immune system.

Methods and Aldorithms: *B. longum* GT15 cells were collected in the middle of the log-phase in both experimental (with IL-6 and TNFα) and control conditions. *L.rhamnosus* K32 cells were collected in the middle of the log-phase in both experimental (with IL-6, IL-8, IL-10 and TNFα) and control conditions. Total RNA extraction and purification was performed using the RNeasy Mini Kit. Ribosomal RNA was removed using the RiboZero rRNA Removal Kit (Bacteria) and libraries were prepared using the NEBNext® Ultra II Directional RNA Library Prep Kit. The Kallisto v0.46.0 software was used for mapping of reads and estimation of transcript abundance. Differential expression analysis was performed using edgeR v3.26.8 package. The plots were generated using the ggplot2 package in R. Differentially expressed genes (DEGs) were then subjected to enrichment analysis of COG functions and KEGG pathways. The construction of PP was performed using the program OrthoFinder v.2.3.7. Identification of TSS for *B. longum* GT15 and subsequent analysis were performed from .sam files with BAC-Browser.

Results: Addition of IL-6 [0,1 ng/ml] and TNFα [10 ng/ml] on the growth medium led to moderate but statistically significant changes in the growth rate of the culture *B. longum* GT15. Genes that were significantly differentially expressed in response to the addition of cytokines were singled out: a total number of 128 DEGs in the sample of *B. longum* GT15 grown with IL-6 compared to the control sample, including 68 downregulated and 60 upregulated genes and 987 DEGs for the sample of *B. longum* GT15 grown with TNFα compared to the control sample, among which 477 genes were downregulated and 510 genes – upregulated. For *B. longum* GT15 the analysis of DEGs

showed, TNFα altered the expression of a higher number of genes than IL-6. The observed effects of cytokines on *B. longum* GT15 were cumulative and prolonged, features that are conducive for the adaptation to the new conditions. Gene (BLGT_RS00625) encoding *hsp20* was upregulated 5-fold exposed to TNFα. Since the response of *B. longum* GT15 could be directed at bringing down TNFα levels, we suspect this mechanism to be one of the many ways used by bifidobacteria to dampen inflammation. As predicted by KEGG, 34 pathways were altered by IL-6 and 87 pathways were altered by TNFα. We used phylogenetic profiling to identify putative operons among the DEGs. The 410 potential TSS were identified for *B. longum* GT15. From them, 38 TSS confirm predicted DEGs operons for TNFα treatment and 7 operons for IL-6 treatment. According to functional analysis (COG), exposure to TNFα altered mainly the expression of genes included in energy pathways. This may be explained by the accumulation of resources caused by inflammation and activation of ATP-dependent ABC transporters to maintain homeostasis. Exposure to TNFα has also stimulated the expression of genes involved in propanoate metabolism is potentially one of the main metabolites accounting for the probiotic action of bifidobacteria. Exposure to TNFα can signal to bifidobacteria an increase in the concentration of ammonia leading to increased expression of genes, involved in nitrogen metabolism.

Interleukins do not affect the growth of the strain *L. rhamnosus* K32. But the analysis of DEGs showed IL-8 and IL-10 altered the expression of a higher number of genes than TNFα (507, 317 and 77 DEGs respectively). IL-6 doesn't affect gene expression *L. rhamnosus K32*. Exposure to cytokines IL-8 and IL-10 at various concentrations (0,1 ng/ml, 1 ng/ml and 10 ng/ml) affects the amount of DEGs, but does not affect their expression. Phylogenetic profiling predicted evolutionarily stable groups of DEGs combined into operons. 17 potential groups were predicted for IL-8 and 10 groups for IL-10. Common groups were found for IL-8 and IL-10 containing 22 DEGs. DEGs of these common operons are involved in the protection and maintenance of cell homeostasis. Most DEGs are ABC transporters. Also 2 DEGs (LRK_05050 and LRK_08445) refer to the XRE family transcriptional regulator that regulate transcription during stress. Functional analysis showed that most DEGs are involved in carbohydrate metabolism and transport. This may be due to intense energy-consuming processes activated by the addition of interleukins and associated inflammation.

Conclusion: We demonstrated how IL6 and TNFα activate their defense mechanisms, and also showed how bifidobacteria can exhibit probiotic effects in conditions of inflammatory processes. There is a single mechanism involved in the formation of resistance to immune response factors in the conditions of the inflammatory process in lactobacillus. Thus, in this study, we predicted the genes involved in a putative signaling pathway underlying the mechanisms of resistance to inflammatory factors in bifidobacteria and lactobacillus. Since bifidobacteria and lactobacillus are a major component of the human intestinal microbiota exhibiting pronounced anti-inflammatory properties, this study is of great practical and scientific relevance.

Acknowledgements: The study is supported by the Presidential Bursaries СП-4751.2021.4.