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Bioinformatics and experimental approaches in the creation of the drug "Superbact" for parkinsonism's treatment

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In recent years, revolutionary changes have taken place in the world in the development and use of pharmacological preparations based on bacteria – inhabitants of the human and animal microbiota and their biologically active ingredients. Pharmabiotics are live biotherapeutic preparations and/or their metabolites and components with established pharmacological ingredients, mechanism of action and aimed at the treatment of specific nosologies. Pharmabiotic preparations are opposed to probiotics, which are used mainly as biologically active supplements and consumed by healthy people.

In this regard, a consensus has formed on the need to study the specific properties of strains and genes that determine the manifestation of the desired effect. In addition to traditional microbiological and biotechnological approaches, a complex of omics technologies – genomic, transcriptomic and proteomic – is used to create pharmacobiotics.

The *Limosilactobacillus fermentum* U-21 strain, due to the high antioxidant activity identified in in vitro and in vivo studies, has shown itself to be a promising candidate for the creation of pharmacobiotic preparations based on it. In particular, the AO activity of its supernatant culture liquid and cell culture was shown. In the model of oxidative stress of the free-living nematode *Caenorhabditis elegans*, the *L. fermentum* U-21 strain increased the median lifespan of the nematode by 25–30 %; in the model of induced parkinsonism in rodents, the introduction of *L. fermentum* U-21 prevented the degradation of brain dopaminergic neurons and pathological changes in internal organs.

To identify specific molecular genetic mechanisms that can affect the manifestation of these properties, genomic and comparative genomic analysis was carried out. Bioinformatics search for genes was carried out using the reference catalog of genes of antioxidant proteins found in various types and strains of lactobacilli [https://github.com/Alexey-Kovtun/Catalog] created by us on the basis of literary sources and a program for searching for genes/proteins with the desired properties. We also used a comparative analysis of the nucleotide sequences of the studied strain with reference strains and analysis of the nearest environment of the found genes using the Sequence Set Browser. A hypothetical operon was considered to be a set of genes located on the same DNA strand no further than 30 bp. from each other and, in the presence of data on the expression of genes that changed the expression under conditions of oxidative stress in one direction.

The genome of the *L. fermentum* U-21 strain was compared with the *L. fermentum* genomes available in the NCBI database as of February 28, 2022, and with the *L. fermentum* 279 and *L. fermentum* 103 strains that do not exhibit antioxidant properties under experimental conditions. The comparison revealed 138 unique genes, some of

which are extremely rare in this species of lactobacilli and two groups of rare genes, one of which is unique for the strain.

In the genome of the *L. fermentum* U-21 strain, 29 genes were identified that determine proteins with AO properties, namely: genes for thioredoxin complex proteins (*trxA* and *trxC*, *tpx (ahpC*), *ahpF*, *nrdH*), which is generally not characteristic of *L. fermentum*; transport protein genes (*cydC*, *cydD*) and glutathione synthesis (*ghsF(AB)*) rare in *L. fermentum*; and also contains a large number of genes for metabolism, transport and binding of heavy metal ions (copA, copB and others) and metal-dependent transcription factors (including copR, copY/tcrY). The cluster including the MFS transporter and ferrochelatase is found only in 7 genomes of strains of this species deposited in GenBank.

The data obtained are the first step in the identification of genes that determine the properties of the *L. fermentum* U-21 strain. Subsequent studies of the transcriptome and proteome, carried out at the Laboratory of Microbial Genetics of the IOGEN RAS, will provide more information about the genes and their products that determine the unique properties of the *L. fermentum* U-21 strain. Taken together, the obtained data demonstrate the unique AO properties of the strain, allowing it to be positioned as a promising candidate for the development of drugs for the treatment and prevention of various inflammatory diseases, including cardiological, autoimmune diseases, and parkinsonism.

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