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Use of Probiotics and Prebiotics in Food Products

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ABSTRACT: The number of available commercial foods with probiotics is on the rise. This mini review aims to identify the importance of probiotics in foods and how they influence the gut health and immunomodulation in human hosts. Emerging approaches like the use of prebiotics and synbiotics are also discussed to enable the reader a lucid understanding of the science behind functional foods.

KEY WORDS: Probiotics, Prebiotics, Synbiotics, Immunomodulation, Functional foods

I. INTRODUCTION

The name "Probiotic" has a Greek origin which means, "for life" and can be considered as possessing the opposite attributes of an antibiotic. A probiotic is defined as, "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" [1]. FAO's description of probiotics as, "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" is currently accepted worldwide. The bacterial genera like Lactobacillus, Bifidobacteria and some species of Bacillus that have the GRAS (Generally Regarded as Safe) status are the commonly employed microbes for the purposes of probiotics. Some of the natural and conventional sources of probiotics are mammalian milk, cow milk, fruit juices, honey, whole fruits, fermented drinks (Kefir) and fermented foods (Pickles). Few unconventional sources like the air in the surroundings of food products and food product preparations; and feces of breast-fed infants have also been reported [2].

Since the times of yore, probiotics have been a part of household diet in many parts of the world (Africa, Asia, China, Sub-Saharan regions, etc.) in the form of yogurt, kefir, koozh, sauerkraut, etc. Broadly, they are vegetable based/ meat based/ milk based/ legume based/ cereal based. In all these products, the natural combinations of the bacterial flora (and sometimes, yeast) which develop in the food after the appropriate duration of fermentation are ingested as such with no further processing. This has been able to maintain a healthy gut for many years in populations.

Since the past two decades, there is an increasing awareness and a call for revival of such traditional foods and beverages. Many research reports elucidate the beneficial effects of probiotics on maintenance and enhancement of human health in ways that include, but are not limited to, immune regulation [3], gut health [4] and cancer mitigation [5]. With the growing changes in human diet and lifestyle, body metabolism is significantly affected resulting in reduced immunity and many lifestyle-associated diseases. Probiotics have been projected as a source of cure for many such health disorders.

II. PROBIOTICS AND THEIR EFFICIENCY

Gut flora (approximately, 10¹⁴ cells in the adult intestine) can be considered as maintaining a commensal relationship with the human hosts. They assist in breaking down the components of foods that are not readily digested by the human digestive system. The authors in [6] have reported that though the infant gut is aerobic at birth, it gradually becomes anaerobic over a few days to accommodate the beneficial flora like Bifidobacteria. The oligosaccharides from human milk (HMO) form some of the initial substrates for the development and proliferation of the colonized beneficial flora. Many of the HMO's are not digestible by the infant digestive enzymes and reach the large intestine as such. Even in



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the adult gut, most of the oligosaccharides except maltotriose are not digestible; and reach the large intestine. They are either excreted or are converted to Short Chain Fatty Acids (SCFA) by the gut flora. Since most of the gut bacteria are saccharolytic, their main end products are SCFA (acetate, propionate and butyrate) and gases (H_2 , CO_2 and CH_4). SCFA are easily metabolized and serve as the energy source to colonocytes to help maintain a healthy gut and therefore, mitigate the risk of colon cancer.

Some of the SCFA's that enter the blood stream have many more functional implications in the human system. Explicitly, acetic acid is absorbed into liver, muscle and other tissues. In the liver, acetic acid is transferred into acetyl CoA which is a precursor for lipogenesis and stimulates gluconeogenesis. Propionic acid increases the ATP production in liver, prevents gluconeogenesis and increases glycolysis. Butyric acid is the major energy substrate for colonocytes and is the source for about 70% of energy of colonocytes. It reduces cell differentiation and stimulates apoptosis in tumour cell lines.

