Systemic and antimutagenic properties of probiotics

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Abstract. Until recently, it was believed that the effects of probiotics are limited to a certain range of specific effects, but more and more studies indicate that their action on the host organism is more complex and systemic. Such effects interference of probiotics in the regulation of various pathways in the host organism. Systemic effect does not mean the sum of many minor impacts but precisely targeted impact on the most sensitive points or metabolic pathways. That allows to achieve a more significant effect in small doses through a cascade of reactions. In this case, probiotics can act both through the effect on other symbiont bacteria and directly on the host. One of the agents of such interactions can be oligopeptides of nonribosomal origin.

1 Introduction

The main mechanisms that ensure the positive effect of probiotics on the host organism are considered to be: 1) antagonism with pathogenic microflora; 2) stimulation of specific and non-specific immunity; 3) stimulation of the growth of normal microflora; 4) the release of digestive enzymes; 5) production of amino acids and vitamins; 6) destruction of xenobiotics (allergens, mutagens), as well as substances that are making it challenging to assimilate of food [1, 2]. However, recent studies increasingly show that not all of the effects of probiotics fit into this pattern. Apparently, the effect of probiotics is systemic, affecting not only individual organs and organ systems but also the whole organism.

Some studies indicate that probiotics affect the mental state of the host [3], interfere with the regulation of metabolism, the work of hormonal systems [4], gene expression [5], and other regulatory mechanisms. The term "systemic" means that the effect cannot be explained by a simple summation of many minor effects. In most cases, the action of probiotics is described exactly this way – as the sum of antioxidant, antagonistic, and many other effects. The systemic principle presupposes an action on critical points and regulators. This signal should be transmitted inside the cell according to the cascade amplification principle, which would explain the effects of minimal doses.

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This work examines the effects of probiotics, which may be associated with subtle regulatory influences, their mechanisms, and their relationship with DNA protection (antimutagenic activity). Our research also shows that the antimutagenic and regulatory properties of probiotics can be the basis of their systemic effects.

2 Probiotics as regulators

In living organisms, there are indeed systems controlled by relatively simple "switches" – regulatory cascades, gene operons, etc. Through these systems, probiotic bacteria can interact with the rest of the microbial community and with hosts. According to the existing point of view, interspecies antagonism of bacteria is based not only on strategies for the destruction of the antagonist but also includes more subtle mechanisms developed over millions of years of interspecies rivalry, including the mechanisms of regulation of its metabolism [6]. One of the fundamental approaches to this seems to include reducing mutagenesis in host cells, especially targeting at mitochondria in eukaryotes. In prokaryotes this mechanism can be the basis of somewhat controversial strategy: decreasing mutation rate in antagonistic species leads to weakening of its evolutionary potential and, as a result, a decrease in the ability to adapt to antibiotics.

2.1 Targets in prokaryotic cells

The following points and processes can be considered as targets for the above-described effects in prokaryotic cells:

1) SOS repair and expression of genes for response to stress responses. The SOS response in bacteria is the most important mechanism of mutagenesis [7]. As shown in our works [8], a cell-free preparation of probiotic strains Bacillus amyloliquefaciens B-1895 and Bacillus subtilis KATMIRA1933 can inhibit the SOS response in E. coli and reduce the number of antibiotic-resistant mutants in gram-positive and gram-negative organisms. Several Lactobacillus strains possesses the same property [9]. The fraction of metabolites isolated by ion-exchange chromatography showed resistance to temperatures up to 80°C and proteinases, as well as a positive reaction to the presence of a peptide bond. Filtration of the fraction through a filter with pores up to 10 kDa did not lead to a decrease in activity, which indicates a small size of the target molecules.

2) Quorum-sensing. This mechanism provides biofilm formation, a manifestation of pathogenic properties, and resistance to environmental factors in many bacteria. In addition, it is known that the rate of mutagenesis in biofilms increases [10], therefore, strategies to decrease mutagenesis can be connected with this mechanism. Peptides of natural origin are often considered as agents that violate the "quorum-sensing" [11].

3) Horizontal gene transfer. This process, being associated with the SOS response at the level of regulation (via the RecA protein), is also an essential mechanism of adaptation to antimicrobial agents [14].

2.2 Targets in eukaryotic cells

In eukaryotic cells the following can be distinguished as target processes for systemic influences:

1) Processes in mitochondria, including the generation of ROS and their inactivation, as well as the expression of mitochondrial genes. There are records that mitochondria, which, according to generally accepted concepts [13], are descendants of symbiotic bacteria, play

the role of a regulatory "command center" in cells [14]. As it turns out in recent years, probiotics can actively interact with the host mitochondria [15].

