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REVIEW ARTICLE: CURRENT ROLE OF PROBIOTICS IN FOOD SAFETY AND HEALTH

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Abstract:

Background*: Probiotics are live microorganisms, which when administered in adequate amounts; confer a health benefit on the host. Subcategories of the general term probiotic include probiotic drugs (intended to cure, treat, or prevent disease), probiotic foods (which include foods, food ingredients, and dietary supplements), direct-fed microbials (probiotics for animal use), and designer probiotics (genetically modified probiotics). As the field of probiotics has advanced, the types of clinical indications tested for probiotic impact and the range of physiologic status of subjects being tested have greatly expanded. A given probiotic, tested in different clinical situations, might exert a beneficial effect, show no effect, or result in an adverse effect. However, a negative or adverse effect in certain situations does not negate probiotic status. Such results do, however, stress the need to be specific about the benefits that are documented for each probiotic and the situations in which use is considered to pose an undue risk. Use of "probiotic" to describe a strain refers to proven beneficial effects of the strain. Furthermore, it should not be presumed that a probiotic will be effective or safe under all conditions of use(1)*

Methods *: Review of different article on google scholar and pubmed.*

Results: *The science around the concept of probiotics continues to expand. Current global research efforts have greatly contributed to the understanding of the role of GI commensal organisms in their extraordinary symbiotic relationship with humans. Continued research into the microbiota will no doubt help lead to an improved insight into the impact of probiotics on human health.*

Conclusion*: Evidence continues to emerge that probiotics have an influence on the immune system and thereby may enhance resistance to infections, particularly those of the GI or respiratory tract, and help to mitigate allergies, particularly in infants and young children. Evidence is gradually developing for the potential for probiotics to impact other conditions of the GI tract. One critically important fact to bear in mind is that reported benefits of probiotics should be considered strain-specific. It must be remembered only a limited number of microbes have been documented as probiotic. In all cases, it is clear that probiotics must be consumed regularly in order to confer ahealth benefit.*

Key words: ***probiotics , probiotic drug,*** *Lactobacillus , Probiotic therapy*

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1. INTRODUCTION:

1.1 What Is a Probiotic?

Probiotics are live microorganisms, which when administered in adequate amounts; confer a health benefit on the host. Subcategories of the general term *probiotic* include probiotic drugs (intended to cure, treat, or prevent disease), probiotic foods (which include foods, food ingredients, and dietary supplements), direct-fed microbials (probiotics for animal use), and designer probiotics (genetically modified probiotics). As the field of probiotics has advanced, the types of clinical indications tested for probiotic impact and the range of physiologic status of subjects being tested have greatly expanded. A given probiotic, tested in different clinical situations, might exert a beneficial effect, show no effect, or result in an adverse effect. However, a negative or adverse effect in certain situations does not negate probiotic status. Such results do, however, stress the need to be specific about the benefits that are documented for each probiotic and the situations in which use is considered to pose an undue risk. Use of "probiotic" to describe a strain refers to proven beneficial effects of the strain. Furthermore, it should not be presumed that a probiotic will be effective or safe under all conditions of use(1)(2)(3)(4).

1.2 Are Probiotics Foods or Drugs?

Probiotic therapy is becoming increasingly common in veterinary and human medicine, and numerous probiotic products are now available commercially. There are controversies in literature as to whether probiotics are food or drugs. According to Weese JS, Probiotics are considered to be food supplements, not drugs(5). However, Passariello A*et al* reported probiotics may be registered as food supplements or drugs(6). The definition above issued as part of an expert consultation that specifically dealt with probiotics in food (including water).However; the definition did not include the term "food." Although almost identical to definition as live microorganisms, which when consumed in adequate amounts, confer a health effect on the host"—the consultation substituted the word "administered" for "consumed," presumably to expand the concept of probiotics to include administration in ways other than by mouth. Given that food must be consumed orally, the consultation apparently intended that the definition not be limited to consumption(7)(8).

1.3 What Is Meant by "Health Benefit"?

Key to definition of probiotic is that it must confer a "health benefit." What might not be as clear is what a "health benefit" encompasses. When broadly interpreted, the term refers to both drug-type effects (eg, mitigation of diarrhea), which may be assessed in healthy or diseased populations, and food-type benefits (such as supporting a healthy immune system), which should be assessed in healthy (including at-risk) populations. Furthermore, from the perspective of the Food and Agriculture Organization of the United Nations (FAO), physiologic effects—generally measured by biomarkers rather than clinical end points—that may correlate with health enhancement(9)(10).

