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Potential Probiotics and Their Application

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Abstract: Probiotics are health promoting viable microorganisms which have useful effects on the health of digestive system when acquired in a defined dosage. Probiotics can be formulated in to different types of products like food, drugs and dietary supplements. Probiotics improve digestion of food, absorption of nutrients, reducing cholesterol levels, pharmaceuticals development, functional food development and preventing growth of undesirable microorganisms in the digestive tract. The selection of probiotics from different sources involving the evaluation of the basic features of these bacteria, including acid, salt and bile tolerance, ability to adhere to the gut, combat pathogens in the GIT, production of antimicrobial substances, ability to stimulate the response and ability to influence the metabolic activities. Potential probiotics has been isolated from human sources such as feces of healthy adults, breast fed infants and human breast milk and animal sources are poultry, pigs and ruminants. Probiotic bacteria isolated from these sources like *Enterococcus faecalis*, *L. salivarius*, *L. rhamnosus* and *L. casei* helps to improve human health particularly today with the increasing threat of antibiotic over usage and the prevalence of antibiotic resistance organisms. The use of growth promoting antibiotics in animal feeds is banded in different countries due to potential risks such as the spread of resistance genes and the contamination of milk and meat with antibiotic residues. As a result, many livestock producers and researches have explored different alternative strategies to enhance animal performance and health. Probiotics are one of the best alternatives to improve animal production. The use of probiotics in animals improves milk production in lactating cows, milk fat, milk protein and SNF contents. Safety is an overarching concern among all probiotic stakeholders, from government regulatory agencies, to consumer advocacy

organizations, to manufacturers. However, still there are not systematic reviews that analyze adverse effects and safety of probiotic administration evidence to date has demonstrated no serious adverse effects. The market of probiotics in global market is rapidly increased. The market in 2015 was $33.19 billion and expected to reach $46.55 billion by 2020 which indicates a compound annual growth rate of 7%.

INTRODUCTION

Probiotics are health promoting viable microorganisms that inhibit the growth of harmful bacteria, stimulate good digestion, boost immune function, increased resistance to infection and favor the intestinal micro flora balance when acquired in a defined dosage[1, 2]. Probiotics can be formulated into different types of products and the delivery is traditionally associated with fermented dairy foods[3]. Probiotic bacteria can be produced in different fermentation methods by using starter cultures[4].

Probiotics used for the development of functional foods is universally accepted and can be introduce to the food in the specified manner and number with their ability to introduce nutritional and health benefits[5]. The most common probiotics bacteria in use are Lactic Acid Bacteria (LAB) which plays an essential role in shelf life and nutritional value improvement and enhances the healthiness and secondary characteristics of functional foods[6]. Traditionally prepared yogurts are the major sources for the isolation of potential probiotic LAB[7].

Probiotic bacteria selection from different sources started by screening of non-pathogenic bacteria evaluation of basic features including acid and bile tolerance, ability to adhere to gut epithelial cells and ability to combat pathogens in the GIT[8]. Use of the different strains, dosage andduration of treatment and smaller size of the trials makes interpretation of the available data more difficult. Current evidence indicates that probiotic effects are strain-specific; they do not act through the same mechanisms nor are all probiotics indicated for the same health conditions[9].

The development of successful probiotic products depends on the selection of probiotic strains for human consumption, proof of a therapeutic effect, strain survival, viability at the time of consumption and storage requirements[10]. Probiotics have a promising role in the inhibition of pathogenic microorganisms, reduction of antibiotic-associated diarrhea and alleviation of acute diarrheal diseases, improving the nutritional status of the population and as a vaccine adjuvant in developing countries[11]. The main objective of this review is to discuss the potential impacts of health promoting probiotic bacteria.

History of probiotics: The word 'probiotic' comes from Greek word to describe substance secreted by one microorganism that stimulates the growth of another. The history of probiotics began with the history of man by consuming fermented foods[12]. They can be formulated into many different types of products, including foods, drugs anddietary supplements. Yogurt, fermented and unfermented milk, miso, tempeh and some juices and soy beverages have potential probiotics[13]. The strains of *Lactobacillus, Bifidobacterium* and *Streptococcus* have traditionally been used in the manufacture of various kinds of fermented dairy products[14].

