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A role of probiotic beverages in human health with special references to probiotic milk

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ABSTRACT

The numerous perceived health benefits and the growing awareness about probiotics have caught the attention of the food industry. Food companies are increasingly manufacturing foods with incorporated probiotic bacteria, which fall under the new category of foods called Functional Foods. Probiotic dairy products such as yogurts containing *L. acidophilus* and *Bifidobacterium* spp. constitute a significant amount among the commercially available probiotic foods. Functional foods have been developed in most food categories and even by conservative estimates, the global market size already exceeds that for organic foods. In addition to providing consumers options for improving their health and well-being, functional foods such as probiotics in dairy products are an attractive market sector, providing new economic opportunities.

Keywords: Probiotics, dairy products, *Bifidobacterium*, probiotic food.

INTRODUCTION

Probiotic food products have been consumed by human beings in the form of fermented foods, for many years [1,2,3]. According to the report by FAO/WHO, probiotics are: "Live microorganisms which, when administered in adequate amounts, confer a health benefit to the host". The most common types of probiotics are Lactic acid bacteria and include specie from the *Lactobacillus*, *Pediococcus* and *Bifidobacterium* genera. Various species including *Lactobacillus rhamnosus* and *Bifidobacterium* have mainly been used as probiotics over the years [4,5]. An important characteristic of probiotic bacteria is that they need to survive through the gastro intestinal track of the host. As *S. thermophilus* and *L. bulgaricus* are not expected to survive and grow in the host's intestinal tract, they are not categoried as probiotics by most scientists

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Probiotic bacteria are grow in the host's intestinal tract, they are not categorized as probiotics by most scientists and are therefore considered as yogurt cultures [6]. Hence a probiotic yogurt will typically contain conventional yogurt starters and additional probiotic bacteria so as to provide health benefits in humans by creating an improved balance in the flora of the intestines [7]. Besides of these advantages of probiotics, and probiotic food there is very less information is available on the synergistic effects of prebiotics and hydrolyzed milk on the growth and survival of bifidobacteria spp., which could lead to new products for the dairy market.

Fermented milk and probiotic bacteria

Fermented milk has been used throughout the history of mankind. Nearly every civilization have developed some type of fermented milk product such as buttermilk, labneh, acidophilus milk and yogurt, which were familiar to many people, although the fermentation process was not defined yet. Yogurt, which came from the Turkish term in the eighth century as- yoghurut [8] and the spelling changed over time to the present spelling in the eleventh century. Yogurt is defined as- product obtained by the fermentation of milk with cultures of streptococcus thermophilus and lactobacillus delbrueckii

ssp. bulgaricus, however, ―yogurt-like products are made by substituting L. bulgaricus by other Lactobacillus species for the fermentation of milk or yogurt containing probiotic bacteria [9].

The fermentation process of dairy products and its bacteria have received great attention over the decades after discovering the importance of viable bacteria in food for health benefits. At the beginning of the last century first scientific work had been done by Metchnikoff to investigate the beneficial effects of fermented milk for human health. Numerous scientific studies have been published describing the health benefit associated with the consumption of fermented dairy products [10].The lactic acid bacteria (yogurt bacteria; Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus) do not inhabit the human and animal intestinal tract, nor do survive passing through the digestive system [11]. Therefore, to get the beneficial health effects associated in the fermented dairy products, there has been and increase interest in the incorporation of the intestinal species Lactobacillus acidophilus and Bifidobacterium species in the fermented milk products [12,13,14], these include fermented liquid milks, ice cream, yogurt, and cheese and in soya milk and soya yogurt [15,16,17]. A probiotic is defined classically as a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract. This definition, however, was initially intended for use with animal feed products. For human nutrition, the following definition has been proposed: Probiotics are viable microorganisms that are beneficial to the host when administered in appropriate quantities [18].

In the last decade, the use of probiotics in Fermented dairy product and feed product applications has a noticeable interest and develop.

