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Fermentation of Bovine, Non-Bovine and Vegetable Milk

Tridjoko Wisnu Murti

Abstract

Fermented milk has been developing alongside the history of human civilization. It is observed having positive effect on gastrointestinal health. It has reaches at the steps of explaining what happens in the process, despite some information is still unclear. Fermentation involves many organisms, technique, biochemical reactions, tool and apparatus as well as cultural diversity among people and regions due to differences and changes in climate. Fermented milks, using milk as the raw material from bovine and non-bovine dairy species, and in some regions, especially in Asia and Africa, they also use materials from vegetable extracts. Some progress in Fermented Milk Science, has explained the role of such fermented foods for human health. These benefits have been more and more progressing to select specific microbes, known as probiotic cultures, which combined with specific substances from vegetable extract (prebiotic) could improve lactose digestion, role anti-cancer, anti-hypercholesteremic and anti-pathogenic bacteria as well as anti-virus were discussed in this article.

Keywords: fermented milks, probiotic and prebiotics, bovine and non-bovine milk, health benefits

1. Introduction

Fermented milk having studying since long time and having the positive effect of selected microbiota on gastrointestinal health on human from different region in the world. Fermented milk acomposed largely lactic acid bacteria with history of save use. Lactic acid bacteria are considered as the best for prebiotic-containing fermented milk while preserving the taste and nutritional properties.

This review is presented since the definition of fermented milk manufacturing source of raw milk material (Bovine, small ruminans milk as well as vegetable milk) and it's benefit role for human being.

2. Definition

Fermentation is a process involving the (re) generation of ATP by microorganisms *via* the transformation of organic substances (in this case, lactose as a sugar in the milk); specific enzymes, produced by bacteria (thermophilic, mesophilic, or intestinal bacteria) or fungi as well as yeasts, acting as catalysts in this reaction. Several categories of fermented milk, which depend on the dominant final products are: alcoholic, lactic, acetic, butyric, propionic. Advances in fermented milk production technology has led to variety of products, naming more than 400

of local fermented milk, i.e.: Kefir, yakult, yoghurt, Ymer, Lang-fii, bifidus milk, acidophilus milk, koumiss, lassi, leben, shrikhand as well as cheese, that suit diverse cultural taste. According to the *Codex alimentarius*, fermented milk is a dairy product obtained by the fermentation of milk, which may have been made from products obtained from milk with or without any modification of their composition (within certain specified limits), *via* the action of corresponding microorganisms and which result in a lowered pH with or without coagulation (isoelectric precipitation).

3. Raw milk materials

Raw materials for fermented milk are milked from different ruminants or other not only limited from cows, buffalo, sheep and goats, but also from mares as well as camel milk [1–3]. The composition of raw milk materials is in **Table 1**. Milk from Buffalo, Sheep and Yak rich in Fat contents, leads to high total solids while, Mare’s milk rich in lactose as Breast milk, considered as the 1st choice to replace or to complete breastfeeding. Soymilk composition contains CP 4.5, Carbohydrate 10.0, Fat 4.3, ash 0.66 and Moisture 80.34%, respectively [6].

Type of fermentation in lactic acid bacteria used are homo- or hetero-fermentation using the main products of lactic acid for homo-fermentation bacteria or lactic acid, acetic acid, and CO₂ for hetero fermentation. The isomers of lactate are L-lactate, D-Lactate or DL-Lactate as in **Table 2** [2]. Isomer L-lactate

Source	Total solids	Fat	Total Protein	Casein	Whey Protein	Lactose	Ash
Cow	12.3–14.5	3.4–5.5	3.0–4.0	2.8	0.6	4.6–7.0	0.7
Buffalo	16.0–17.0	6.0–7.5	4.3–4.7	3.6	0.9	4.3–4.7	80.9
Goat	11.5–13.5	3.4–4.5	2.8–3.7	2.5	0.4	3.9–4.8	0.8
Sheep	16.0–20.0	6.0–8.5	5.5–6.5	4.6	0.9	4.0–4.7	1.0
Camel	13.5–16.0	5.0–5.5	3.5–4.5	2.7	0.9	5.0–6.0	0.7
Mare	10.0–12.0	1.0–2.0	1.6–1.8	1.3	1.2	6.0–7.0	0.5
Yak	17.8–18.0	6.5–9.0	5.5	NA	NA	5.0–6.0	0.9

[4, 5].