1.4 Composition of Probiotics

Probiotics in theory can be composed of any live microbe. A large number of probiotics hail from the *Lactobacillus* or *Bifidobacterium* genera. Also popular is *Saccharomyces boulardii* (a yeast). Less commonly used are strains of *Escherichia coli* or *Bacillus coagulans*. One category of microbe that is typically not considered to be a probiotic is a virus. Live viruses have been administered as vaccines, but such use is generally considered to be outside the realm of probiotics. A distinction should be made between a probiotic and a live, active culture. Fermented foods, especially fermented dairy products, frequently contain live, active cultures. As it is essential that probiotics be documented to have a health benefit, and given that live, active cultures are generally tested only for food fermentation properties and not health benefits, equating live, active cultures and probiotics is not correct. Until the live cultures are shown to confer a health benefit, they should not be called probiotic. Therefore, not all fermented foods, even those retaining live cultures, should be considered to be probiotic foods. Another misuse of the term *probiotic* comes from equating probiotic with native beneficial bacteria. Given that probiotics must be isolated, characterized, demonstrated to have a health benefit, and then administered, it is not correct to talk about "native probiotic bacteria(11)(12)(13)(14).

1.5 Not All "Probiotics" Are the Same

Products contain different genera, different species, or even different strains of the same species, and not all products should be expected to work the same. Therefore, claims of efficacy should be target specific and should be made only for products that have been found efficacious in carefully designed studies. The marketplace has many examples of different strains of the same species: *Lactobacillus acidophilus* NCFM and La-1; *L. rhamnosus* GR-1 and GG; *Lactobacillus casei* Shirota and DN-114 001; *Lactobacillus reuteri* RC-14 and ATCC 55730; and *Bifidobacterium lactis* HN019 and BB-12. Each of these strains has a unique dossier to document

individual health benefits. It is noteworthy, however, that among dozens of European commercial products, the same biotype (based on pulsed-field gel electrophoresis of chromosomal DNA) was predominant among *Bifidobacterium*containing products, suggesting that *Bifidobacterium* strains used commercially may not be so diverse(15)(16)(17)(18).

1.6 Dose

Product effects are dose specific. It is not possible to provide one "minimum dose" that applies to all probiotics because different probiotics are effective at different levels. Some products are effective at 50 million colony-forming units (CFUs)/day to more than 1 trillion CFU/day. This huge range in effective doses likely reflects differences in strains, clinical end points, and perhaps the best guess of the researcher of what level would be sufficient. Dose-response studies are not common in the probiotic. As far as the final product is concerned, the probiotic dose levels should be based on the ones found to be efficacious in human studies and the colony forming units per gram of product is an important parameter. Although the information about the minimum effective concentrations is still insufficient, it is generally accepted that probiotic products should have a minimum concentration of 10^6 CFU/mL or gram and that a total of some 10^8 to 10^9 probiotic microorganisms should be consumed daily for the probiotic effect to be transferred to the consumer. Furthermore, the strains must be able to grow under manufacture and commercial conditions and should retain viability under normal storage conditions. Viability is by definition a prerequisite for probiotic functionality as it potentiates mechanisms such as adherence, reduction of gut permeability, and immunomodulation and constitutes an industrial challenge. Nevertheless, certain studies have demonstrated that viability is not necessary for all probiotic effects as not all mechanisms nor clinical benefits are directly related to viability and that even cell wall components on some probiotic bacteria or probiotic DNA may have significant health effects. Thus for certain probiotic strains optimal growth during the initial production steps might be sufficient and they may not need to retain good viability during storage(1)(8).

1.7 Label

The label should disclose the genus, species, and strain designation of each probiotic strain contained in the product. This approach provides a level of confidence that the product manufacturer is formulating the product with specific strains consistently over time. Furthermore, strain designations tie the product content back to the scientific publications that document claimed health effects. The product label should also indicate the number of live microorganisms that are delivered in each serving or dose, as well as an expiration date. Levels are typically communicated as CFUs. The suggested serving size or dose should be indicated. Labels should describe health benefits that have been substantiated for the product. Finally, proper storage conditions and corporate contact should be indicated.(1)(19)(20)

2 Burden of food borne disease

The foodborne disease is a major cause of morbidity and mortality in the world's population, causing death of about 1.9 million children worldwide each year, even though most of these diarrheal deaths occur in developing countries, although not limited to these countries. It is estimated that in the United States, foodborne diseases are 76 million people sick with 325,000 hospitalizations and 5,000 deaths each year.New forms of transmission of foodborne and increased antibiotic resistance by pathogens, are evading the conventional control measures(21).

