NOVEL USES OF PROBIOTICS IN HUMAN HEALTH

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Abstract: *Probiotics are live organisms which, when administered in adequate amounts, confer a health benefit to the host. They are found in breast milk, dairy products, fermented vegetables, peas, etc. The obtained results of using probiotics to treat some disorders, especially of the gastrointestinal (GI) tract, but of the skin or the liver as well, make Metchnikoff's perspectives extend and the abundance of commercial probiotic found on the market is the proof that there are other, non-invasive, ways to treat diseases. The mechanism behind their effect is the following: by using probiotics, the number of selected bacteria will grow exponentially so the pathogens will not be able to colonize the GI tract, a process called "competitive exclusion". The main effects of probiotic use were found in improving the patient's gastrointestinal health, but also some promising results on anti-hypertensive activity; skin, oral and urogenital health; immune and liver function and cancer.*

Probiotics (lat. "pro" – for, gr. "biotic" – "bios" – life) have been defined in 2001 by a Joint FAO/WHO group of experts as live microorganisms which when administered in adequate amounts confer a health benefit to the host.(1) The introduction of the probiotic concept in 1907 by Nobel Prize winner Elie Metchnikoff, who suggested that "the dependence of the intestinal microbes on the food makes possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (2), lead to various definitions of the term "probiotic". This was firstly used in 1965 by Lily and Stillwell to describe "substances secreted by one organism which stimulate the growth of another" (2), followed by Parker, who in 1974 defined them as "organisms and substances which contribute to intestinal microbial balance". In 1989, Fuller suggested that probiotics are "live microbial supplements which beneficially affect the host animal by improving its microbial balance" (3), followed by Salminen et al. who stated that probiotics are "foods containing live bacteria which are beneficial to health".(2) The 2001 Guidelines for using probiotics conceived by FAO/WHO underlined that the benefits of one line or strain does not necessary apply to the other ones.

Most probiotics are bacteria – both lactic acid bacteria (LAB) and non-lactic ones – but also yeasts.(4) The most commonly used probiotics are: ***Lactobacillus*** – found in kefir, yoghurt, and other fermented foods; ***Bifidobacterium*** – found in some dairy products; ***Saccharomyces boulardii*** – yeast probiotic; ***Streptococcus salivarius subsp. thermophilus*** – found in some dairy products, it generates large amounts of lactase; ***Enterococcus faecium*** – found in human and animal intestinal tract; ***Leuconostoc*** – LAB found in sauerkraut and other pickled vegetables.(4) The most used LAB are *Lactobacillus acidophilus, L. bulgaricus, L. plantarum, L. casei, L. pentoaceticus, L brevis* and *L. thermophiles*, as well as other Gram-positive bacteria introduced in food fermentation.(5) A new and unconventional probiotic source is green peas (*Pisum sativum*), due to the large amounts of *Leuconostoc mesenteroides* contained inside the beans.(6)

One of the first reviews of the probiotic action of lactic acid bacteria (LAB) was written in 1999 by Naidu A.S. et al. (7) and it shows their ability to „prevent adherence, establishment and replication of several enteric mucosal pathogens, while releasing different enzymes into the intestinal lumen" and to manifest „synergistic effects on digestion and alleviate symptoms of intestinal malabsorption". Consuming dairy products fermented with LAB can have potential antitumor effects, because it may inhibit "the mutagenic activity; decrease several enzymes implicated in the generation of carcinogens, mutagens, or tumor-promoting agents; suppression of tumors; and the epidemiology correlating dietary regimes and cancer".

The specific cellular components in LAB strains seem to induce strong helpful effects, including the „modulation of cell-mediated immune responses, activation of reticulo-endothelial system, augmentation of cytokine pathways and regulation of interleukins, and tumor necrosis factors".