Table 1.
Milk composition of different dairy animals.

Genus	Fermentation type	Main products	Lactate isomers
<i>Streptococcus</i>	Homo	Lactate	L
<i>Pediococcus</i>	Homo	Lactate	L, DL
<i>Lactobacillus</i> :	Homo	Lactate	L, D or DL
• obligate homo	Homo	Lactate	L, D or DL
• Facultative hetero	Hetero	Lactate, Acetate	L, D or DL
Obligate hetero	Hetero	Lactate, Acetate, CO ₂	L, D or DL
<i>Leuconostoc</i>	Hetero	Lactate, Acetate, CO ₂	D
<i>Bifidobacterium</i>	Hetero	Lactate, acetate	L

[2].

Table 2.
Types of fermentation in lactic acid bacteria.

is more acceptable for infants. Genus bacteria, which are in fermented milks, i.e.: Lactobacillus (*L. delbrueckii* subs. *Bulgaricus*, *L. acidophilus*, *L. helveticus*, *L. brevis*, *L. fermentum*, and *L. kefir*), Streptococcus (*S. thermophilus*), Leuconostoc (*Ln. mesenteroides*, and *Ln lactis*), *Pediococcus* (*P. acidilactici*, and *P. pentosaceus*), Acetobacter (*A.aceti*), and Bifidobacterium (*B. breve*, *B. adult*, *B. infantis*, *B. longum*, *B. bifidum*, and *B. pseudolongum*).

While for yeast included *Torulaspora delbrueckii*, *Kluyveromyces marxianus* subsp. *marxianus*, *Candida kefir*, and *Saccharomyces cerevisiae*. Some bacteria are considered as thermophilic or mesophilic starters, and other as intestinal bacteria, depended on optimal temperature of growth or source location [7].

4. Manufacturing of fermented milks

Characteristics of naturally fermented milk depend upon the availability of the milk in respective regions. However, fermented milk like Zabady, Laban, Rayeb, and Shubat from Northern Africa (Egypt, Morocco, and mid-west Asian countries) and Shoyu from Himalayan region as well as flmjölk and långfl from Sweden have same characteristics of fermentation [5, 7].

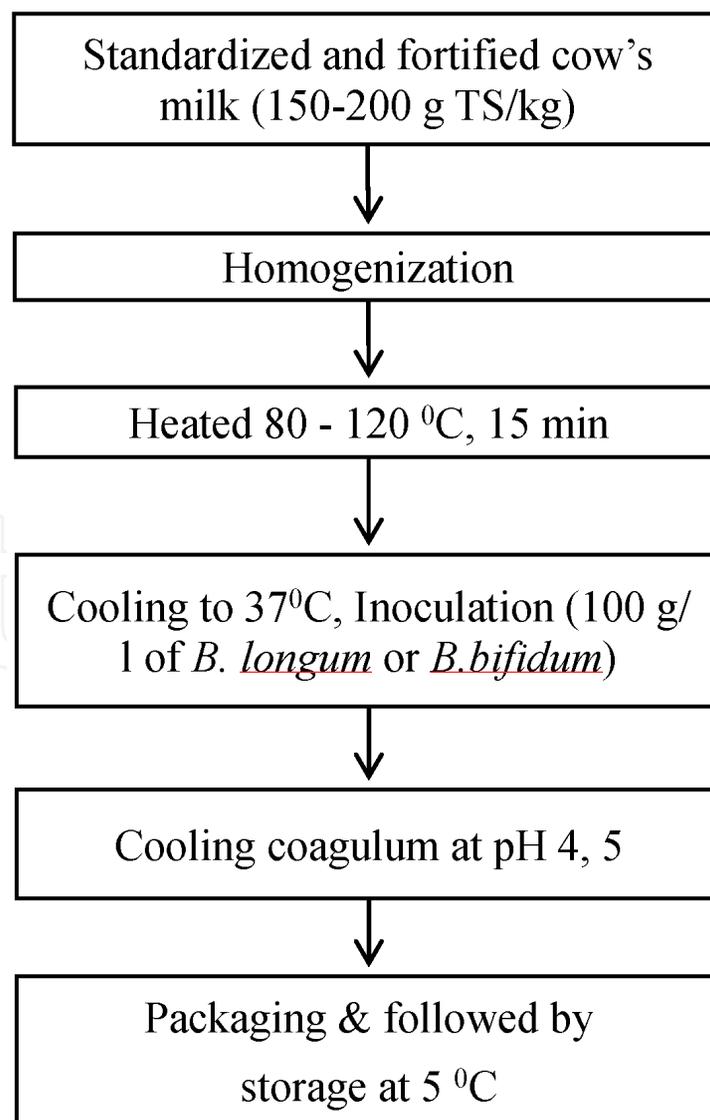


Figure 1.
General steps for manufacturing of bifidus milks.