2) Expression of nuclear regulatory genes that trigger large cascades, for example, the p38 MAPK pathway [2, 16]. The expression of genes responsible for the response to stressful influences, particularly oxidative stress, is most often affected. Activation of these cascades promotes the organism's adaptation to stressful conditions and can lead to an increase in life expectancy [16]. It should be noted that these effects were observed both under the action of living cells and under the influence of cell-free preparations [2].

These critical points are schematically indicated in Figure 1.

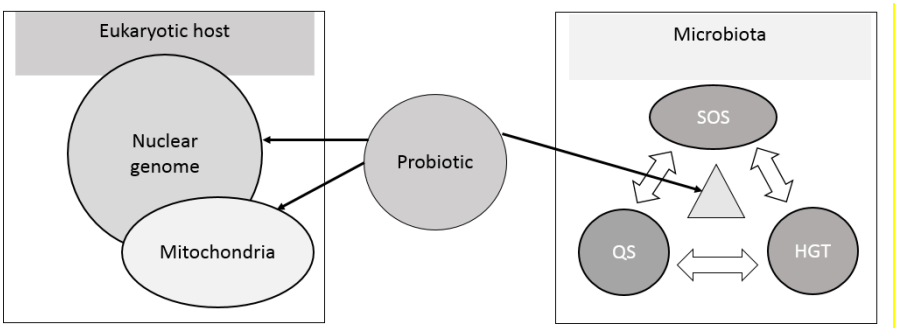

Fig. 1. Targets for regulatory action of probiotics.

3 Searching for possible systemic regulators

In the projects of our team, special attention was paid to the anti-mutagenic effect of probiotics. We didn't try to study all systemic effects, and focused on the study of the following phenomena: A) some probiotics are able to reduce the level of antibiotic resistance mutations in other microorganisms; B) the same probiotics appear to have the ability to prolong lifespan of the host.

Both of these effects can be attributed to antimutagenic properties.

In our study of the biological activity of two *Bacillus* strains, that was carried out on chickens, it was found that a preparation based on the *Bacillus subtilis* KATMIRA1933 strain caused a decrease in the number of mitochondrial DNA damage (by 34% compared to the control).

The probiotic supplement was found to increase the transcriptional activity of vitellogenin synthesis genes. Vitellogenin is a precursor protein of egg yolk; however, it is also known that vitellogenin acts as an antioxidant that promotes longevity in queen bees - they have a higher expression of the vitellogenin gene compared to workers and are more resistant to oxidative stress [17]. It is also was discovered to be influencing the immune system [18]. In birds, active synthesis of vitellogenin is a marker of the reproductive period.

There were also multiple improvements in physiological and biochemical parameters [19], that can be described as systemic effects, such as:

The body weight gain in all experimental groups was higher than in the control.

- The weight of the ovary and the length of the oviduct of the chickens in the experimental groups significantly exceeded the control (by values up to 9.8%).
- Improvement was observed in biochemical blood parameters; the quality of the sperm of the roosters, egg production of laying hens, morphological and biochemical parameters of eggs (in particular, shell thickness).
- Increase in egg fertilization and decrease in embryo death in the first 7 days of incubation.

However, the most interesting effect was the following: the inevitable decrease in egg production in laying hens in one of experimental groups occurred more slowly than in control. The rate of reproductive aging of chickens in comparison with the control slowed down by 2.1%, though the general lifespan was not prolonged.

Molecules that could provide the above-mentioned systemic and regulatory effects should have a number of specific properties: 1) small size (which gave them an ability to penetrate membranes); 2) resistance to proteinases and other environmental factors; 3) affinity for protein receptors or similarity with protein factors involved in regulatory cascades; 4) existence in a multitude of isoforms and the possibility of a quick rebuilding of the structure.

Based on the properties of the metabolites isolated by us, these are small peptides. For *Bacillus* sp. is characterized by such secondary metabolites as nonribosomal synthesized peptides (NRPs). They do not exceed several kDa in size, do not denature when exposed to temperature, and are not hydrolyzed by proteinase K. Such stability is provided by atypical amino acids and stereoisomers in the structure [20, 21]. NRPs are often considered antimicrobial and antifungal agents, but recently there are data on their participation in regulatory processes [22]. NRPs meet all of the above criteria. It should be noted that oligopeptides as regulatory molecules are characteristic of most living organisms. Peptides produced, among other things, by the human body have features of similarity to NRPs [2], which suggests that these molecules can interact with receptors with a similar configuration.