These organisms play significant role in lowering the pH of the large intestine through the release of lactic and acetic acid [19]

.A large number of probiotic strains Isolates from food, especially dairy products, but also from many applications samples for human, and nutrients for animals. All commercially available isolates of the genera Lactobacillus and Bifidobacterium were identified and compared with reference strains from official culture collections (ATCC, DSMZ, LMG, CCUG, and others).

As bifidobacteria are used as probiotic cultures in commercial dairy products, the questions arose , which species are used? [20,21]. In general, there are 56 species of lactobacillus and 29 species of Bifidobacteria, which used worldwide in dairy products [14].

However, the main species of lactobacillus and Bifidobacteria had been reported in literature such as; L. lactis, L. casei, L. paracasei and more. B. longum, B. infantis, B. breve, B. lactis and B. bifidum [14]. Recent experience revealed that B. animalis had been applied frequently in fermented dairy products due to the fact that this species is somewhat less sensitive against acidification (pH below 4.2) and elevated oxygen tolerance, which is remarkable within the Bifidobacteria [22, 23].

Bifidobacteria are unique in many other respects.

Human related sequence of bifidobacteria differs from both homo and hetero-fermentative bacteria in that they metabolize carbohydrates by aspecial enzyme called Fructose-6-Phosphate Phosphoketolase (F6PPK), which also metabolize Fructooligosaccharides (FOS) which are polymers of fructose and cannot be metabolized by either human digestive enzymes or most undesirable gut microorganisms.

The use of Bifidobacteria in fermented milk shows that it has very slow properties and it requires specific growth factor, known as bifidogenic factors, these are carbohydrates such as N-acetyl-D- glucosamine and lactulose found in human milk [24] and processed milk products, respectively. ― Unlike yogurt bacteria, probiotic bacteria grow very slow. Moreover, it has shown that breast-fed infants have higher numbers of Bifidobacterium than bottle-fed ones [25].

The use of bifidobacteria in fermented milk has many benefits to human and animals by several mechanisms [26, 25,27,28].

Therefore, it has been very important to determine the efficacy of the product containing probiotics, such as the acceptance of the product by the consumers and the survival of probiotic microorganisms during its production. In general, the food industry has applied the recommended level of 106 CFU/g at the time of consumption for Lactobacillus acidophilus, bifidobacteria and other probiotic bacteria [14,29]. Although milk contain the essential nutrients for the growth of bifidobacteria, but the level of amino acids and peptides insufficient to provide the ideal condition for rapid their growth and survival [30,31].

In consequences bifidobacteria have been used only to limited extent in commercial fermented milk [31,1] in spite that fermented milk is the preferred carrier for bifidobacteria.

The growing understanding of the relationship between diet and health increased the demand for food with specific benefit beyond their basic nutrition such as improving the health and well being of human.

This food is called functional food However, Functional food has defined as one, which provides a specific health benefit over and above its normal nutritional status [32]. Moreover, the functional foods must remain as foods (not capsules, etc.) and they must also reveal their effects in amount that can usually be expected to be consumed in the diet.

It has been suggested that food will use as functional when it has shown beneficial effect on one or more target in the body and that beside their nutritional effects such as well-being and health of the host [33].

The old generation of functional foods indicates of using supplements to the food to increase their nutrition and health effects such as vitamins and micronutrient. However, in the new concept of functional foods there was more interest in the gastrointestinal interactions [34], that led for more interest in the dominated organisms in the gastrointestinal tract (indigenous microflora) which found to beneficially effect human health, which known as probiotic bacteria. Therefore, the use of probiotic micro-flora was one of the most promising areas for the development of functional foods in the recent studies [35] because of what probiotics has established a great benefit to human health.

Bifidobacteria were the most dominated organisms in the gastrointestinal tract and their viability and metabolic activity have shown very beneficial effects on the health of the gastrointestinal tract [36] and that always related to the presence of a suitable environment and nutrients, which are very important for the viability and activity, for bifidobacteria to use it in the bowel as carbon and energy source, these compound were referred to as ―bifidogenic factors[37].