Exploring prophylactic and therapeutic applications of probiotic LAB for reducing the addiction to synthetic antimicrobial substances and to control the continuously growing immune-compromised host population, resulted in a great success after 143 human clinical trials during 1961-1998, on a total of 7,526 subjects studied, where no one reported an adverse effect after oral intake of LAB; moreover LAB were well tolerated and proven to be safe for consumption. The research team stated that new functional probiotic foods will include baby formula, children food, fermented fruit juices, cereal and fermented soy bean products, but also disease specific clinical food containing viable LAB, prebiotic precursors and/or pro-bio-active cellular components.(7)

Another important review regarding the importance and effects of probiotics or products containing probiotics has

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also been published in 1999, by Sanders Mary Ellen.(8) The most common effects were reduced lactose intolerance or diarrhea syndromes, encouraging anticarcinogenic and immunomodulation results, a strong decrease of the acute toxic effects of the intestinal flora metabolism during an exponential growth of gut bacteria or hepatic disease. By using *Lactobacillus salivarius* WB1004 the infection of human and murine gastric cells with *Helicobacter pylori* was inhibited both *in vitro* and *in vivo.* Sanders also mentioned the benefits of oral or local intake of probiotics for improving women's urogenital tract by modifying the local microflora and decreasing the recurrence of *Candida* infections or bacterial vaginosis.(8)

Probiotics like *Lactobacillus* and *Bifidobacterium* had good results over intestinal diseases, stimulating the immunity. Until Sanders' review (8), probiotic bacteria showed sufficiently positive results over antibiotic-associated diarrhea, toxic amines in the blood stream of chronic kidney and liver disease patients with small bowel bacterial overgrowth, diarrhea, bowel disorders and pseudomembranous colitis.

This review focuses on the newly discovered and prospective uses of probiotics in the 21st century in human health. Based on the target, we can classify the effects as it follows:

Gastrointestinal disorders

Because they have demonstrated a high adherence to the intestines epithelial cells and then a great resistance in the GI tract, the most common probiotic strains are part of *Lactobacillus, Streptococcus and Bifidobacterium* genus, closely followed by enterococci and yeasts.(9) Gastrointestinal disorders may be classified by their impact, from annoying to fatal.

Adults tend to be unable to digest lactose because of the deficit of lactase, the lactose metabolizing enzyme. Those who have lower levels of lactase tolerate lactose from yoghurt better than that from milk, probably because the small intestines microbiota increases lactose digestion, which may enhance the contact between lactase and lactose.(10)

Diarrhea can have microbiological, immunological or physiological causes, some being linked to normal microflora modifications. After administrating probiotic bacteria the host's organism was relieved of: antibiotic-associated diarrhea, the presence of toxic amines in the blood flow at patients with chronic renal and liver diseases due to the bacterial overpopulation of the small intestine, travellers' diarrhea, irritable bowel syndrome (IBS), inflammatory intestinal diseases, lactose intolerance, rotavirus or other pediatric diarrhea symptoms, pseudo-membranous colitis after *Clostridium difficile* infection.(5,8)

Lactobacillus rhamnosus GG (LGG) combined with *L. acidophilus, L. bulgaricus* LB1, *L. reuteri* and *Bifidobacterim lactis* gave encouraging results in lowering the rotavirus infection period which developed into pediatric diarrhea, combined with *L. acidophilus, L. bulgaricus* LB1, *Streptococcus thermophilus* and *Bifidobacterim bifidum* helped prevent various forms of acute diarrhea.(5,11)

The GI disorder caused by an immunological response after gluten ingestion is known as "celiac disease" and it affects 2% to 5% of genetically susceptible individuals. It has been considered that some micro-environmental conditions such as gut microbiota may influence this response. By performing tests on a humanized mouse model of gluten sensitivity, a new study published by Elsevier found that gut microbiome can play an important role also in the body's response to gluten.(12)

Other disorders treated with probiotics were Crohn's disease and diverticulitis, but although they seem safe in a clinical setting, the situation may be different for hospitalized immunocompromised patients who have a higher risk to develop sepsis – generalized inflammatory reaction.(13)