CDC defines a foodborne disease outbreak as the occurrence of two or more similar illnesses resulting from ingestion of a common food. During 2009-2010 in the United States, a total of 1,527 foodborne disease outbreaks (675 in 2009 and 852 in 2010) were reported, resulting in 29,444 cases of illness, 1,184 hospitalizations, and 23 deaths. Among the 790 outbreaks with a single laboratory-confirmed etiologic agent, norovirus was the most commonly reported, accounting for 42% of outbreaks. *Salmonella* was second, accounting for 30% of outbreaks. Among the 299 outbreaks attributed to a food composed of ingredients from one of 17 predefined, mutually exclusive food commodities , those most often implicated were beef (13%), dairy (12%), fish (12%), and poultry (11%). The commodities in the 299 outbreaks associated with the most illnesses were eggs (27% of illnesses), beef (11%), and poultry (10%). Public health, regulatory, and food industry professionals can use this infor-mation when creating targeted control strategies along the farm-to-table continuum for specific agents, specific foods, and specific pairs of agents and foods(22).

During 2009-2010 in the United States, 1,527 foodborne disease outbreaks were reported, of which 7,089 cases were caused by *Salmonella* and 651 cases by *Escherichia coli* O157: H7(22). *Salmonella*

enteritidis var Typhimurium is a facultative intracellular bacterial pathogen that infects, replicates and persists in macrophages. This pathogen can cause severe intestinal infections. On the other hand, *Escherichia coli* O157: H7 can cause bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. This strain has a unique capacity to survive in an acidity that is lethal to other *Enterobacteriaceae*(23)(24). As a consequence of the indiscriminate use of antibiotics to treat human and animal microbial infections, some bacteria have developed new resistances(21, 25). In order to avoid the use of antibiotics and to control efficiently the proliferation of gastrointestinal disease-causing bacteria, probiotics are successfully employed (26).

Probiotics are live microorganisms that confer a health benefit on the host when administered in appropriate amounts; consequently their use in the formulation of foods is very common and is still increasing. Probiotics genus of *Lactobacillus* and *Bifidobacterium* belong to the gastrointestinal microflora and are utilized in the manufacture of dairy products.Probiotic prophylaxes and therapies are gaining wider acceptance as more scientific data emerge regarding their interaction between pathogen and beneficial microbes in the human intestinal tract and molecular mechanisms of probiotics' action. Probiotic bacteria which confer beneficial effect for the host and have pronounced antagonistic activity against food borne pathogens are expected to present a clear alternative in the prevention and treatment of foodborne infections (27)(28).

Many studies have attempted to identify specific positive health effects of probiotics on human health. It has been revealed that different species or even strains belonging to the same species exert different effects on human health. Several health benefits have been claimed for probiotic bacteria, which include anticarcinogenic properties, lactose digestion, serum cholesterol reduction and immune system stimulation. Probiotics have also preventive and therapeutic effects on several types of diarrhea of different etiologies. Probiotic bacteria are increasingly used for food and pharmaceutical applications to restore disturbed intestinal microflora and related dysfunction of the human gastrointestinal tract. Probiotic bacteria affect growth of microbial pathogens and favor commensal's microflora proliferation, by synthesizing antibacterial compounds (including bacteriocins, non-bacteriocins and organic acid molecules) and by decreasing pH(29)(30)(31)(16)(32)(33)(34).

In the United States, consumption of chicken and turkey continues to increase and there has been a shift in the dynamics of poultry production. With these significant changes, effective strategies for intervention are required to maintain the food safety of these products to protect public health. In recent years, there have been growing concerns regarding antibiotic resistance, prohibition of growth promoters, and consumer demand for antibiotic or chemical-free produce. Such factors are critical in identifying potentially safe and alternative strategies in bird production. In this context, considering the use of probiotics in poultry production would be prudent as food safety remains a contemporary issue. Their implementation has great potential in delivering promising results by reducing the intestinal pathogenic load and thereby reducing the subsequent contamination in poultry production. Several mechanisms of action have been proposed including resistance to colonization, competitive exclusion, production of toxic and inhibitory compounds, competition for nutrients and stimulation of the immune system. Probiotics also offer potential host-protective health effects and chicken growth benefits by modulating the gut microflora(35).

