DOI 10.18699/SBB-2022-281

Omix technologies and bioinformatics: an example of use in creating a pharmabiotic based on the *Limosilactobacillus fermentum* U21 strain

Poluektova E.U.[1]*, Mavletova D.A.[1], Odorskaya M.V.[1], Marsova M.V.[1], Klimina K.M.[1, 2], Koshenko T.A.[1], Yunes R.A.[1], Vatlin A.A.[1]*, Danilenko V.N.[1]

[1] *Vavilov Institute of General Genetics, RAS, Moscow, Russia*

[2] *Federal Research and Clinical Center for Physical and Chemical Medicine of the Federal Medical and Biological Agency of Russia, Moscow, Russia*

**epolu@vigg.ru vatlin; alexey123@mail.ru*

Key words: Pharmabiotics, antioxidants, omics technologies, lactobacilli

Motivation and Aim: Today's world is undergoing revolutionary changes in the development and use of pharmacological preparations based on bacteria and their biologically active ingredients. Such preparations are increasingly referred to as pharmabiotics, as opposed to probiotics, used primarily as dietary supplements and consumed by healthy people. Pharmabiotics are live biotherapeutic drugs and/or their metabolites and components with established pharmacological ingredients, mechanism of action and intended for treating specific diseases. In order to develop pharmabiotics, aside from traditional microbiological and biotechnological approaches, a complex of omics technologies can substantially facilitate the process. Lactobacilli are the important component of the human microbiota and, due to the active synthesis of biologically active compounds and bidirectional communication with the host organism, they can affect the state and antioxidant (AO) status of the macroorganism. The strain of *Limosilactobacillus fermentum* U-21 showed high AO activity on *in vitro* and *in vivo* models [1, 2]. In this work, we used a combination of genomic, transcriptomic, and proteomic technologies to identify genes and proteins that potentially determine the unique AO properties of the strain.

Methods and Algorithms: The DNA of the *L. fermentum* U21 strain and the comparison strains *L. fermentum* 103 and *L. fermentum* 279 was sequenced and deposited in the NCBI GenBank (WGS PNBB01, PGGI01, PGGE01). The search for genes exhibiting AO properties in the genome of *L. fermentum* U-21 was carried out using the reference catalog of genes of antioxidant proteins found in various species and strains of lactobacilli [3; https://github.com/Alexey-Kovtun/Catalog] and the developed algorithm for their search [4]. RNA was isolated on a King Fisher automated station with the MagMAX™ mirVana™ Total RNA Isolation Kit (Thermo Fisher Scientific). Ready libraries were sequenced on an Illumina HiSeq 2500. Kallisto v0.46.0 software was used to map reads and evaluate transcript abundance. Differential expression analysis was performed using the edgeR v3.26.8 package integrated into the Degust v4.1.1 web tool. The proteins of the cell-free culture supernatants of *L. fermentum* strains were separated in SDS-PAGE. For mass spectrometric analysis, the colored protein bands were excised and the peptides were separated using the Ultimate 3000 Nano LC system connected to a Q Exactive HF mass spectrometer through a nanoelectrospray source (Thermo Fischer Scientific) based on the mass spectrometry group of the Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry. RAS. LC-MS/MS data analysis was performed using PEAKS Studio 8.0 build 2016-0908 software. The primary structures of the generated peptides were analyzed based on the UNIPROT KB protein sequence database (07.2016).

Results: Genomic analysis of the strain made it possible to identify 29 genes, the products of which are likely to exhibit antioxidant properties. The most important genes are those encoding proteins of the thioredoxin complex and those encoding the metabolism and transport of heavy metals. Hydrogen peroxide was chosen as a stress inducer. We used a peroxide concentration that did not affect the viability of the cells of the strain in a given period of time (10 mM, 30 min). 380 genes showed an increase in expression and 370 genes showed a more than twofold decrease in expression upon exposure to hydrogen peroxide. The greatest increase in expression was observed in genes involved in the production of ammonium as a source of nitrogen (14–24 times), various oxidoreductases, catabolism of disaccharides, as well as enzymes that protect proteins and nucleic acids from oxidation and stress proteins common to different types of stress. The expression of genes encoding mobilome elements, IS elements, type II toxin-antitoxin systems also increased. The expression of genes encoding subunits of ATP-binding cassette transporters (ABC-transporters), the phosphotransferase system for sugar transport and fatty acid biosynthesis was sharply reduced. Among the antioxidant protein genes, the greatest increase in gene expression was noted for the operon containing the genes encoding thioredoxin reductase and cysteine synthase and the operon containing the genes for the P-type ATPase heavy metal transporter and the protein containing the domain associated with heavy metals. Proteomic analysis of the exoproteome of the strain enabled the identification of the ClpB protein belonging to a chaperone complex, which can play a key role in refolding the misfolded proteins as a result of oxidative stress in various tissues and organs of the animal body.

Conclusion: The use of a complex of omics technologies to characterize the therapeutic properties and mechanism of action of the *L. fermentum* U21 strain is one of the first attempts in this field of research. The data of genomic and transcriptomic analysis indicate that the main defense against oxidative stress in this strain is the thioredoxin system, which functions through the regulation of the dithiol/disulfide balance. The process of metal chelation also makes a significant contribution to the antioxidant properties of the strain. The results of transcriptomic analysis of a strain of lactobacilli under conditions of oxidative stress, as well as the data of genomic and proteomic analysis, make it possible to significantly expand the range of bacterial enzymes involved in the response to stress. These data can be used to select probiotic strains with antioxidant activity, as well as to develop additional components in probiotic preparations aimed at treating diseases associated with oxidative stress, primarily inflammatory diseases.

Acknowledgements: The study is supported by State task No. 0092-2022-003.

References

1. Marsova M., Abilev S., Poluektova E., Danilenko V. A bioluminescent test system reveals valuable antioxidant properties of lactobacillus strains from human microbiota. *World J Microbiol Biotechnol*. 2018;34(2):27. doi: 10.1007/s11274-018-2410-2.
2. Danilenko V.N., Stavrovskaya A.V., Voronkov D.N., Gushchina A.S., Marsova M.V., Yamshchikova N.G., Ol'shansky A.S., Ivanov M.V., Illarioshkin S.N. The use of a pharmabiotic based on the Lactobacillus fermentum U-21 strain to modulate the neurodegenerative process in an experimental model of Parkinson's disease. *Ann Clin Exp Neurol*. 2020;14(1):62-69. doi: 10.25692/ACEN.2020.1.7
3. Averina O.V., Poluektova E.U., Marsova M.V., Danilenko V.N. Biomarkers and Utility of the Antioxidant Potential of Probiotic Lactobacilli and Bifidobacteria as Representatives of the Human Gut Microbiota. *Biomedicines*. 2021;9(10):1340. doi: 10.3390/biomedicines9101340
4. Kovtun A.S., Averina O.V., Zakharevich N.V. et al. *In silico* identification of metagenomic signature describing neurometabolic potential of normal human gut microbiota. *Russ J Genet*. 2018;54(9):1101-1110. doi: 10.1134/S1022795418090089.