Crohn's disease and ulcerative colitis are chronic diseases of the GI tract with no exact known cause that have similar symptoms, such as inflammation and diarrhea. When patient is suffering from Crohn's disease the inflammation is located in the colon serosa, submucosa and mucosa and in some cases to the entire GI tract, but in ulcerative colitis only the submucosa and mucosa are inflamed. Crohn's disease together with ulcerative colitis are referred to as inflammatory bowel disease (IBD). The Crohn's disease patient also experiences weight loss and abdominal pain; and if ulcerative colitis – bleeding. Clinical placebo controlled studies showed that probiotics can help ease the associated IBD symptoms.(10)

S. boulardii and other LAB or combinations between these were proven to help in ameliorating the symptoms of antibiotic-associated diarrhea. In this respect, literature (13) mentions using VSL#3 for diverticulitis and maintenance of remission of chronic relapsing pouchitis after inducing the remission with antibiotics and *B. infantilis* 35624 for IBS. *E. coli Nissle* 1917, LGG and VSL#3 are as effective in inducing or maintaining the remission state for ulcerative colitis or Crohn's disease like the standard therapy with mesalamine – prolonged release anti-inflammatory agent. If they are applied simultaneously their effects will not cumulate.(13) VSL#3 is a highly-concentrated probiotic made of eight bacterial species normally found in the GI microflora – four lactobacilli: *Lactobacillus casei, L. plantarum, L. acidophilus and L. delbrueckii subsp. bulgaricus*; three bifidobacteria*: Bifidobacterium longum, B. breve and B. infantis* and *Streptococcus salivarius subsp. thermophilus.*

A spectacular breakthrough in GI treatment with probiotics is represented by the use of HuMiX – human-microbial crosstalk – which is a modular microfluidics-based model developed for co-culturing human and microbial cells under *in-vivo*-like conditions, specific to the GI human-microbe interface.(14) This device allows the re-enactment of transcriptional, metabolic or immunologic conditions after adding *Lactobacillus rhamnosus* GG. A transcriptional response will appear only if another culture is added (*Bacteroides caccae*) along with LGG.

HuMiX consists of three chambers (the top one with growth media that circulates among the other chambers, the middle one with a membrane that holds the human intestinal cells, and the lower chamber, being a bacteria growing area) with the ability to recreate the internal aerobic and non-aerobic conditions of the human GI, but also giving the researchers the possibility to monitor oxygen level in real-time, access to every cell compartment and to observe everything through state-of-the-art "microscopes or high-resolution multi-omic analyses".(14)

Immune function

Probiotics give different results depending on strain or administration method, dosage or the immunological state of the patient. *Lactobacillus acidophilus, Lactobacillus paracasei, Lactobacillus casei, Lactobacillus rhamnosus, Bifidobacterium longum, Lactobacillus johnsonii EM1, Lactobacillus gasseri, Bacillus clausii, Escherichia coli Nissle (EcN)* 1917 were firstly used alone but then in combination with other strains (*LGG* with *L. gasseri, L. acidophilus* and *Bifidobacterium lactis* or the probiotic mix VSL #3) to test their effect on allergic rhinitis. Probiotics from *Lactobacillus*

and *Bifidobacterium* gave notable results both because of the fast observed effects but also because of the immune-modulating properties shown in co-culture with mononucleic human peripheral blood cells.(15)

A recent double-blind human trial was conducted to determine a connection between changes in the gut microflora and birch allergy symptoms, and whether probiotic administration has any effect on any of the two. Forty seven allergic children were randomly given either a combination of *L. acidophilus* NCFMTM and *Bifidobacterium lactis* B1-04, or a placebo, the results being monitored during the four months of the experiment. After analyzing their blood and nasal secretions (for cytokines and eosinophils) and their feces (both calprotectin and IgA and for microbiota changes), the study concluded that administrating those probiotics reduced the symptoms associated with birch pollen allergy by lowering the eosinophils levels from nasal secretions and the IgA from patients fecal samples.