Antimicrobials are delivered to animals for a variety of reasons, including disease treatment, prevention, control, and growth promotion/feed efficiency. Antimicrobial growth promotants (AGPs) were first advocated in the mid-1950s, when it was discovered that small, subtherapeutic quantities of antibiotics such as procaine penicillin and tetracycline (1/10 to 1/100 the amount of a therapeutic dose), delivered to animals in feed, could enhance the feed-to-weight ratio for poultry, swine, and beef cattle. For many years, the positive effects of this practice were championed, while the negative consequences went undetected. But microbiologists and infectious disease experts facing antibiotic resistance questioned the possible harm from this use .Consumers may be exposed to resistant bacteria via contact with or consumption of animal products—a far-reaching and more complex route of transmission. There is undeniable evidence that foods from many different animal sources and in all stages of processing contain abundant quantities of resistant bacteria and their resistance genes. The rise of antibiotic-resistant bacteria among farm animals and consumer meat and fish products has been well documented. Demonstrating whether such reservoirs of resistance pose a risk to humans has been more challenging as a consequence of the complex transmission routes between farms and consumers and the frequent transfer of resistance genes among host bacteria.

Such correlations are becoming more compelling with the advent of molecular techniques which can demonstrate the same gene (or plasmid) in animal or human strains, even if the isolates are of different species(36).

3.Desirable Probiotic Properties

In order for a potential probiotic strain to be able to exert its beneficial effects, it is expected to exhibit certain desirable properties. The ones currently determined by in vitro tests are (i) acid and bile tolerance which seems to be crucial for oral administration, (ii) adhesion to mucosal and epithelial surfaces, an important property for successful immune modulation, competitive exclusion of pathogens, as well as prevention of pathogen adhesion and colonisation, (iii) antimicrobial activity against pathogenic bacteria, (iv) bile salt hydrolase activity. Nevertheless, the value of these parameters is still under debate as there are matters of relevance, in vivo and in vitro discrepancies, and lack of standardization of operating procedures to be considered. As there are no specific parameters essential to all probiotic applications, the best approach toestablish a strain's properties is target population and target physiologic function (37)(9).

Some authors have suggested that the strong antimicrobial activity of *Lactobacillus* and *Bifidobacterium* strains to inhibit intestinal pathogens, included *Salmonella* Typhimurium and *E. coli* O157:H7 is due to the organic acid production, particularly lactic and acetic acids(38)(39)(40)(41).

Recent study by Naderi et al. reported no antagonistic activity in *Lactobacilli* suspension against test on *Enterococcus* and *Enterobacter* strains and *K. pneumoniae,* which were resistant to most antibiotics. However, an inhibitory effect was observed for *E. coli*(42).*Arias O., A. B. et al.* reported that *L.acidophilus* and *L. rhamnosus* supernatants were effective to inhibit the pathogenic strains growthpresenting a greater effect on the inhibition of *Salmonella* Typhimurium *L. acidophilus* and *L. rhamnosus* supernatants showed a greater reduction of *Salmonella* Typhimurium population (about 6 - 7 LOG CFU reduction) compared to *E. coli* O157: H7 (3 - 5 LOG CFU reduction). In fact, *Salmonella* Typhimurium population became undetectable after 4 and 8 h of culture with *L. rhamnosus* and *L. acidophilus* supernatants, respectively. Moreover, in *Arias O., A. B. et al*, *E. coli* O157: H7 population was reduced 5 and 3 LOG by *L. acidophilus* and *L. rhamnosus* respectively(43).

Microbes, or micro-organisms, include bacteria, fungi, yeasts and algae. They can be found everywhere on Earth, including hostile environments like volcanoes, the ocean bed and deserts. They are incredibly diverse and have adapted over millions of years to occupy their own particular niches. As far as humans are concerned, microbes are best known for their role in causing disease, but their power has also been harnessed for millennia to the benefit of humankind. They are used in the production of fermented foods including dairy products, breads, and vegetables and, of course, wines and beers to name but a few. Owing to their potential for very selective action, microbes are also crucial to the development and production of pharmaceuticals such as antibiotics and to the production of food ingredients such as vitamins and citric acid. Microbes are also involved in the production of many other chemicals and enzymes and are used in waste processing(2, 44, 45).