At present, probiotics products and especially probiotics dairy foods are marketed successfully all over the world because of their acceptance of consumer and the awareness about their positive aspect for the health benefits.

Health benefits for human

HEALTH BENEFITS

Bacteria have a reputation for causing disease, but a growing body of scientific evidence suggests that you can treat and even prevent some illnesses with foods and supplements containing certain kinds of live bacteria. For example, Metchnikoff related the longevity of Bulgarians to the present of Lactobacillus bulgaricus in the souring milk -yogurt. Moreover, in the Northern Europeans consume a lot of these beneficial microorganisms in the dairy products, because of their tradition of eating foods fermented with bacteria, such as yogurt and cheese, these beneficial bacteria have named later as probiotics.

Probiotic microflora displays numerous health benefits beyond providing basic nutritional value. The health benefit reported of probiotics is the improvement in gut health and the prevention of intestinal infections and stimulating the immune system [38]. Infection prevention is increasingly preferred over using the traditional action by chemotherapy with antibiotics, that rise the concern over development of antibiotic resistance has placed probiotics at the fore.

The principal mechanism for this action is known as competitive colonization or competitive inhibition [39,40,41,42]. This is described as the creation of probiotic bacteria in the human intestine, which acts as a vital barrier to invasion by pathogens in the gastrointestinal tract of the human host. Over 90% of the total cells in the body are present as bacteria in the colon, getting 1012 CFU (Colony Format Unite) for every gram of large intestinal contents [43,44]. Under natural conditions, a protective gut microflora develops and there is no need for a bacterial supplement [45] but the changing food habits and lifestyle force us to take processed food, which affects our access to, and colonization, by probiotics. Moreover, we also consume antibacterial substances ranging from vinegar to antibiotics. To reside in the gut, when ingested, probiotic bacteria are resistant to gastric acidity and bile salts [46] and therefore pass through the upper gastrointestinal tract and have the ability to adhere to the intestinal mucosa. Further, the probiotics‘ secretion of by products such as lactic acid and acetic acid lower the pH in the intestine and producing hydrogen peroxide inhibiting the growth of pathogens and helped to speed pathogens through the intestines [47,48].

The enhancement of the immune system is another reported health benefit of probiotics [49,50], as it appears that this effect by balancing control of pro-inflammatory and anti-inflammatory cytokines [51,14] and therefore

probiotics are considered as immune-stimulatory agent and an important tools to prevent intestinal inflammation, stop gut mucosal dysfunction and decrease hypersensitivity [52].

Recently, there is a relatively large volume of scientific literature basis supporting the use of probiotics for diarrhoea has started to become established. Probiotics have been shown effectiveness in the prevention of several types of diarrhea, including antibiotic-associated diarrhea, bacterial and viral diarrhea (including travelers' diarrhea), as well as that caused by lactose intolerance [53]. It is thought that this action is due to the secretion of antimicrobial polypeptides known as bacteriocins and that by; reduction in gastrointestinal pH through stimulation of lactic acid-producing bacteria; a direct antagonistic action on gastrointestinal pathogens; competition with pathogens for binding and receptor sites; improved immune function; and competition for limited nutrients [54). It seems that the effect of probiotics on travellers diarrhea depends on the bacterial strain used and the destination of the travelers [55].

Observational data suggest that consumption of fermented dairy products is associated with a lower prevalence of colon cancer, which is suggested that probiotics are capable of decreasing the risk of cancer [56].

The Mechanisms described by Inhibition of carcinogens and pro-carcinogens, Inhibition of bacteria capable of converting pro-carcinogens to carcinogens [57]. Moreover, probiotic and especially bifidobacteria has shown to increase the α- and β-galactosidase activities in the faecal samples after feeding with the fermented milk containing probiotics, which is considered to be an important probiotic quality, as it supports lactose digestion in the intestine and compensates for lactase-deficiency [14,58,59].