In general, manufacturing process of fermented milk is in **Figure 1**. The technology and biochemistry of fermentation have been extensively investigated and varied among countries, regions or for special purposes. However, the principal stages of production of any type of fermented milk are included: standardization of fat content, fortification of the milk solids, homogenization, deaerization (optional), and high heat treatment have much in common reviewed. Fermented dairy products, included probiotics cultures, are generally not commercialized as in single cultures, except Yakult and Bifidus milk or acidophilus milk. However, Fermented milks contain mixed cultures.

5. Fermented non-bovine milk and vegetable extract

Fermented non-bovine milks grew along with fermented cow milk, despite its limited numbers.

Four major ruminant species (cow, goat, sheep, and buffalo) contribute raw milk for their manufacturing, on which the research has been focusing since a long time. Currently, the worldwide production of non-bovine milk reaches 133 million tons per year, accounting for more than 17% of all milk production. Of this amount, 13.5% accounts from goat milk production. Goat milk is one of the major contributors to non-bovine milk production [8]. In recent years, the social transformation underway in poor and developing countries, climatic changes, and increased attention to animal welfare are highlighting on some minor animal species that have a “local dairy concern” such as equines, camels, and yaks [9]. It has studied fermented bovine and non-bovine milk (goat and mare’s milk) using raw milk or whey liquid added by probiotic bacteria, either single or multiple probiotic culture [3]. Goat milk possesses beneficial characteristics that make it potentially useful in human medicine or nutrition. Fermented Goat Milk developed in the presence of *L. casei* [10]. As well as present of *Streptococcus thermophilus* or mix probiotic cultures could well accepted by consumers [11].

The so-called vegetable milks are in the spotlight as the beverage that is lactose-free, animal protein-free and cholesterol-free features which fit well with the current demand for healthy food products [12–14]. There is an increasing demand for non-dairy probiotic foods (both fermented and non-fermented) including fruit and vegetable juices, soy and certain cereal products due to vegetarianism [15]. Soy Milk is rich in essential and branched amino acids except Met, and good source of B- vitamins especially, niacin, pyridoxine and folic acid/folacin [13]. Asia represents high demand of vegetable beverages, included fermented vegetable milk as Soymilk [12]. The food market reflects to an ever-greater degree the consumer demand for healthy food products, especially fermented beverage using cereals, nuts as almonds, peanuts, as well as fermented soymilk. France may be a country that considers, it is not milk, but extract [16]. There are two large different groups of vegetable milks or plant milks: nut and cereal milk that can be broadly classified by [12].

6. Probiotics and health benefits for human being

Fermented milks or vegetable extracts offers tremendous potential for promoting health, improving nutrition, and reducing the risks of various diseases. Lactic acid bacteria, both mesophilic and thermophilic or intestinal bacteria may be considered as probiotic cultures which improve health status of the host. Infants, children, adults, and elderly can consume fermented milks for their good taste, health

benefit and their general nutritional value, reducing the incidence of malnutrition, lactose intolerance and diarrhea. Those with special medical needs can switch to fermented milks to provide added nutrition, solve intestinal disorders, improve immune function, and optimize gut ecology. Offering general health benefit effects for human indicated the role of probiotic bacteria. Modern production makes these products available to world population, resulting in the consumption of fermented milk products will reach new milestone.