The reason the authors used these specific strains was that they were either known for their anti-inflammatory effects or presumed to stimulate Th1 (type 1 helper T cells) response, and their combination might optimize these two effects. The first observed effect was the colonization of the gut microbiota and a high density of these probiotics in the analyzed fecal samples, indicating a positive response to this treatment.(16)

LAB strains act as competitive inhibitors, competing with other bacteria for growth along the digestive tract, thus improving immunity by raising the IgA-producing plasma cells, the number of T lymphocytes and Natural Killer (NK) cells and enhancing phagocytosis.(17)

The ESKAPE group (Gram-positive and Gram-negative bacteria like *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginos* and *Enterobacter* strains) is studied intensively. Probiotic therapy was studied on the ESKAPE pathogens – lactobacilli, bidifobacteria and propionibacteria were used against *S.aureus* and *P. aeruginosa,* and a study from 2014 proved that *Propionibacterium acnes* killed planktonic *S. aureus* by lowering the bacterial cytoplasms pH: moreover, the propionic acid showed a wide spectrum activity against *E. coli* and *Candida albicans,* inhibiting their growth.(18)

Skin health

By influencing the cytokines – inflammatory mediators – some LAB have been proved to reduce inflammation and hypersensitivity.(5,19) The healing process of a wound consists in inflammation, cell proliferation (derma fibroblasts and keratinocytes) and extracellular matrix remodeling.

A chronic wound has a chronic inflammation and so the cellular proliferation and healing take a very long time. The bacterial film has a great significance in the dynamic of the chronic inflammation that occurs in non-healing wounds, studies indicating that over 60% of these wounds have a biofilm (20), and it can eventually lead to the host death because of the infections associated with it. Knowing the interactions between the harmful bacteria and the host, hence the benefic role of the pH and probiotic use is important for the wound management. The pH increases from acidic towards alkaline because of the low oxygen level, healing stage, debridement, worm therapy and bacterial colonization.(21) By manipulating the wound with probiotics it seems that pH drops to normal, the oxygen tension raises, the inflammation is reducing and the pathogen infection is prevented or stopped. Because probiotics also demonstrated their ability to deactivate pathogen bacterial viral factors, Sonal SM (19) suggests using them to treat the diabetic feet ulcers.

An Argentinian study compared the effects of applying either *L. plantarum,* or silver sulfazidine cream (plus clorhexidine baths) on infected 2nd degree burns and non-infected 3rd ones, observing the number of bacteria and the healing rate. Although there were no significant differences between the two patient groups, there were no observed cases of sepsis either, suggesting that *L. plantarum* might be used as an alternative because it works similar to the classical treatment for the slow healing burns (22), without being invasive. The same strain was tested against diabetic and non-diabetic ulcers and, although there were no significant differences between the two study groups, *L. plantarum* topical treatment reduced bacteria, neutrophils, apoptotic/necrotic cells and promoted wound healing.(23)

Diabetes

Recent clinical trials performed in Denmark before 2010 showed a connection between the intestinal microbiota and metabolic diseases such as diabetes or obesity. The microbiota consisted mainly in Firmicutes (*Lactobacillus* being the most important), Proteobacteria and Bactereodetes (pathogens known for causing tooth decay and gum disease). The researchers discovered that a high amount of Bactereodetes and a low amount of Firmicutes "will lead to decreased glucose tolerance which is key problem with diabetes mellitus".(24) By administrating probiotics, the gut microflora is supposed to be balanced once again and therefore the insulin resistance (with *Bifidobacterium spp.*) and hypertensive conditions can be lowered.(24)

Cancer

Laboratory studies included *Lactobacillus delbrueckii* subsp. *bulgaricus* that manifested a binding behavior with the heterocyclic amines (carcinogenic), but also one human trial that suggested an anti-carcinogenic activity of LABs because they reduce the β-glucuronidase enzyme, known for its ability to create colon cancer producing colloids in the digestive system.(25) Furthermore, people consuming dairy products shown a lower incidence of colon cancer compared to those who do not.