Playne[60] suggested that Health benefits imparted by probiotic bacteria are strain-specific and not species- or genus- specific. For example the strains *Lactobacillus rhamnosus* GG (Valio), *Lactobacillus paracasei* Shirota (Yakult), and *Bifidobacterium Lactis* Bb12 (Chr. Hansen), *L. acidophilus* La5 (Chr. Hansen) have the strongest human health efficacy data, against some or all of: lactose intolerance; rotaviral diarrhea; antibiotic-associated diarrhea and some other bacterial diarrheas and infections. *B. animalis* found to stimulate the immune response *in vivo* [61,62]. Moreover, it has improved in control study the useful of *Bifidobacterium lactis* strain Bb12 in prevention of acute diarrhea in infants [63,64,65] and same by *Lactobacillus* GG [66].

Bifidobacterium lactis Bb12 well known in their ability to improve the growth of children when supplement in their formula. Candida can also be suppressed or controlled by viable *L. acidophilus*. It is also thought thatnon-viable forms exert such control but to a lesser extent [67].

The only prebiotics for which sufficient data have been generated to allow an evaluation of their possible classification as functional food ingredients are the inulin-type fructans, which include native inulin, enzymatically hydrolyzed inulin or oligofructose, and synthetic fructooligosaccharides [68,69,72].

Inulin

Inulin is natural food ingredient, which found in many vegetables and obtained industrially from chicory roots [70]. Usually GFn is the symbolized use for inulin as polysaccharides mixture, where G is the glucose moiety, F is the fructose moiety and (*n*) equals the number of fructose moieties linked by β (2→1) linkages (Figure 2.1). This bonding renders it resistant to hydrolysis, both in the stomach and the intestine [71]. The degree of polymerization (DP) of inulin typically ranges from 2 to 60. Usually 10% of the inulin extracted from chicory roots is sucrose and fructose, and 30% is oligosaccharide (DP=10). The production process of inulin naturally from chicory roots is by diffusion in hot water, followed by refining and spry drying.

The nutritional properties of inulin as a supplement to the healthy foods is obtained when inulin is not broken down in the upper digestive systems of human and should be given energy value of 6.28 kj/g [71], fermented by the intestinal flora causing increase in the biomass, producing of short chain fatty acids and decrease in the pH, and significant increase of bifidobacteria in the colon and inhibits the growth of less beneficial bacteria, [72]. So using these ingredients in food allows improving the nutrition value of the products, by reducing the calorie content and increasing the bifidus-promoting capacities.

Inulin has natural taste, colourless and minimal influence on the natural characteristics of the products. Inulin is highly soluble fiber ingredient, which gives its more importance in the dairy industries such as milk drink, yogurt, and cheeses. Combining inulin with sweetness improves the taste of products giving more sugar-like sweetness. Atconcentration of 40-45% inulin form a fatty tasting solution, which make it highly used in the free-fat products to give the smooth creamy texture and taste; Inulin has successfully applied in fat-reduced table

spread, cheese products, meat products, frozen desserts, fillings, sauces and meat replacers. [73,74].

Using the food as vehicles for inulin considering in its ability to keep the inulin save to the intestine where the bifidobacteria inhabit, but yogurt has shown to be the best choice for that purpose. However, bio-yogurt - with inulin showed problem in transferring the short chain length of the inulin because of the possibility to interfere too much with the inter-micellar bonding of the casein, that why it is important to watch the optimum level of casein to give maximum gel to natural yogurt with inulin. Moreover, the different effect of the yogurt culture or *Bifidobacterium spp*. on the different levels of oligosaccharides which generate undesirable acids when utilize some of the inulin and the effect of the inulin on the gel strength, viscosity and flavour of the natural yogurt.

Oligosaccharides

Oligosaccharides of various types found in common foods such as fruit, vegetables, milk and honey [75]. Oligosaccharides often have between 2-10 polymers of sugar. It is often contain low levels of monosaccharide and di- or polysaccharides. They have various properties depended on their molecular- weight; low molecular-weight can used in food freezing temperature and the high once use for thickness and increase body and mouth feel.