Most of the 10^{14} bacteria in the gut are found in the large intestine (colon) and, over the past 30 years or more, interest in the gut microbial population – the microbiota – and its environment has intensified. Numerous research studies have shown that, far from being passive inhabitants of the gastrointestinal (GI) tract, the habitual residents of the gut (commensal micro-organisms) interact with their host in a very intricate manner. They may modulate the effect of potentially harmful bacteria, impact the host's GI tract, digestion, metabolism and immune system, and might even influence functions beyond the gut(46).

The concept that food-borne bacteria can be beneficial to health emerged at the turn of the twentieth century and is usually attributed to Nobel Prize-winning Russian scientist Ilya Metchnikoff who hypothesised that consuming large amounts of fermented milk products that contained *Lactobacillus* bacteria ("soured milk") could prolong and improve the quality of life because these bacteria entered the colon and limited the activities of undesirable microbes. Metchnikoff therefore saw the intestinal tract as an organ that could be manipulated to improve health by adding exogenous bacteria to the gut. As a result, commercial yogurts and fermented milks gained some popularity after the First World War, but it was not until the 1980s that the sales of products containing probiotics began to grow rapidly - first in Japan and then extending to Europe during the 1990s(8).

Probiotic bacteria may be defined as '*live micro-organisms which, when administered in adequate*

amounts, confer a health benefit on the host'. They can interact with commensal bacteria and can also have a direct impact on the host. Disentangling these interactions is one of the key challenges for future research. Other key challenges are to understand their mechanisms of action, to elucidate more specifically which probiotic strains can offer which health benefits and to define the intake levels needed to achieve those effects(2).

Today, over 60% of functional food products are directed towards digestive health, with prebiotics and probiotics probably being the most common, worldwide. Probiotics and prebiotics target the host through the gut by distinct as well as complementary mechanisms of actions. The concepts of probiotics for use in the human diet and will explore the scientific basis for potential human health benefits. In general, research to date indicates that these food ingredients offer possible health benefits and do not pose any risks to health. Indeed, a range of naturally occurring probiotics, primarily from the genera *Lactobacillus*, *Bifidobacterium* and *Saccharomyces*, have long been consumed throughout the world either as part of traditional diets or in the form of modern functional foods(11, 47, 48).

4. THE PROBIOTIC CONCEPT

4.2 Definition and history

The word "probiotic" (origins: Latin *pro* meaning "for" and Greek *bios* meaning "life") was first used in 1954 to indicate substances that were required for a healthy life. Out of a number of definitions, the most widely used and accepted definition is that proposed by a joint FAO/ WHO panel (FAO/WHO, 2001): *"Live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host"*(1).

"The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" and "systematic investigations should be made on the relation of gut microbes to precocious old age, and on the influence of diets which prevent intestinal putrefaction in prolonging life and maintaining the forces of the body."Until recently, high quality scientific research supporting the purported benefits of probiotics was limited, partly because the complexity of the gut ecosystem was largely underestimated. In the last three decades, research has progressed and, with the application of molecular techniques, major advances have been made in the characterization of specific probiotics as well as in our understanding of their mechanisms of action and health effects(49)(50)(33).

4.3 Selection of probiotic candidates

Beyond safety, the selection of a probiotic strain is driven primarily by its potential to confer a health benefit for humans. It is commonly considered that, for food applications, probiotics need to survive until they reach the part of the GI tract where they exert their intended effect. For example, to be active in the colon, probiotics must resist salivary enzymes, stomach acid, small intestinal secretions of bile and enzymes as well as the pH changes and chemical milieu of other foods and beverages they will encounter during their passage along the GI tract. In addition, they need to compete with the resident microbiota. Finally, a selected strain has to fulfil a number of technological requirements, such as culturability on a large scale, genetic stability and maintaining viability in a food product or supplement. Thus, the identification of suitable probiotic strains worthy of further study is a very complex and detailed process that can take substantial research effort(51)(40).

The most commonly used probiotics in foods are species from the genera *Lactobacillus* and *Bifidobacterium*, but yeasts such as *Saccharomyces* spp. have also been used. There are a number of important steps required to characterize each strain. Modern molecular methods should be used for species and strain identification because they are far more reliable than phenotypic methods. Thanks to recent progress in technology, sequencing the full genome of a new strain is no longer very expensive or time-consuming and this opens the way for detailed characterization of a specific strain and comparison with its close relatives. Through assessing phenotypic and genotypic properties, microbial taxonomy groups together related species into one genus and, further, related strains into one species. Nevertheless, even when belonging to the same species, different strains can be distinguished by unique genetic and physiological properties(16, 29, 52, 53).