Another mechanism that encourages probiotics ability to fight cancer is altering the intestinal transit time, hence facilitating a faster elimination of mutagenic agents; mechanical studies suggest that probiotics act on epithelial colon cells kinetics, so they do not allow cancerous cells to proliferate.(5)

Liver disease

Any change of gut microbiota modifies liver function and could lead to the beginning of a liver disease such as Hepatic encephalopathy (HE), cirrhosis, spontaneous bacterial peritonitis, etc.

These diseases may appear due to pathogenic bacteria growing due to a modified intestinal permeability or a weak immune system. This can be avoided by using probiotics, because of their microbiota regulatory function, immunity boosting capacity and also because probiotics were proven by Solga AF (26) to slow the development of Hepatic encephalopathy.

Hypertension

Hypertension is mainly caused by lipid abnormality, hypercholesterolemia and obesity. After Mann and Spoerry first stated that lactobacillus-fermented milk has hypo-cholesterolemic effects, scientists have shown that bifidobacteria also reduce the serum cholesterol, as stated both by Sanders (8) by using *L. casei* YIT9018, *L. helveticus*, and by a human randomized, crossover, and placebo controlled

design trial, performed in 2002, by administrating yoghurt containing *L. acidophilus* and *B. longum.* The result consists in a significantly increased HDL (high-density lipoprotein) with beneficial effects in decreasing blood pressure.(24)

Urogenital heath

Women's urogenital tract is susceptible to infections because of the modifications to the internal microflora, especially when Gram-positive cocci, Gram-negative rods or yeasts replace some or all the normal lactobacilli, thus repopulating the natural environment with lactobacilli could improve their health.

Other disturbing agents that can affect the inner layers on the urogenital tract are excessive intimate hygiene, birth-control methods, antibiotics, menstrual cycle, menopause, pregnancy, diabetes, antifungal agents.(2) These factors will increase pH level above 4.2 and an affected vaginal lining, but after using probiotics (localized treatment) the lining is secured by the protection and barrier double-mechanism and the pH goes back to a normal 3.8-4.2.

Oral health

Cavities appear because of the acidic environment created by bacteria inside the oral cavity. One of the most harmful bacteria that resides in the oral cavity is Gram-positive anaerobe *Streptococcus mutans.* A group of scientists from the University of Florida discovered a new strain of *Streptococcus*, called *S.* A12 which breaks arginine to ammonia and therefore increase the pH level and inhibits the growth of *Streptococcus mutans*.

The researchers are now exploring the possibility of implanting this probiotic in a healthy child or adult who might develop cavities, but the frequency of this procedure is yet to be established.(27)

Most of the periodontal diseases – gingivitis, periodontitis and pregnancy gingivitis – can be cured or alleviated by using *L. acidophilus*. Gingivitis is treated by using *L. brevis*, *L. casei, L. salivarius, L. reuteri*, and *Bacillus subtilis. L. brevis* has anti-inflammatory proprieties and it also inhibits collagenase (MMP) activity. *B. subtilis* decreases the number of pathogens in periodontal tissues.(28)

Studies should be continued to see how probiotic intake can influence human health, whether the focus is on cancer, chronic wounds management, gastrointestinal problems, obesity, etc. by using more strains, by modulating the exposure time, administration technique, target place, study method and the simultaneous interaction between all of the above.

CONCLUSIONS

The complexity of the modern life and the higher health standards have led to a cautionary look on "classical" treatments, i.e. surgery and chemical treatments, and to look closer to "unconventional" treatments, although this refers to thousand years of experience and wisdom.

Among these methods, probiotics have already proven their efficiency in certain diseases, and the researchers strive to discover new uses of these beneficial organisms. The researches should continue, in order to confer probiotic academic and then public acceptance.

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