Probiotic Bacteria

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Probiotic bacteria are widely accepted as therapeutic agents against inflammatory bowel diseases for their immunostimulating effects.

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1. Introduction

In the last two decades, the field of human gut microbiota (HGM) has been marked by intensive and fruitful research. Today, the HGM is defined as a community of commensal, symbiotic and pathogenic microorganisms residing both inside the body and on its surface and that have adapted in the process of evolution to humans. The HGM consists of viruses, archaea, protozoa, fungi and bacteria, with the latter representing the largest and most studied group [1]. The HGM contains the most dense microbial population compared to other organs, which translates into high compositional and functional diversity [2]. Gut bacteria participate in the metabolic processes occurring in the intestines, such as the metabolism of carbohydrates and proteins, bile salts and polyphenols. They protect the human body from pathogens and maintain the integrity of the intestinal barrier. Finally, they synthesize hundreds of biologically active substances, such as lactic and acetic acids, hydrogen peroxide, bacteriocins, short-chain fatty acids, vitamins, proteins, peptides, cell wall components and neuroactive compounds, all of which shape and ensure the normal functioning of the immune, nervous and endocrine systems [2][3][4]. Of particular interest are the immunomodulatory properties of CpG motifs, as seen in bifidobacteria characterized by notoriously high GC content genomes. CpG motifs—stretches of DNA rich in their contents of the deoxyoligonucleotides cytosine and guanine—were shown to activate both the innate and adaptive immune responses via Toll-like receptor 9 (TLR-9) [4]. Today, the microbial composition of the human gut is an important health indicator. Many studies have found correlations between the HGM composition and human diseases (obesity, hypertension, cardiovascular disease, diabetes, cancer, inflammatory bowel disease (IBD), gout, depression and arthritis). The HGM also correlates with overall infant health and longevity [5].

As our knowledge of the composition and function of the HGM has expanded, it has become clear that certain health-conferring bacteria can be put to use for therapeutic purposes. Probiotics are defined as live microorganisms that, when administered in adequate amounts, exert beneficial effects on the host organism. Since the observation made by the Russian scientist Elie Metchnikoff regarding the positive influence of lactobacilli on health [6], probiotic properties have been uncovered in many microbial genera and species: *Bifidobacterium*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Bacillus*, *Escherichia coli* M-17, *Saccharomyces boulardii* and others. Probiotics are especially effective in treating chronic inflammatory bowel diseases and the metabolic syndrome while virtually showing no side effects [7]. The prospect of using certain probiotics as an alternative treatment for depression was delineated many years before the term psychobiotics was coined [8][9]. Numerous studies have followed and further strengthened the concept of psychobiotics by demonstrating the salubrious effects of bacteria-based therapy on neurodegenerative and psychiatric diseases such as depression, schizophrenia, epilepsy, cerebral ischemia, Parkinson's disease, Alzheimer's disease, insomnia, autism spectrum disorder and others. Nevertheless, the bulk of the research in this field has been carried out on animal models, mimicking, to a certain extent, the symptoms of these diseases. Moreover, the majority of studies were not followed up in humans; among the strains that were tested in clinical settings, few were proven effective [10][11][12].

Depression persists in today's world as the most common psychiatric disorder. The numbers we have today remain a rough estimate. According to the World Health Organization, some 300 million suffer from depression; the actual number is likely higher than that [13]. Depression qualifies as a socially significant disease, since it significantly reduces the quality of life of patients. Depression is no less a burden for patients than it is a burden for their families. Some of the major symptoms of depression include a lingering bad mood, anhedonia, fatigue, lethargy and anxiety and, if left untreated, can become a high risk factor for suicide. The current nascent paradigm stipulates that depression is accompanied by chronic

subclinical inflammation that stems from an aberrant gut microbiota, increased permeability of the gut barrier and impaired immune status [14]. There is sufficient evidence that bacterial metabolites are implicated in the pathogenesis of depression [15]. Since antidepressants are effective only in a subset of patients and are also plagued by their infamous side effects—insomnia, fatigue, anxiety, confusion, gastrointestinal symptoms, dry mouth, skin redness, itch and photophobia—there is an ever-increasing demand for safer drugs, which could be satisfied by probiotics [16].