4.4 Safety

The safety of probiotics is tied to their intended use, which includes consideration of potential vulnerability of the consumer or patient, dose and duration of consumption, and both the manner and frequency of administration. Unique to probiotics is that they are alive when administered, and unlike other food or drug ingredients, possess the potential for infectivity or in situ toxin production. Since numerous types of microbes are used as probiotics, safety is also intricately tied to the nature of the

specific microbe being used. The presence of transferable antibiotic resistance genes, which comprises a theoretical risk of transfer to a less innocuous member of the gut microbial community, must also be considered. Genetic stability of the probiotic over time, deleterious metabolic activities, and the potential for pathogenicity or toxicogenicity must be assessed depending on the characteristics of the genus and species of the microbe being used. Immunological effects must be considered, especially in certain vulnerable populations, including infants with undeveloped immune function. A few reports about negative probiotic effects have surfaced, the significance of which would be better understood with more complete understanding of the mechanisms of probiotic interaction with the host and colonizing microbes. Use of readily available and low cost genomic sequencing technologies to assure the absence of genes of concern is advisable for candidate probiotic strains. The field of probiotic safety is characterized by the scarcity of studies specifically designed to assess safety on the one hand contrasted with the long history of safe use of many of these microbes in foods on the other hand(54).

Many probiotic organisms belong to genera represented in the functional group of bacteria known as lactic acid bacteria, which have been safely consumed for many years and as such are presumed to be safe ingredients in the food supply. To formalize and underwrite this concept, a system for a pre-market safety assessment was proposed that leads to a 'Qualified Presumption of Safety (QPS)' in the European Community. In summary, a safety assessment of selected groups of micro-organisms from a defined taxonomic group (e.g. genus or group of related species) can be made on the basis of four pillars of information (identity, body of knowledge, possible pathogenicity and end use). If the taxonomic group and characterization to strain level do not raise safety concerns or if any safety concerns can be defined and excluded, the organism may be granted QPS status. Thus, for any strain of micro-organism that can be unequivocally demonstrated to be from a qualified QPS group (such as *Lactobacillus* or *Bifidobacterium*), further safety assessment is limited to tests for antibiotic resistance. If a microbe is not covered by QPS, then a comprehensive assessment of safety is likely to be required before it can be used in the food supply(11)(55)(54)(56).

4.5 Application of probiotics in food

Probiotic organisms are used in a variety of foods, the main category being dairy products, but they are also present as food supplements in capsule or tablet form. Since viability is an essential property of a probiotic, the final product must contain an adequate amount of living probiotic(s) until the end of its shelf life. A health claim for the addition of probiotics to foods or food supplements should only be made if there are documented benefits based on good quality human trials conducted with the relevant food product containing the specific strain that is the subject of the claim and using relevant endpoints. These studies should also be able to demonstrate the safe, effective dose of the probiotic organism in food. Like legislation on food safety, regulation of health claims for foods varies by country or region and any claims on commercial products containing probiotics must adhere to requirements, which in some cases include pre-market approval of the claim by the regulatory authorities(57)(2)(11).

4.6 Mechanisms of Probiotic Activity

Probiotics have various mechanisms of action although the exact manner in which they exert their effects is still not fully elucidated. These range from bacteriocin and short chain fatty acid production, lowering of gut pH, and nutrient competition to stimulation of mucosal barrier function and immunomodulation. The latter in particular has been the subject of numerous studies and there is considerable evidence that probiotics influence several aspects of the acquired and innate immune response by inducing phagocytosis and IgA secretion, modifying T-cell responses, enhancing Th1 responses, and attenuating Th2 responses (8).

4.7 Immunological functionality

The immunological benefits of probiotics can be due to activation of local macrophages and modulation of IgA production locally and systemically, to changes in pro/anti-inflammatory cytokine profiles, or to the modulation of response towards food antigens(58)(29).

4.8 Production and secretion of antimicrobial metabolites

Many of the probiotic organisms that produce antimicrobial substances often times will have an advantage over organisms that grow and compete vigorously for intestinal sites for colonization. Antimicrobial substances produced and secreted by natural inhabitants of the intestinal tract can either kill or inhibit growth of pathogens. Generally, most bacteria produce agents that either kill or inhibit related species or even different strains of the same species of bacteria. Some of the inhibitory products produced by probiotic bacteria include the short chain volatile fatty (lactic, propionic, butyric, and acetic

acids), hydrogen peroxide, and diacetyl and each has a different mode of action. It is recognized that each strain has unique and different properties and that the probiotic effects of a specific strain must not be extrapolated to other strains(55) .