MECHANISM OF ACTION AND EFFECTS OF PROBIOTICS

Mechanism of action of probiotics: The mechanisms by which probiotics exert their health promoting effects have been studied *in vitro* in animal models and in human intervention studies. The mechanism action of probiotics on the improvement of health includes; settlement and intestinal proliferation, regulation and control of microbial flora of intestine, prevention and activity against pathogenic agents, prevention of the side effects of antibiotics treatment and enhancing the power of the body's immune system[15]. Generally, probiotics might have the modes of action in production of anti-pathogenic substances, competition of available nutrients, enhancement of the immune system and competition of colonization sites[16].

Probiotics used for humans: Nowadays, consumers are aware of the link between lifestyle, diet and good health. Probiotics are one of the fastest growing categories within food for which scientific researchers have demonstrated therapeutic evidences[17]. The health properties of probiotics are strain specific[18]. Potential probiotics for human have been isolated from human sources such as feces of healthy adults, breast-fed infants and human breast milk[19]. Probiotic *Lactobacillus* genuses are commonly isolated from human breast milk[20] while *Bifidobacterium* is isolated from the feces of healthy human. Few studies reported probiotic *Enterococcus faecalis* found in human feces[21] and *Pediococcus* probiotic, can be isolated from healthy human breast milk[22, 16].

Health professionals and pharmaceutical companies need to objectively help and guide their clients and consumers toward appropriate prophylactic and therapeutic uses of probiotics. Available data from traditional medicine and clinical use clearly state that probiotics have great health potential, particularly today

with the increasing threat of antibiotic over-usage and prevalence of antibiotic resistant microorganisms[13]. Recently, there has been increasing interest in their use to prevent, mitigate or treat specific diseases. A multitude of clinical trials have investigated the use of probiotics for diseases ranging from necrotizing colitis in premature infants to hypertension in adults[23].

Probiotics used for farm animals: In livestock production, probiotics can be employed to control pathogenic bacteria in the intestine tract, reduce methane emissions and enhance growth performance and immune regulation. Probiotics can be presented in the form of capsules, paste, powder and granules and it may consist of single or multiple strains. The appropriate use of probiotics increased milk production of lactating cows[24]. Direct-Fed Microbial (DFM) are dietary supplements that inhibit gastrointestinal infection. Microorganisms that are used in DFM are *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus* and *Propionibacterium*, strains of *Megasphaera elsdenii* and *Prevotella bryantii* and yeast products containing *Saccharomyces* and *Aspergillus*[25].

Currently, the main sources of probiotics for use in various animal species are mainly the GIT of the same animal species and it may also isolate from an animal species and other sources of fermented plants and animal products. *Propionibacterium freudenreichii* isolated from dairy products has been used to reduce enteritis and to improve health in pigs[26]. Milk fat, milk protein and SNF content tended to be higher in cows supplemented with probiotics preparations. Microbial food supplements can also be used as probiotics which benefits the host animals by improving their intestinal microbial balance. The quality animal production in the target species can be achieved through reduced morbidity, stimulating forage digestion and augmenting the quality and quantity of milk, meat and egg.

Thus, future research and applications in field trials are necessary to look for new combinations with the aim to produce standard safe compositions at a high functional level. A continuous inclusion of a good blend of probiotics at 10^8 CFU mL^{-1} dose in drinking water may successively improve the performance and gut health of commercial broiler chicks[23]. It can be recommended that using a freshly produced liquid microbial growth promoter in the non-medicated and de-chlorinated water could prove highly beneficial for the local broiler producers. It could be suggested that further research work should be performed to comparatively evaluate the effectiveness of freshly produced liquid live microbial cultures with other powder forms both as applications in drinking water and rations as-post pellet applications[27].

In livestock production: Health benefits of probiotics have been studied in controlled human trials which document a diversity of benefits[28]. Probiotic supplements are now widely used in quality animal production and accepted as agents that can bring significant health benefits such as enhancement of the immune system, antimicrobial effects inhibiting intestinal and food poisoning pathogens, reducing constipation and improving intestinal mobility, management of diabetes and prevention of osteoporosis, improved nutrition through the enhanced breakdown of vitamins, minerals and amino acids and their absorption through the intestinal walls and Prevention of infection by harmful bacteria [16].

