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Probiotics Review and Future Aspects

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Abstract:- Probiotics are live microorganisms that benefit human health when administered in sufficient dosages, and are widely used as therapy to re-establish unbalanced human microbiota. They are composed of various microorganism strains, most commonly lactic acid bacteria such as Lactobacillus, Bifidobacterium, and Streptococcus, or yeast Saccharomyces boulardii, which are beneficial for human health. These microorganisms have the ability to resist bile salts, gastric acids, and pancreatic enzymes, as well as to colonize the intestinal tract. Thus, they are the optimal choice for probiotics. They can also be ingested through daily food intake. Fermented food, especially yogurt, is rich in probiotic microorganisms. Recently, probiotics are being used to effectively treat certain acute and chronic diseases as intestinal disorders are highly related to irregular gastrointestinal microbiota. Irritable bowel syndrome is the most regularly diagnosed gastroenterological disorder. In addition, diarrhea can be reduced or even treated with the proper usage of probiotics. The approach of treatment of lactose intolerance and certain types of cancer by probiotics is currently being researched. Probiotics are also used to prevent pathogenic bacteria from colonizing the intestine. The proof of the connection between diseases or certain medical conditions and probiotics as therapeutic agents is interpreted by mechanisms of action. Currently, probiotics are a very popular field among researchers. The main aim of this research is to establish and review potential microorganisms that have probiotic bacterial properties.

Keywords:- *Probiotics; Microorganisms; Treatment; Lactobacillus; Bifidobacterium.*

I. PROBIOTICS AND THEIR HISTORY

Probiotics are defined as ‘microorganisms, which, when ingested, may have a positive effect in the prevention and treatment of a specific pathologic condition’. This definition was declared by Charteris et al. [1]. The word probiotic is derived from Latin “pro” and Greek “biotic”- meaning for life [8].

The history of probiotics is assumedly as old as is human history. The theory of probiotics was firstly suggested by Nobel Prize-winning Russian scientist Eli Metchnikoff in 1908. He stated that long life was associated with the consumption of fermented milk products [2]. Lilly and Stillwell firstly used the term probiotic in 1965 to define stimulation of growth of one microorganism, by substances that were secreted by another [9]. In 1974, Parker improved the description by specifying that probiotics are ‘organisms and substances which contribute to intestinal microbial balance’[7]. The definition was then altered in 1989, by Fuller to ‘a live microbial feed supplement which beneficially affects the host animal by improving its microbial balance’. This was crucial because the term substance is too broad to be linked to probiotics. Fuller fortified the theory of way of action of probiotics in a way that probiotics are used as treatments for re-establishing natural condition that exists in an organism, which has been disrupted in any way. One of the modern definitions was proposed by Salminen et al, in 1998, and defined probiotics as ‘foods which contain live bacteria which are beneficial to health’. Furthermore, in 2002, Marteau et al, defined probiotics as ‘microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being’. The up-to-date definition of term probiotic is represented by Charteris et al. [1][2]

II. PROBIOTICS IN FOOD

Functional food is any healthy food that is consumed usually and is physiologically beneficial property (health-promoting or disease-preventing), apart from the basic function of supplying nutrients. One category of functional food is food containing probiotics. Their beneficial effects are supporting the health of the intestinal tract and boosting immunity. One example of this category of food is fermented dairy products, which include yogurt, kefir, kombucha, sauerkraut, pickles, miso, tempeh, kimchi, sourdough bread, some cheeses, cultured milks, even some juices, snacks and bars, all containing strains of beneficial microorganisms, vitamins, minerals, and natural fibers. Overall, yogurt is weighed as the most valuable probiotic food. The concentration and type of species of bacteria differ from one yogurt to another but ranges from 90 billion to 500 billion colony-forming units (CFU) that stands for

how many bacteria are capable of dividing and forming colonies [6]. Also, all cereals are broadly suitable substrates for human-derived probiotic strains. However, they are controlled by numerous factors including composition, processing, substrate formation for the growth of probiotic strain, and so on [3].