Grittenden and Playne [76] have selected food grade oligosaccharides and listed them: Fructo-oligosaccharides (FOS), Galacto-oligosaccharides (GOS), Lactulose, Lactosucrose, Malto-oligosaccharides, Isomalto-oligosaccharides, soybean- oligosaccharides and xylo-oligosaccharides in the commercial productions. Of these food grade oligosaccharides there are some of them, which have the bifidogenic effect and health promoting properties; such as Fructo-oligosaccharides and Galacto-oligosaccharides.

Galactooligosaccharides

Galacto-oligosaccharides (GOS) are found in the human milk and proved to have the bifidogenic promoting effect for the breast-fed infants [77,78,79]. GOS is primary production from Cow‘s Whey-derived lactose [80]. The lactose solution is converted to GOS by the action of β-galctosidase, which has transgalactosylation activity (Figure 1). GOS have a DP of 2 to 10 units, which are able to pass through the upper gut without hydrolysed by digestive enzymes, to reach the large intestine and beneficially affects the host by stimulating the growth and/or activity of target flora only.

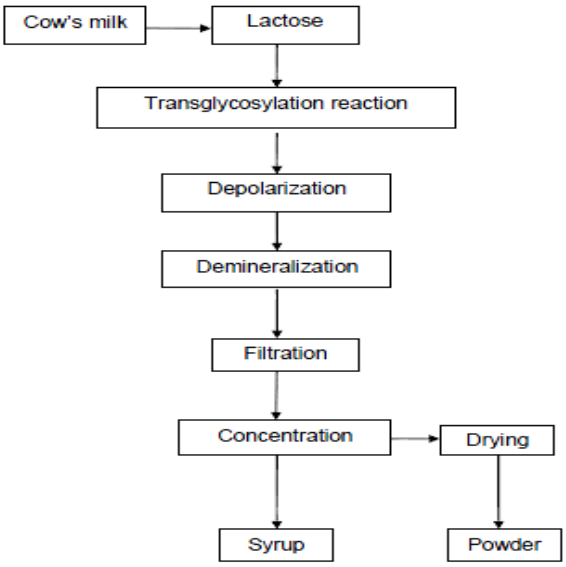

Figure 1: Production of galactooligosaccharide from cows‘ milk

GOS found in the breast milk at level of 1g/L [81]. Therefore, it is considered as bifidogenic factors in fermented milk products and baby foods. GOS consist of number of β1-6 linked galactosyl residues bound to a

terminal glucose unit via α1-4 linkage [82], that make GOS utilized by some probiotics (stimulating their growth) depend on their enzymes and especially Lactobacillus group [83]. GOS can be used in food contributing to both thickness and mouth-feel, sweeteners as it sweetness is 35% of sucrose and source for fiber. They have calorie value of 1.7Kcal/g [84]. ***Fructooligosaccharides***Fructo-oligosaccharides (FOS) and Inulin are the most classified as bifidogenic oligosaccharides in productions. Fructooligosacchrides (FOS) contains between 2- 4 of β (2→1) linked fructose units linked to the terminal α-D-glucose residue. The natural way of FOS productions is by enzyme hydrolysis of Inulin (Figure 2). Therefore, there is no difference from the chemical view between inulin and FOS, but the DP for FOS is between 2-10 units with an average of 4 [85].