The ability of probiotics to decrease the incidence or duration of certain diarrheal illnesses is perhaps the most substantiated health effect of probiotics[13]. There is preliminary evidence that use of probiotic *lactobacilli* and metabolic byproducts potentially confer benefits to the heart, including prevention and therapy of various ischemic heart syndromes and lowering serum cholesterol. Consumption of certain *lactobacilli* may reduce blood pressure in mildly hypertensive people. Probiotic also stimulate certain cellular and antibody functions of the immune system. Results accumulated so far suggest that probiotics may provide an additional tool to help your body protect itself and manipulation of the gut flora with the right probiotic bacteria may improve gastrointestinal tract oxalate levels and decrease oxalate absorption[28].

Development of functional probiotic food: Probiotics as functional food components are recognized as safe microorganisms of viable single or mixed cultures with claimed health promoting effects on their host by improving the properties of the indigenous intestinal micro-flora[29]. An increase in knowledge of functional foods has led to development of foods with health benefits beyond adequate nutrition. The last 20 years showed an increase interest among consumers in functional food which containing probiotics[10]. The technology aims to combine the nutritional value of milk and the health benefits of the bacteria with resulting in nutritionally healthy and desirable products. For the commercial production of probiotics, enriched fermented milk products with carefully selected probiotic starter cultures are required[30].

Selection criteria of probiotics: Novel probiotics must be tested for beneficial properties in both *in vitro*and *in vivo* models. The novelty of probiotic strains is considered together with safety requirements which comprise a complete genome description and annotation, knowledge regarding the transferability of antibiotic resistance, selection of the proper *in vivo* model, toxicological studies and designation of the target population[31]. Other features such as production of bile salt hydrolase and production

of Exopolysaccharides (EPS) have been considered when selecting probiotics. Selection of LAB for use as potential probiotics begins by screening for EPS producing bacteria in all EPS-producing strains[32, 33].

Probiotics isolated from intestines in both humans and animals have some probiotic property differences from dairy products. Intestinal probiotic isolates are more likely to be resistant to low pH, high concentrations of bile and exhibit higher adhesion activity than isolates from dairy origins[34]. Several factors must be considered when using probiotic bacteria in fermented products and must be viable in high count at time of consumption. Prebiotic sources improve the stability and sensory characterization probiotic products[35]. Some supplements such as whey and whey protein concentrate have been improved probiotic viability, physicochemical and sensorial characteristics of probiotic products[36]. From the safety point of view, the probiotic microorganisms should not be pathogenic, no ability to transfer antibiotic resistance genes and able to maintain genetic stability[37].

Isolation and identification of probiotics: Identification of strains is achieved by biochemical and morphological tests such as Growth test at 4 and 15°C in tubes containing MRS broth and the fermentation of carbohydrates. The isolates obtained from various samples mostly show gram positive, non-spore forming rods, showing test negative results for oxidase and catalase[38]. Morphological, physiological and biochemical characteristics helps to isolate the potential probiotic bacteria[39]. The isolation, identification and probiotic characterization of lactic acid bacteria has been emphasized in recent years, due to their multiple beneficial effects[40]. Molecular characterization by analyzing the sequence of 16S rRNA confirms the probiotic characteristics of the potential bacteria[41].

SAFETY PROPERTIES OF PROBIOTICS

Safety is the main concern among all probiotic stakeholders from government regulatory agencies to consumer advocacy organizations and manufacturers[42]. Genetic stability of the probiotic over time, deleterious metabolic activities andthe potential for pathogenicity must be evaluated depending on the characteristics of the genus and species of the microbe[37]. The popularity of probiotics is increase; therefore, concerns about the safety of probiotics with the associated live microbes are measured. A safety assessment, must consider the nature of the specific microbe being consumed, how it is prepared, how it is administered, what does is delivered, duration of consumption, manner and frequency of administration and the health status of the consumer[43].