III. INTESTINAL MICROFLORA AND MICROORGANISMS

The large intestine is a huge surface area that has the opportunity to influence the immune system since it is constantly exposed to different antigens. It is the location in the body with the highest microbial concentration about 10^11 – 10^12 cells per gram of intestinal content. Bacteria dominate the gut microbiota, while fungi and archaea represent only 1% of the species [7]. For an organism to be considered as a probiotic microorganism, some criteria have been proposed. Some of the features of the criteria are: harmless for the host organism, resistant to gastric acid and bile salts, ability to colonize the gastrointestinal tract by adhering to the epithelial tissue, ability to prevent adhesion of pathogenic microorganisms, tolerance to nutrient supplies, and antimicrobial activity [1].

The most frequently used probiotics are strains of lactic acid bacteria *Lactobacillus*, *Bifidobacterium* and *Streptococcus.* The first two are considered to resist bile salts, pancreatic enzymes, gastric acid and have the ability to adhere to colonic mucosa and colonizing the intestinal tracts. Moreover, they have demonstrated the skill to inhibit the in-vitro growth of many enteric pathogens including *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli, Clostridium perfringens* and *C. difficile.* The yeast that is most commonly used in probiotics, *Saccharomyces boulardii*, possesses the property of a probiotic agent, by inhibiting the growth of several microbial pathogens. It is not affected by antibiotic treatments and can survive transit through the gastrointestinal tract [1].

A. Bifidobacterium

This genus represents the major group of saccharolytic bacteria that are found in the large intestine. These bacteria produce vitamin, mainly group B, and digestive enzymes such as casein phosphatase and lysozyme. In addition, they produce metabolic products that lower pH in the local gut environment and are producing an unfavorable environment for bacteria growth. The metabolic products can be acetate, lactate or protein that can inhibit the adhesion of pathogenic Escherichia coli. Some of the bacteria that belong to this genus are: *Bifidobacterium bifidum, B. breve, B. infantis, B. longum*, etc. [9].

B. Lactobacilli

The genus Lactobacilli lack plasmids which makes them suitable for preventing the transfer of antibiotic resistance. They are also able to inhibit the growth of other bacterial species such as Clostridium, Pseudomonas, Staphylococcus, Streptococcus, and Enterobacteriaceae. This ability depends on the hydrogen peroxide production that decreases pH and oxygen concentration. Also, one of the agents of this ability to inhibit the growth of bacteria is the production of bacteriocins. Initially, *Lactobacilli casei GG (LGG)* was selected as a therapeutic bacterium because it is resistant to gastric acid and bile digestion. Another lactobacilli, *L.acidophilus*, has also shown the ability to inhibit the growth of pathogenic bacteria such as *Yersinia enterocolitics, Bacillus cereus, E.coli, Listeria monocytogenes,* and *Salmonella*. What is more, *L. casei* and *L.acidophilus* enhance immunoglobulin A production from plasma cells. Cells of the gastrointestinal tract upon exposure to the lactobacilli are triggered to express interleukin-12 (IL-12), IL-6, tumor necrosis factor (TNF) mRNA and cytokine excretion [9].

IV. PROBIOTIC AS A THERAPY IN GASTROINTESTINAL DISEASES

Recently, clinical trials assessed the ability of a probiotic to effectively treat certain acute and chronic diseases, since many intestinal disorders have been related to an unbalanced gastrointestinal microbiota [7][9]. Scientific evidence support several roles that probiotics can act in alleviating symptoms of several diseases [7].

The cause of lactose intolerance is the reduced production of beta-galactosidase. This is a normal condition in all adult healthy mammals and should not be considered as a disease, but people from northwest European descent are an exception. In people who are intolerant to lactose, consumption increases the osmotic load in the small intestine with the secretion of fluids that can cause diarrhea. The fermented milk products do not cause the occurrence of the symptoms as opposed to the consumption of milk. This can be explained due to the presence of beta-galactosidase in the bacteria fermenting the milk. Upon entering the body, bacteria are lysed by bile and the enzyme beta-galactosidase is released that is able to degrade lactose. The fermented milk products have a longer transit time through the gastrointestinal tract and even more aid digestion of lactose. It is reported that *L. delbrueckii, L. bulgaricus* and *S.thermophilus* are associated with fermented products and are more effective. Certain probiotic bacteria might be a way of therapy in individuals with lactose intolerance, but this approach is still uncertain [5].