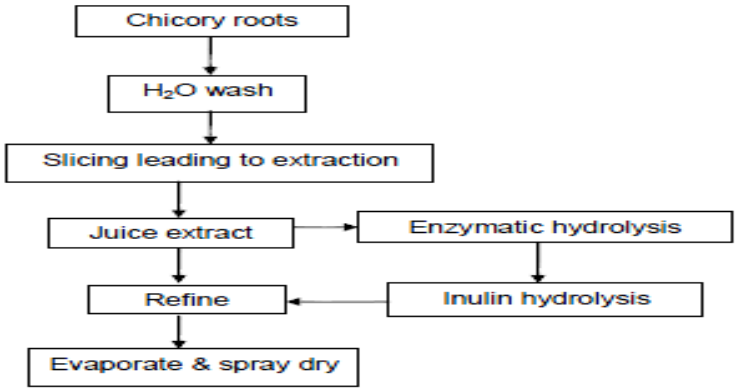

Figure 2: Inulin and oligofructose manufacturing process

Oligofructose is more soluble than sucrose and Inulin, provides 30-50% of the sweetness of table sugar. It is considered to be low-calorie ingredients which around 1.5 Kcal/g. Inulin and FOS are widely found in nature and manufactured by companies such as Orafti, Sensus and Cosucra. A lot of scientific studies *in-vivo* have shown that FOS (RAFTILOSE® P95) and Inulin (RAFTILINE® ST) from ORAFTI have bifidogenic effect on host; When consumed at a dose of 5g/day for oligofructose and ≤ 8 g/day for inulin, they significantly modify the composition of the intestinal (faecal) flora, selectively increasing the numbers of Bifidobacteria and reducing the harmful bacteria [86,87,88,89,90, 91](Figure 3).

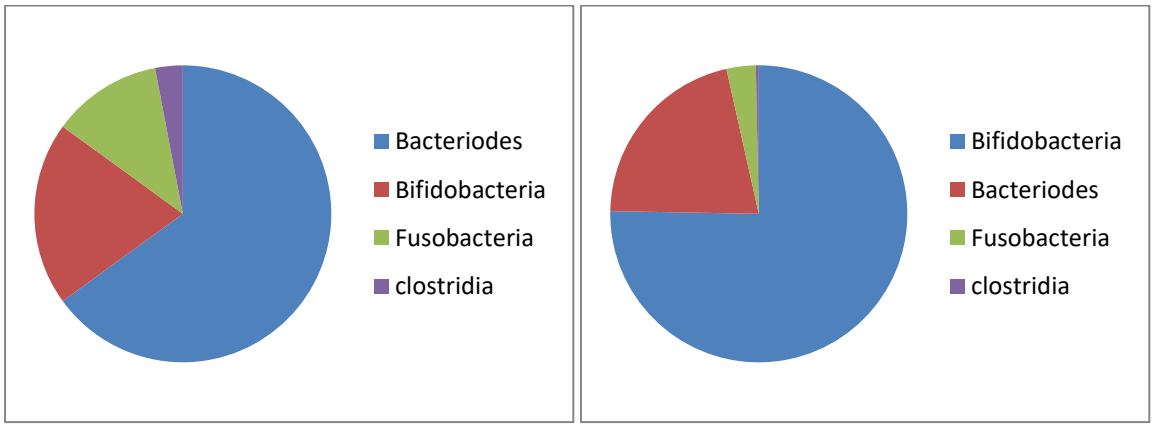

Figure 3: Increase in *Bifidobacteria* after intake of 15g/day of inulin (92. Adapted from Gibson *et al*., 1995)

Food for Specified Health Use (FOSHU) is unique system in Japan for approving ingredients/foods with a functional claim [93,94]. Prebiotic containing foods are most common in this category because the most FOSHU food designed for the gastrointestinal health benefits. The survival of probiotics including *L. acidophilus, L. Casei* and *Bifidobacterium spp*. was improved by adding of FOS (P95, from ORAFTI) at 1.5% w/v to yogurt containing these microorganisms [95].

Inulin and oligofructose have found to improve the bioavailability of minerals such as calcium, magnesium and iron and they increase the absorption of calcium and magnesium in the large intestine [96,97]. Use oligofructose in the diet by 15g/day produce substantial changes in the intestinal microflora and increase the faecal bifidobacteria count and reduce the potential pathogens such as *Clostridium, E. coli*. It has found that symbiotic supplements of Oligofructose and *L.rhamnosus* and *B. animalis* subsp. *Lactis* improved the immune responses and stopped colon tumors. Prebiotics have shown resistance to gastrointestinal infection because of their stimulatory effect on *Bifidobacterium* spp., which produces several anti- pathogenic mechanisms [98].