As the bacterial metabolism increases at a steady state, the concentration of short chain fatty acids in the intestine increases, an acidic pH prevails in the intestine. *Bifidobacteria* and *Lactobacillus* are resistant to acidic pH whereas *Clostridium* species are sensitive to this pH and are therefore suppressed. These fatty acids can thus stimulate the growth of *Bifidobacteria* while suppressing growth of unfavorable ones like *E. coli* or *Clostridium* sp. Increased number of Bifidobacterial colonies is accompanied by the production of nitrogen derivatives like ammonia, indole, phenol, skatole and also by the resulting elimination of carcinogens [7]. The probiotic bacteria contain enzymes like β -fructosidase (that catalyzes hydrolysis of FOS) that are lacking in the human digestive system. Therefore, beneficial bacteria like *Bifidobacterium* gain over pathogenic strains in the gut.

Probiotics have also been reported to reduce the risk of infectious bacteria by producing substances like defensis, bacteriocins, nitric oxide and hydrogen peroxide [8]. It has also been reported that infants harboring certain species of Bifidobacteria and Bacteroids have more IgA and IgM cells circulating in the blood. Authors in [9] have reported the effects probiotics have on B-cells, T-cells, NK cells, macrophages, epithelial and endothelial cells. Molecular studies like comparative genomic hybridization revealed that the bacterial genes stimulating interleukin synthesis in the host are involved in encoding N-acetyl glucosamine / galactosamine phosphotransferase system, components of bacteriocin biosynthesis and transport pathways [10]. It follows therefore that, if there can be a genetic modification in the probiotic genome, the responses in host can be changed accordingly. Mechanism of immunomodulation by bacteria in the host is well studied. It is a result of direct methods (activation ofintracellular signaling pathways, toll-like receptors) and indirect methods (development of a favorable environment for the growth and proliferation of regulatory T –cells).

With these increasing reports on the efficacy of probiotics and probiotic-derived factors on the homeostasis in the host, probiotics are loaded in increasing amounts in foods (yogurt, juices, etc.) to ensure their presence in sufficient numbers in the human digestive tract. These foods have been termed, "Functional Foods" as they are being ingested for a specific purpose of action in the host. According to [11], milk remains the most favored medium for developing probiotic-rich foods due to its buffering capacity in the GI tract. Still, fermented food products are preferred as there is traditional wisdom associated with them that catches the attention of the consumers. Furthermore, the bacteria that are part of the fermentation process and its products can also be ingested as probiotics along with the externally added strains. For commercial production of yogurt two batches of milk are mixed; one of which is inoculated with the standard starter culture strains and another with the probiotic strains. Use of probiotic strains alone was reported to negatively affect the flavor of the yogurt as there could be unfavorable changes in the pH. Authors in [12] have reported the successful development of chocolates containing Lactobacilli derived from yogurt. The number of viable bacterial colonies in 50% yogurt-saturated chocolates was 3.37 log cfu/g and did not increase much when the saturation was 100%. This could have been due to the sensitivity of the bacteria to changes in the surrounding medium as a result of processing. Many such considerations have to be taken into account which will depend on the product being produced. For example, probiotic ice cream production should be able to maintain the smoothness of the end product and the temperature at which the ice creams are stored should not affect the survival and metabolism of the probiotic; and therefore the end product. Similarly, probiotic cheese production should take into account the churning process and the amount of oxygen available in the medium. Although the dietary fiber and protein in the juices protect the bacteria from degradation, the pH and secondary metabolites in fresh fruit juices should be taken into account while supplementing with probiotics [13].

The current recommended level of probiotics in foods is 10^6 cfu/g and intake of at least 100 g of probiotic foods is suggested to maintain a healthy gut. Nevertheless, it would be prudent to utilize probiotics on a person-to-person basis



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since the gut population is unique to an individual. The composition of the gut flora depends on various factors like diet, exercise, genotype, stress, hormones, disorders, etc. of the individual. So, a personalized daily dosage of probiotic intake would lead to targeted therapeutics that can be highly beneficial to the host. Such a schematic would require knowledge about the microbiology, genetics and biochemistry of the host.