Infectious diarrhea can be treated and infectious gastroenteritis can be prevented by probiotics. There are shreds of evidence that show the reduction of symptoms duration by 17 hours and the frequency of diarrhea when treated with probiotics. The therapeutic capability of probiotics has been shown to be effective in the treatment of rotavirus diarrhea when treated with oral rehydration therapy and Lactobacillus rhamnosus GG (LGG). LGG is able to reduce the duration of symptoms of rotavirus and prevent viral diffusion. Also, the probiotics are efficient in preventing community-acquired and nosocomial infectious diarrhea. In contrast, probiotic microorganisms did not show any improvement in the treatment of non-rotavirus gastroenteritis, but new probiotic bacteria, *L.paracasei, L*

rhamnosus GG, L.reuteri, L.casei, and B.lactis, shows the ability to successfully treat non-rotavirus diarrhea [5][9]

Diarrhea can occur due to the administration of antibiotic therapy and is called antibiotic-associated diarrhea (ADD). Even though most antibiotics are related to ADD, those that act against anaerobic bacteria are mostly the cause of diarrhea and the risk increases with the use of aminopenicillins. It occurs in 5-30 % of the general population and in most cases no infectious agents are found. *Clostridium difficile* is mostly associated with ADD and the prevention includes usage of probiotics since diarrhea is correlated with the change in the normal composition of the intestinal microflora. *S.boulardii* has been recognized as the most effective probiotic organism. This yeast preparation inhibits the growth of pathogenic bacteria. Its optimal living temperature is 37°C and resistance to digestion makes it a good choice for treatment since it is able to reach a colon in a viable state. *S.boulardii* is not affected by antibiotic treatment and is rapidly eliminated after the therapy is completed. It inhibits toxins A and B from *C.difficile* by releasing protease, but a certain risk of diffusion of *S.boulardii* in immunocompromised patients is present [9].

Probiotic therapy has been administered in the past few years in inflammatory bowel disease. It has shown several mechanisms of action that can be used in the management of clinical inflammatory bowel disease [9].

In the epidemiological study, Bifidobacterium number was decreased in patients with Crohn's disease and since antibiotics are widely used in the treatment of this disease, probiotics are suggested as a new treatment. Lactobacilli that produce an immunomodulatory effect on cytokine production can be beneficial in reducing the inflammation that is a form of a Crohn's disease [9].

The most frequently diagnosed gastroenterological disorder is irritable bowel syndrome, which is characterized by abdominal pain and irregular bowel movements. The increased gas production as one of the symptoms is suspected to be due to the change in the intestinal microflora. It has been also associated with the lower lactobacilli and bifidobacteria colony number and increase anaerobic Clostridium spp. in place of anaerobic Bacteroides spp. and Bifidobacterium spp. Clinical trials using probiotic supplements have suggested that probiotics can be a promising therapy [9].

Pathogenic agents, that can cause gastroduodenal ulcers, increase the risk for gastric adenocarcinoma and lymphoma in humans is *Helicobacter pylori*. Colonization by this pathogen results in chronic gastritis and the current treatment includes a combination of antibiotics and a proton pump inhibitor. Due to the antibiotic resistance, this treatment does not treat all patients and they comply with the diverse side effects such as vomiting, diarrhea, and abdominal pain. Recently, alternative therapeutic treatments are emerging such as usage of probiotic supplementation. Various modes of action of probiotic microorganisms account for the reduction of colonization. Lactobacilli have been used to treat *H.pylori* infection alone and together with other agents. Probiotics, according to the clinical trials, do not eliminate *H.pylori* but help maintain lower levels of *H.pylori* in the stomach [9].

V. PROBIOTIC THERAPY USED FOR THE TREATMENT OF VARIOUS CONDITIONS

A dose of microorganisms administered vary between 1 × 109 to 10 × 109 colony forming units per dose and there are no uniform therapeutic schedules. Usually, probiotic therapy is administered twice a day or in recurring weekly schedules. 109 live cells of probiotic microorganisms daily are sufficient to temporarily colonize the gastrointestinal tract [9].