Studies of antimicrobial resistance of probiotic microbes have reported depending on isolation sources and antibiotic tested groups. Broad spectrum antibiotics such as tetracycline and chloramphenicol have been detected antibiotic resistant genes as a horizontal gene transfer in LAB probiotics[14]. LAB can be resisted to tetracycline that they may acquire the resistance gene from other bacteria[44]. Probiotics have the theoretical risk of transfer of antibiotic-resistance genes to pathogenic bacteria. Many *Lactobacillus* strains are naturally resistant to vancomycin which raises concerns regarding the possible transfer of such resistance to more pathogenic organisms, particularly enterococci and *Staphylococcus aureus.*

Although current FDA safety standards for foods, food additives and drugs appear largely adequate for probiotics, some aspects of probiotic safety regulation could be improved[42]. No specific regulation exists for probiotics within the EU and the use of probiotics is not recommended[45]. Nevertheless, the QPS approach envisioned by the EU and EFSA is a promising alternative for safety assessment of probiotics and it includes additional criteria to evaluate the safety of probiotics. There are no systematic reviews that analyze safety and adverse effects of probiotic administration but evidence to date has demonstrated no serious adverse effects[46].

Challenges and Risks of probiotics: Several properties that are thought to be important for the probiotic effect as they can modify the survival capacity of the strain *in vivo* clearly differ between strains of similar species. They include tolerance to acid and bile salt, adherence to epithelial cell; enzymatic activity and production of antimicrobial compounds. The lack of detail in the description of administered probiotic organisms in most studies hindered evaluations of safety. There is a lack of assessment and systematic reporting of adverse events in probiotic intervention studies and the interventions are poorly documented[46]. In spite of the problems with dosage and viability of probiotic strains, lack of industry standardization and potential safety issues, there is obvious considerable potential for the benefits of probiotics over a wide range of clinical conditions. Ongoing basic research will continue to identify and characterize existing strains of probiotics, identify strain-specific outcomes, determine optimal doses needed for certain results and assess their stability through processing and digestion[13].

Regulation and marketing of probiotics: There is no common worldwide safety assessment standard with legal status for dietary supplements containing probiotics. Approaches in the United States, the European Union (EU) andthe member states of the EU differ significantly. Food deliberately containing live bacteria is not regulated and is subject only to the general requirement that food must not impose a risk to human health. Safety aspects are regarded with respect to the target animal species, the

final consumer, the environment and the safety of workers in contact with the microorganism during production or application. The current regulatory framework does not address the role of foods in treating, mitigating, or curing disease[42]. FDA regulates products by category (foods, drugs, dietary supplements, medical devices and cosmetics). Products containing this label, therefore, currently are not obligated to meet any standards unique to probiotics. There is however, a growing understanding of this term among consumers and healthcare professionals. It is unfortunate that products currently can be labeled as probiotics but be neither well defined nor substantiated with controlled human studies.

Probiotics market: The global probiotics market was thought to be worth $45.6 billion in 2017 with predictions of $65 billion by 2024[47]. Market analysis forecasted that, the global market for probiotics as ingredients will grow from $1.71-3.56 billion during 2016-2025 and the probiotic dietary supplements sector will grow from $3.3 billion to $7.0 billion[48]. The future probiotics market of Asia-Pacific is projected to be dominated by China, Japan and India[49]. The market and investments in probiotics have been growing rapidly in recent years throughout the world. All probiotic products have not the same function since they depend on quality, safety, tolerability, price and efficacy[50]. But probiotics have been introduced into the market as nutritional supplements with lower standards and lesser guarantees[51].

CONCLUSION

Probiotics are live microorganisms that promote the health of animals and human being when they are administered in appropriate amount. The use of probiotics for human use and animal health is continuously increased. The main source of probiotics for human use are dairy products and they improved the health of human being whereas the animal probiotic sources are the own digestive tracts. Probiotic LAB from fermented dairy product plays essential roles in fermentation, improvement of the shelf life, nutritional value improvement, enhances the healthiness and secondary characteristics of functional foods and also it improves the digestive system, inhibit the pathogenic bacteria, boost the immune system, lowers the cholesterol level, removal of carcinogens and increase the resistance to infections. Resistance to bile salts and high pH, strain survival, non-pathogenicity, ability to adhere the epithelial cells and anti-microbial effects are the main selection criterions of probiotic bacteria. The demand of probiotics in the international market is continuously increased but there is a big concern on the safety of probiotics from the government to the consumers because there are not legal national and international regulations about safety of probiotics. Therefore, there should be national and international regulations about the safety of probiotic products on human consumption and ongoing basic research will be continued to identify and characterize existing strains of probiotics, identify strain-specific outcomes, determine optimal doses needed for certain results and assess their stability through processing and digestion.

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