Modulation of host immunity is one of the most commonly proposed benefits of the consumption of probiotics. *Lactobacillus* and *Bifidobacterium* strains used as probiotics have been acknowledged for their role in preventing and treating acute gastrointestinal infections, allergy and atopic diseases and inflammatory bowel diseases(59).Probiotic lactic acid bacteria can signal the immune system through innate cell surface pattern recognition receptors or via directlymphoid cell activation. Some strains of lactobacilli tointermittently translocate across the intestinal mucosawithout causing infection. Further employment for the use of immunomodulatoryprobiotics in health care is in the control ofmicrobial pathogens. orally delivered probiotics can combat infectious diseases and several potential mechanisms have been proposedto support this phenomenon, including: that localised lacticacid production by probiotics in the GI tract can limitpathogen growth; that anti-pathogen substances secretedby the probiotics (e.g. bacteriocins) are directly microbicidal;that seeding the gut mucosa (albeit transiently) withde novo 'friendly' bacteria can limit pathogen attachment(i.e. competitive exclusion); or that immunomodulatorysignals generated by probiotics can stimulate host immunitysu/ciently to a!ord a degree ofenhanced protectionagainst pathogens. While none ofthese theories is mutually exclusive. suggestive evidence of a rolefor probiotic-mediated immunomodulation in the controlof microbial pathogens (60).

4.9 Microorganisms used as probiotics

Lactobacillus species	Bifidobacterium species	Others
L. acidophilus *L. rhamnosus* *L. gasseri* *L. casei* *L. reuteri* *L. delbrueckii* subsp. *bulgaricus* *L. crispatus* *L. plantarum* *L. salivarius* *L. johnsonii* *L. gallinarum* *L. plantarum* *L. fermentum* *L. helveticus* *L. oris*	*B. bifidum* *B. animalis* *B. breve* *B. infantis* *B. longum* *B. lactis* *B. adolescentis*	*Enterococcus faecalis* *Enterococcus faecium* *Streptococcus salivarius* subsp. thermophilus *Lactococcus lactis* subsp. lactis *Lactococcus lactis* subsp. cremoris *Propionibacterium freudenreichii* *Pediococcus acidilactici* *Saccharomyces boulardii* *Leuconostoc mesenteroides* *Weissella cibaria* *Weissella confusa*

Adapted from(8)

5. Basis of the biological effect of probiotics

The health benefits from probiotic products and applications are extremely diverse and are continuously expanded with new insights and scientific developments.

5.1 Microbiological functionality

The ultimate goals of microbiological interventions through probiotics may be to stabilize or improve microbial homeostasis in a body environment and to lower pathogen invasion and colonization. The resilience of a microbial community against invasion by exogenous strains largely depends on the availability of non-occupied functional niches. If not all functional niches are occupied by the endogenous microbial community, there is an increased risk for pathogen invasion in the ecosystem, colonization, and subsequent infection.

Probiotic microorganisms can be used to improve or restore microbial homeostasis in two scenarios. Firstly, they may occupy functional niches that are left open by the endogenous community, thereby preventing (opportunistic) pathogens from occupying that niche. Such process is often referred to as competitive exclusion, and primarily targets the competition for nutrients, physical sites (e.g. mucus adhesion) or receptors. The second scenario is more of an antagonistic nature as probiotics may actively lower (opportunistic) pathogen invasion or development into the ecosystem. Such approach primarily targets: i) the production of short chain fatty acids and other organic acids (e.g. lactic acid) by probiotics, thereby lowering the pH and increasing the bacteriostatic effect of organic acids towards pathogens; ii) the production of bacteriocins, which are small microbial peptides with bacteriostatic or bactericidal activity; and iii) the production of reactive oxygen species, such as hydrogen peroxide, that are highly reactive and increase oxidative stress for pathogens in micro-environments(54)(17).

5.2 Nutritional functionality

Specific microbial groups produce vitamins and may thereby contribute to vitamin availability to the human host. Apart from vitamin K, vitamin B12, and pyridoxine,other vitamins, such as biotin, folate, nicotinic acid, and thiamine, can be produced by gut microorganisms. This type of activities may affect host health and may therefore be considered as potential probiotic effects. Lactase deficiency causes lactose intolerance, which results in abdominal cramping, nausea, and bloating. Probiotic strains that are lactase-positive have been successfully applied to relieve discomfort from lactose intolerance(8).