Besides using probiotics in treating different gastrointestinal diseases, with the emergence of autoimmune and atopic diseases, a probiotic treatment has been evaluated as one type of therapy for these conditions, too. Several factors can be involved in developing an allergy or autoimmune disease. One of these factors is reduced microbial concentration during infancy and early childhood, which is important in postnatal maturation of the immune system. The diet directly influences the development and composition of the gastrointestinal tract since the intestines are sterile at birth. Probiotics may influence the composition of the microflora and establish the normal gut flora [9]. Also, immunoinflammatory response to the antigens from ingested food in allergic individuals has been shown to be reduced by the probiotics by promoting the production of anti-inflammatory cytokines [5].

Lactobacilli are dominant bacteria in the female urogenital tract and during infection of the vagina or urinary tract by faecal microorganisms the number of lactobacilli decreases. Antibiotics are the treatment of choice but frequently can cause gastrointestinal symptoms. What is more, antibiotic resistance may occur which makes antibiotics unable to restore a natural barrier to infection. Some probiotics can be used as an alternative strategy for urogenital tract infection treatment and protection. The ability of probiotic bacteria to adhere to the uroepithelial cells and produce inhibitors of pathogenic growth makes them able to compete with the pathogenic bacteria for the attachment to the epithelial layer [9].

VI. PROBIOTICS AND CANCER

Hypothetical mechanisms are stated that could exert an anticancer effect, particularly in colon cancer. Some of these factors are binding mutagenic compounds and stimulating their secretion, inhibiting the ability of pathogens to convert procarcinogens into carcinogens using different enzymes, causing deactivation of carcinogens by protecting DNA from damage by bacterial invasion of intestinal stem cells and stimulating immune surveillance and suppress inflammatory processes. Even though these modes of action may sound like a good proof for using

probiotics in cancer prevention and in, they are insufficient. In contrast, probiotic supplements have the potential to cause infections in individuals with compromised mucosal epithelia. Epidemiological studies have reported that probiotics demonstrated low infection in countries where the use of these therapeutic agents is not used frequently. Primarily, immunocompromised patients have acquired an infection after using probiotic therapy [9].

VII. HOST-PROBIOTIC INTERACTIONS AND MECHANISMS INVOLVED

Mechanisms by which probiotics act are: (i) interference with pathogenic bacteria by competing with nutrients and adhesion sites, (ii) improvement of the barrier function of the epithelial lining, (iii) immunomodulation, and (iv) influence on other organs of the body through the immune system and neurotransmitter production [7].

Besides its ability to adhere to the intestinal epithelium and prevent enteropathogens from invasion, probiotics also produce antimicrobial substances, for instance, bacteriocin and short-chain fatty acid (SCFA), which inhibit the growth of pathogens. Intestinal microbiota, as well as some of the emerging probiotics, have genes that code for proteins that are responsible for biochemical pathways that are not present in the human host, such as degradation of complex carbon source or vitamin and other essential compound production [7].

Probiotics can interact with the host at 3 different levels of the human GIT, such as mucus layer, epithelial layer and gut-associated lymphoid tissue (GALT). The mucus layer is subdivided into two sublayers: outer and inner. The outer sublayer is gel-like, and it has a presence of high numbers of bacteria, antimicrobial peptides, and secreted immunoglobulin A, aimed to limit the amount of colonizing this location by commensal bacteria. The epithelial layer has different cell subpopulations and lines the whole intestine lumen. Enterocytes and colonocytes are specialized for nutrient absorption, Goblet cells for the secretion of mucin, major component of mucus, Paneth cells for the release of antimicrobial molecules, and M cells, specialized in transepithelial transport, which are cellular mediators between the molecules and microorganisms present in the intestine lumen and innate immunity. The interaction of the probiotic and host organism at this level is of high importance for keeping the barrier function, since the tight junctions, location in which two adjacent epithelial cells join together, seal the lateral surface of the enterocytes. The major point of interaction between the human host and the probiotics is the GALT, which us mucosal-associated lymphoid tissue and is located in the lower part of the GIT. GALT is composed predominantly of Peyer's patches and plasma/lymphoid cells. The cells of GALT are responsible for the defense and immune-modulatory functions [7].