2. Selection of Potential Antidepressants among Bacteria

The first step in selecting for psychobiotic strains is to conduct a wide-scale in vitro assessment of the general probiotic properties of bacteria. Bacterial strains should be selected based on their ability to withstand the lethally acidic environment of the stomach and the bile salts in the duodenum. At this stage, the strain's ability to adhere to intestinal epithelial cells and to suppress the growth of pathogenic bacteria should be investigated as well. Then, preliminary safety studies are needed to confirm the absence of mobile genes, accounting for transferable pathogenicity and antibiotic resistance. The next step is to look for other properties, such as the production of active compounds—namely, bacteriocins, organic acids and exopolysaccharides—and the conversion of carbohydrates, proteins and other food components into short-chain fatty acids and other molecules. Expanding our knowledge of the technical characteristics of potential psychobiotics is crucial for production. The viability of the strains in the end product depends on their ability to withstand the freeze-drying process. Just as important is to test the viability of the strains during long storage. There are tests specifically designed to check the activity of the strain at all stages of the manufacturing process. After all these steps are completed, the strain's specific activity, whether antidepressant, anti-inflammatory, anticarcinogenic or immunomodulatory, should be studied in both in vitro and in vivo systems. Finally, perhaps the most necessary and revealing part of this protocol is to validate the strain's effects in human volunteers [17].

The antidepressant potential of bacteria has been attested to by hundreds of studies conducted on laboratory animals—mainly rats and mice. The bacterial strains tested in these studies belonged predominantly to the genera *Lactobacillus* and *Bifidobacterium* [18]. These studies also emphasized the strain-specific rather than species-specific nature of these properties [19]. Most experiments are designed similarly, and they involve the administration of a single or a mixture of strains of bacteria to a control group made up of conventional or gnotobiotic animals as opposed to a control group receiving a placebo. At the same time, the animals are exposed to a stressor for the duration of the experiment and tested afterwards in relevant behavioral tests, such as sucrose preference, forced swimming test, elevated plus maze, open field and others. The biochemical results show that specific probiotic strains can impact the HPA (hypothalamic–pituitary–adrenal) axis by lowering the corticosterone and ACTH (adrenocorticotropic hormone) blood levels. The levels of dopamine, serotonin, tryptophan and other neurotransmitters are also normalized in various regions of the brain and the blood serum. Other effects involve the regulation of the activity of gamma-aminobutyric acid (GABA) receptors in various regions of the brain and the level of the brain-derived neurotrophic factor in the hippocampus and blood serum. Most importantly, some probiotic bacteria can reduce inflammation by inhibiting the release of a C-reactive protein and other proinflammatory cytokines (IL-6, IFN-γ, TNF-α and IL-1β) and upregulating anti-inflammatory cytokines such as IL-10 in the blood plasma and the spleen. A recent in vitro study conducted by Dyakov et al. demonstrated that the protein FN3 produced by bifidobacteria possesses TNF-α-binding properties. *Bifidobacterium longum* contains the gene cluster PFNA, which is presumably involved in species-specific communication between the bacteria and their hosts. The gene cluster PFNA consists of five genes, including *fn3*, which codes for a protein containing two fibronectin type III domains. *E. coli* BL21 (DE3), containing the recombinant plasmid pET16b:fn3, was genetically engineered to produce a recombinant FN3 protein [20]. Probiotic bacteria are also known for their ability to activate many antioxidant enzymes in the brain and blood serum—namely, superoxide dismutase and glutathione peroxidase. The relationship between the gut microbiome-derived enzymes and the human organism remains largely uncharted territory. Enzyme preparations, for instance, work differently in the human body. The most important question is how enzymes/metabiotics penetrate human cells. These could be type III secretion systems, bacteriophage infections, Gram-positive bacterial extracellular vesicles, ergothioneine and microbe-derived metabolites such as *L. reuteri* [21][22][23][24]. Unfortunately, none of the abovementioned mechanisms were described in other species. Probiotics can interact with the cell components; it is also plausible that probiotics interact with the metabolites of human cells, thus giving rise to new compounds. However, these are often contradictive and speculative pathways that will not be addressed in this study. Probiotics can achieve this in many ways, including by the direct production of antioxidant enzymes or by reduction of the lipid peroxidation levels in the blood [12][25]. Aside from that, the role of probiotic bacteria is central to sustaining the integrity of the gut–brain barrier and the balance of the gut microbiota composition [18].

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