Although prebiotics can help to increase the beneficial bacteria in the gastrointestinal tract, a general increase in the beneficial bacterial population may however not necessarily contribute to increased health effects, as it is strain related.

Biochemistry study of the fermentation process of bifidobacteria in milk

The common result of the bacterial fermentation process is lactate. It is referred to the bacteria, which produce lactate in their fermentation as Lactic Acid Bacteria (LAB). Some of these organisms require very complex nutrition, which is related to their environments such as plant, milk and intestinal of animals or humans. Lactic Acid Bacteria are strictly fermentative and some are oxygen tolerant, such as *Streptococcus*. Others are obligate anaerobes such as *Bifidobacteria* spp. but some can tolerant oxygen in the presence of carbon dioxide (CO_2) [99]. Bifidobacteria naturally inhabitants the gut of animals and humans and their sensitive to oxygen is strain related and the less sensitive strain appears to possess weak catalase activity that removes H_2O_2. Hydrogen peroxide inactivates Fructos-6-phosphate phosphoketolase F6PPK [99], a key enzyme of bifidobacteria in carbohydrate metabolism [100,101]. Bifidobacteria do not produce CO_2, Butyric or propionic acid [102]. Their optimum growth temperature is 37 ^{O}C to 43 ^{O}C, and the optimum pH is 6.5 to 7.0 [103]. Bifidobacteria also produce thiamine, riboflavin, and vitamin B and K [104,105,106,107**]**. These genera are also unique by producing the lactic acid in the form L (+)- lactic acid that easier to metabolize by infants in compare with the D (-)- lactic acid form which produced by *L. acidophilus* and *L. bulgaricus* [108]. There are three pathways has been suggested for the fermentation process of LAB of the carbohydrates to lactate.

The homofermentative pathway, which yields 2 mol of lactate per mol of glucose:

$$\text{Glucose} \xrightarrow{\text{Homofermentative pathway}} 2 \text{ lactate}$$

The heterofermentative, which yields 1 mol of each lactate, ethanol, and CO_2 per mol of glucose:

$$\text{Glucose} \xrightarrow{\text{Heterofermentative pathway}} \text{lactate} + \text{ethanol} + CO_2$$

The bifidum pathway, which yields acetate and lactate in ratio of 3 to 2 respectively:

$$\text{Glucose} \xrightarrow{\text{Bifidum pathway}} 2 \text{ lactate} + 3 \text{ acetate}$$

Bifidobacteria metabolized carbohydrate as main carbon source is through fructose-6-phosphate shunt by using Fructos-6-phosphate phosphoketolase (F6PPK), which is distinguishing bifidobacteria from *lactobacilli*. This pathway produces L (+) lactic acid and acetic acid in ratio 3:2, some species produce formic acid and ethanol as well.

Bifidobacteria utilize lactose, galactose and fructose beside glucose, and has the ability to metabolize oligosaccharides beside the simple sugars such as Inulin, FOS etc. This ability is strain related, for example it has found that *Bifidobacterium Lactis* Bb12 possessed the enzymes required to utilize somekind of sugar such as raffinose family and lactose which are unable to metabolism by other strain of bifidobacteria [109]. These enzymes which were found in most strains of bifidobacteria and not found in the lactic acid bacteria [110], *Bifidobacterium Lactis* Bb12 were found to possess the highest activity of such like enzymes which found with low activity in other strains, these enzymes such as *β*–glucosidase, *α*–glucosidase, D-glucosaminidase and *β*–galactosidase, which are very important in the fermentation process with bifidobacteria [111,109]. *Bifidobacterium Lactis* Bb12 has the ability to grow faster in milk because of possessing the highest activity of leucine aminopeptidase to help the hydrolysis of milk proteins, which stimulate their growth [110,109].

CONCLUSION

Probiotics are bacteria, often called "good bacteria," found mainly in milk-based products. These products include yogurt, fermented, unfermented milk and other cultured milk products. However, in the case with fermented milk products, many times the probiotics are added after the fermentation process. While the full benefits of probiotics have not been fully explored, there are still many professionals who advocate for their use.

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