6. CONCLUSIONS:

Probiotic agents are living microorganisms belonging to the normal flora, with low or no pathogenicity and a positive effect on the health and well-being of the host. Probiotic therapy uses bacterial interference and immunomodulation in the control of several infectious, inflammatory, and immunologic conditions. An impressive list of health effects is attributed to probiotic agents, but scientific methods to select and evaluate potential microbial strains with probiotic characteristics are limited. Careful selection of agents and dose standardization of bacterial strains for commercial and scientific use are required. Studies have shown potential medical benefits of use of probiotics for the treatment and prevention of a variety of infections that involve mucosal surfaces, including pediatric gastroenteritis and vaginitis. The future of probiotics will depend in part on further elucidation of basic mechanisms, allowing scientists and clinicians to maximize their health benefits.

However, the risk of transferring antibiotic resistance from probiotics to virulent microorganisms requires more evaluation. High-risk populations, such as immunosuppressed individuals and elderly persons, need special precautions, and critical evaluation of the safety of probiotic strains is required. New challenges in infectious diseases may expand the role of probiotic agents in the prevention of STDs, transmission of HIV-1, and the control of multidrug-resistant organisms. The stimulatory effect of probiotics on mucosal and systemic immunity and their effect as immunomodulators suggest that probiotics may also be useful in the development of vaccines. With further research, this traditional medical therapy may still prove to be one of our most effective tools against new and emerging pathogens that continue to defy modern medicine in the 21st century

The science around the concept of probiotics continues to expand. Current global research efforts have greatly contributed to the understanding of the role of GI commensal organisms in their extraordinary symbiotic relationship with humans. Continued research into the microbiota will no doubt help lead to an improved insight into the impact of probiotics on human health.

Probiotics are designed to provide added functions that can compensate for, substitute for, or add to the gut microbiota, and therefore impact the host directly

or indirectly through "cross-talk" with the gut microbiota and/or the host. In addition, the effects may be local in the GI tract or systemic.

Evidence continues to emerge that probiotics have an influence on the immune system and thereby may enhance resistance to infections, particularly those of the GI or respiratory tract, and help to mitigate allergies, particularly in infants and young children. Evidence is gradually developing for the potential for probiotics to impact other conditions of the GI tract.

One critically important fact to bear in mind is that reported benefits of probiotics should be considered strain-specific. It must be remembered only a limited number of microbes have been documented as probiotic. In all cases, it is clear that probiotics must be consumed regularly in order to confer ahealth benefit.

7. FUTURE DIRECTIONS

The following are some of the future possibilities for these biological products in the field of infectious diseases.

7.1 Mucosal vaccines and immunomodulation.

The use of LAB as live vectors for oral immunization appears to be an exciting approach, on the basis of their safety, ability to persist within the indigenous flora, adjuvant properties, and low intrinsic immunogenicity.Both macrophage activation and IL-12/g-IFN pathway stimulation are promising areas of research with regard to resistance to intracellular pathogens by enhancement of mucosal and systemic immunity. More experimental and clinical studies are needed to clarify the role of probiotics as immunomodulators, not only in infectious diseases of the GI tract, but also for inflammatory and allergic conditions(61).

7.2 Prevention of transmission of AIDS and STDs

The role of the vaginal microflora in determining the efficiency of HIV and other STD transmission is still not well understood. *Lactobacillus* plays a critical role in the regulation of the vaginal microflora. It has been suggested that the production of H2O2, rather than a particular species of *Lactobacillus,* is essential in the regulation of the vaginal flora. This toxic molecule is the most potent local microbicide present in the human vagina. The findings of experiments have suggested that LB_ given at high concentrations is viricidal for HIV-1(62).

7.3 Infection control programs and eradication of multidrug resistant microorganisms

The alarming increase of inappropriate antibiotic use and bacterial resistance, along with renewed interest in ecological methods to prevent infections, makes probiotics a very interesting field for research.

7.4 Antibacterial effects

In vitro studies suggest multiple specific activities of different probiotic agents against several pathogens, including *Listeria monocytogenes*(63), *Salmonella thymurium* (15), *E. coli*(64)(65), and *H. pylori*(66), among others. Therefore, probiotic agents may provide prototypic antimicrobial substances that will be useful for pharmaceutical companies in the development of new antibiotics.

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