III. ALTERNATIVE APPROACH OF PREBIOTICS

Prebiotics are gaining ground very recently as they are identified as the specialized components of plants/plant products that allow selective growth of probiotics. Gibson and Roberfroid in [14] gave details of a prebiotic as, "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". They also specified three criteria that can classify them as prebiotics - (a) resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption; (b) fermentation by intestinal microflora; and (c) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and recognized oligosaccharides well-being. Most commonly prebiotics are (fructooligosaccharides, galactooligosaccharides, xylooligosaccharides) and large polysaccharides like inulin, resistant starch, dietary fiber, cellulose, hemicelluloses, etc. A hypothesis has been presented in [15] for categorizing poorly absorbed but highly fermentable oligosaccharides and polyols that reach the small intestine and lumen rapidly as FODMAP (Fermentable Oligo- Di- and Mono- saccharides And Polyols). Prebiotics also enhance vitamin absorption, fecal bulking, etc. by way of selectively promoting the growth of probiotics. In [16], the authors have presented a detailed account on the role of every prebiotic component in maintaining a healthy host gut.

The natural sources for prebiotics are fruits, vegetables, cereals, milk, etc. (for example, chicory and Jeruselem artichoke are good sources of FOS and inulin; milk is a source for GOS). Commercial production of FOS is achieved by transfructosylation of sucrose by β -fructosidase. The resulting mixture contains compounds with the general formula GF_n. Some such sugars are kestose, nystose and fructosylnystose. All these sugars are found in commercial prebiotic products. Commercial GOS are produced by transgalactosylation (by glycoside hydrolases) of lactose and is a major component of infant milk formulae. Recently, XOS is gaining importance as a prebiotic and is produced from lignocellulosic materials by chemical or enzymatic treatments. Many microbial systems are being employed to produce oligosaccharides on a large scale with minimal costs. The research team in [17] has presented a detailed account on sea weeds and microalgae as useful sources of prebiotics. Pallavi et al. in [18] have presented an account on identification, extraction and quantification of prebiotics from a custard apple seeds that are generally thrown away as waste.

The average recommended dietary intake of prebiotics varies from 5 to 15 g/day depending on the type of prebiotic and the gastrointestinal health of the person. There are many foods available in the markets that contain prebiotics isolated from different sources, as supplemental ingredients (biscuits, drinks, yogurts, etc). This would prove to be more beneficial as their effect is selectively induced on the probiotic flora already well established and colonized in the gut of each individual host. Perricone et al. in [13] have reported the beneficial effects of fortification of fruit juices with probiotics. They are reported to enhance flavor and enrich the juices with vitamins, minerals, amino acids and anti-oxidants.

IV. INTEGRATED CONCEPT OF SYNBIOTICS

The most recent development in the area of functional foods is the development of Synbiotics which is a combination of both probiotics and prebiotics. Synbiotics can be powder based, oil based, etc. depending on the best and affordable delivery system for the probiotic. Immobilization and encapsulation of the probiotics in a suitable medium is gaining importance in this regard to ensure continuous delivery of the bacteria into the gut. Safe consumption levels of probiotics and prebiotics have to be well determined and established on a case to case basis before recommending regular dosages.

Mitropoulou et al. in [19] reported the importance of viability of the probiotics while food manufacturing and processing. The processing and storage of probiotic foods also affects the growth of the bacteria in it. The decreased pH levels, dissolved oxygen, hydrogen peroxide, etc., all effect the survival of the bacterial cells. It is also important to ensure the maintenance of texture and taste all through the shelf-life of the product. Microencapsulation of the bacteria in biopolymers and calcium alginate complexes ensures that they are protected from causing drastic physiological



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changes in the product. Whey protein-gel isolate is also a promising carrier in this regard. Most efficient approach would be to use natural sources like inulin, dietary fibers isolated fruits, seeds, cereals and vegetables that are also 'recognized prebiotics'. Besides acting as carriers, the matrices also provide nutrition to the probiotics. Lyophilized powders of such synbiotic combinations are also a suitable alternative as they can have a longer shelf-life.

V. CONCLUSION

There is an increasing demand for the identification of new and ingenious food supplements that would promise a healthy lifestyle. The science of pro-, pre- and synbiotics has a huge potential in this regard that is yet to be completely explored. With a huge regard to our nature's supplies and the knowledge obtained from advances in microbial and preservation technologies, novel methods can be identified to assist the world populace.

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