Probiotic bacteria have well-conserved molecules known as microbial-associated molecular patterns. They act by interacting with specific pattern-recognition receptors, which are present on the surface of epithelial and dendritic cells in the organelle membrane. This interaction is responsible for the beneficial effects of probiotics, and for the normal interaction of the intestinal microbiota with the human host [7].

VIII. FURTHER RESEARCH

As we are approaching the age in which we have more affordable and practical tools for genome and metagenome sequencing as well as editing and modifying bacterial genomes, we suppose that the development of probiotics will address the specific needs of customers and issues that customers and scientists are facing. Knowledge of the structure and function of the human gut microbiome, which is also enhanced through massive simultaneous sequencing, has significantly increased the number of species with potential health benefits, although many of these are still at the very early stage of a research study. Future prospective of probiotic microorganisms will suggest the uses of more different species for the production of probiotics. Researching through literature about understanding the role and action of probiotics as a therapeutic agent, granted the remark that *Lactobacillus* and *Bifidobacterium* were mostly used as probiotic microorganisms. Nowadays, the most effective way approaching probiotics should be finding different species and genus of organisms that have a beneficial effect on human health. Some of these new and yet not sufficiently researched organisms are: *Bacteroides xylanisolvens, Bacteroides ovatus, Bacteroides dorei, Bacteroides fragilis, Clostridium butyricum, Faecalibacterium prausnitzii, Lactococcus lactis.* Some of them are isolated from humans while some are genetically modified organisms. All of these mentioned are in the early phase of development and their development is controversial [4].

IX. CONCLUSION

Probiotics are described as "live microorganisms that confer a health benefit on the host when administered in adequate amounts." [1]. These microorganisms are accepted to be safe for usage and are assumed to be old as human history is [2]. The probiotic species that appear in probiotic products came primarily from the human gut or conventional fermented foods [3]. Therefore, most of the probiotics used, both as commercial therapeutic agents and in research, come from two genera *Lactobacillus* and *Bifidobacterium,* in particular. The yeast that is most commonly used in probiotics, *Saccharomyces boulardii,* possesses the property of a probiotic agent by inhibiting the growth of several microbial pathogens. Other probiotics currently available in the marketplace include Saccharomyces, *Bacillus spp.*, Escherichia coli, enterococci, and *Weissella spp* [1]. Several research articles have validated the usage of probiotics as a therapy for gastrointestinal diseases. Gastrointestinal diseases, treated with probiotics, vary from irritable bowel disease, Crohn 's disease to gastroduodenal ulcers caused by *H.pylori*. Diarrhea symptoms and occurrence were reduced for 17 hours if probiotic therapy was induced. Because of

antibiotic resistance, not all conditions can be cured and patients often complain about side effects. Recently alternative medical therapies, like the use of probiotic supplements, have arisen in clinical cases. The reduction of colonization the intestine by pathogenic bacteria is observed during the use of the probiotics, as well as maintaining the colonization of *H.pylori* under the control. Lactobacilli have an immunomodulatory effect on the development of cytokines and help reduce the inflammation that is one of the symptoms of many diseases. Probiotics as well can help in some of the cancer treatments which have been proved through researches [9]. The mechanisms of action of probiotics serve as proof of all the connections between diseases and different conditions and probiotics as therapeutic agents [7]. In our opinion, these microorganisms are prospective in treating several diseases as gastrointestinal diseases, cancer, allergies, autoimmune disease and clearance of many infections caused by a pathogenic microorganism. Also, they are potential agents with multiple targets. The introduction of new probiotic products may be difficult since the target population might be sceptic about new probiotic microorganism. Nevertheless, another difficulty may be proving the safety and benefits of these microorganisms. It can be assumed that an enormous amount of research about the beneficial effect of the microbiome to human health and well-being, might lead to the discovery and creation of novel and more developed microorganisms. These microorganisms may have an even higher rate of survival in the transit through the gastrointestinal tract, which is one of the main components when administering probiotic therapy. In many instances, these may belong to rare and previously uncharacterized microorganisms with uncommon properties, or may even be microorganisms that were previously thought of as pathogens or pathobionts. Novel approaches in probiotic research may present major challenges for science, industrial management, and regulatory agencies.

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