A fraction with SOS-inhibitory and antimutagenic activity was isolated, and an oligopeptide fraction with thermal stability and a mass of less than 3 kilodaltons was isolated by spectrometry. These properties correspond to the spectrum of nonribosomal peptides secreted by the genus *Bacillus* representatives. In spectrophotometric analysis, the samples showed an absorption peak at 200-220 nm, which indicates the presence of compounds with peptide bonds.

For mass spectrometric analysis, two samples were taken, representing the fractions with the highest antimutagenic activity. In general, the mass spectra were represented mainly by singly charged ions with m/z not more than 1000 for almost all chromatographic peaks.

The spectrum of the sample is shown in Figure 1. It shows that the sample contain a significant number of multiply charged ions, suggesting the presence of peptides weighing more than 1500 Da.

The main peaks presented in the spectrum are a peak at m/z 545.81 with a charge state defined as 3+, a peak at m/z 755.32 with a charge state defined as 3+, and there is also a peak at m / z 1103.44 with a charge state defined as 2+.

In general, the data of mass spectrometric analysis allow us to conclude that the active substances in the isolated fractions are of a peptide nature and sizes of no more than 3 kDa. The peptide nature of the samples can be judged by the presence of ions with a charge state of more than 2+ from the spectra of collisional fragmentation (MS/MS spectra) characteristic of peptides.

Bioinformatic analysis of the *B. subtilis* strain genome shows that this strain is capable of synthesis of 4 NRP that are:

- glu leu leu val asp leu leu
- mal* mal mal gly
- nrp* gly thr
- glu orn tyr thr glu val pro glu tyr ile phe

*mal - unspecified malonylated polyketide, nrp - unspecified amino acid

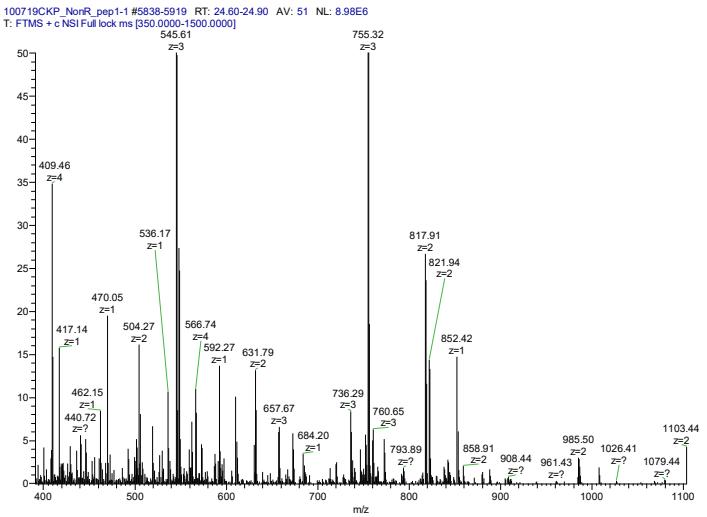

Fig. 2. Mass spectrum of a fraction with antimutagenic activity

The mass of one of them corresponded to the peak found by mass spectrometry, and it seems to be a surfactin-like molecule. The molecule with a calculated mass of 814 is similar to a group of surfactin-like peptides (PubChem CID: 91974341). Modified with different lengths of fatty acids, it forms the basis for a spectrum of products with m / z> 1,000 produced by *B. subtilis* strains. Surfactin is known as an antifungal compound as well as a QS regulator.

It should be noted that some antimicrobial molecules can have the additional functions that can manifest themselves in subinhibitory concentrations. In particular, bacteriocins have a dual action and can also be used by microorganisms as signaling molecules at naturally achievable subinhibitory concentrations [22]. Therefore, we can assume that some of systemic effects of *B. subtilis* strains can be provided by surfactin-like NRPs.

4 Conclusion

Thus, the probiotic properties of bacteria of the genera *Bacillus* and others can be provided, in addition to well-known mechanisms, by interactions with regulatory processes in antagonist microorganisms (in particular, inhibition of the SOS-response and a decrease in adaptability to antimicrobial agents), as well as the effect on the expression of host genes, including regulation of the intensity of oxidative stress, and a decrease in the frequency of mutagenesis. One of the agents of such interactions could be oligopeptides of nonribosomal origin.

The existing paradigm of probiotic perception, in our opinion, overlooks significant systemic effects that occur in the host's body. The new concept of interaction of probiotics with eukaryotic hosts will allow revising approaches to the practical application of probiotics in medicine and veterinary medicine and creating new strategies for the search and development of new strains and probiotic preparations.

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