Probiotics beverages belong to fermented milks are known as health promoting foods which have market estimated at USD 49.4 billion in 2018 and projected to grow reaching 69.3 billion in 2023. The increasing use of yeast *Saccharomyces boulardii*, follows a success story of probiotic containing yoghurt in China, Brazil, and India. Japan, which has increasingly aging population, consider health benefits associated with probiotic-fortified foods and beverages. Therefore, Japan may have new paradigm Smart community 5.0 to replace Industrial Revolution 4.0, which dehumanized people and communities. Scientists have considered that the minimum effective dose of a probiotic is approximately 10^8 – 10^9 cells per mL despite there is no evidence-based consensus on the optimal concentration of bacteria pro-dose [17]. The fresh products, including probiotics, contain live starter culture bacteria, while the extended shelf-life products are in doubts of their content of live microorganisms. The most commonly used bacteria, which are found in various probiotic-dairy products, are Bifidobacteria and Lactobacilli. In addition to their desired health and clinical properties, probiotics must meet several basic requirements for the development of marketable probiotic products. The most important requirements are that probiotic bacteria must survive in sufficient numbers in the product, which their physical and genetic stability must be guaranteed during the storage of the product [18].

In recent years, the proposal of new probiotic-related concepts has gained attention. This is because it has been recognized that some mechanisms and clinical benefits cannot be directly related to the live microorganisms [19]. To survive in milk, they need some supplements that act as a growth promoter. Fermented soy milks can be developed using some probiotic cultures as *L. acidophilus*, *L. casei* as well as *Bifidobacterium pseudolongum* [14, 16, 20], due to the presence of oligosaccharides, belong to non-digestible starch, as Raffinose and Stachyose [21, 22]. These are prebiotic substances (**Figure 2**), which support the growth of probiotic microorganisms (growth promoter). Prebiotics include fructans, oligosaccharides, arabinooligosaccharides, isomaltooligosaccharides, xylooligosaccharides, resistant starch, lactosucrose, lactobionic acid, galactomannan, psyllium, polyphenols, and

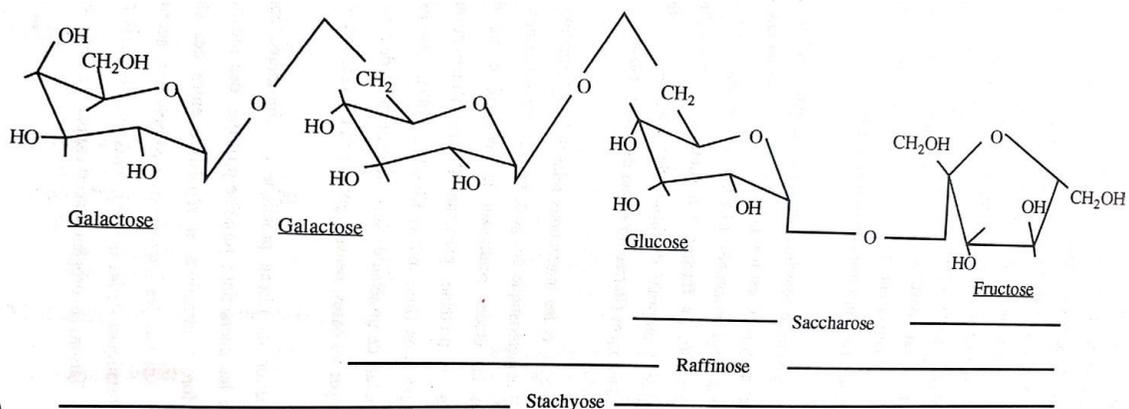


Figure 2.
Structure of Raffinose and Stachyose of soybean.

polyunsaturated fatty acids [23]. Soy flour contains fiber as much as 12 g/ 100 g products, equivalent to 1/5 of 60–80 g/ day recommended to maintain a large bowel flora of 10^{14} microorganisms, typically for a healthy human colon.

Lactobacillus casei and especially Bifidobacterium are among the probiotic cultures that defined by Fuller as a live microbial feed supplement which beneficially affect the host (animal) by improving its intestinal microbial balance. However, this definition of Fuller was improved by [24] as “One product contains specific live microbes at the sufficient numbers and change the microbial balance leading to health effect for the host.” Probiotic’s definition is living microorganisms, which when ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora [25]. At present, [2, 26] explained that the most frequently utilized species for promoting the health were:

1. Bifidobacterium: *B. bifidum*, *B. longum*, *B. infantis*, *B. breve*, *B. adolescentis*
2. Lactobacillus: *L. acidophilus*, *L. casei*, *L. rhamnosus*, *L. fermentum*, *L. reuteri*
3. Streptococcus: *S. faecium*
4. Propionibacterium: *P. freudenreichii* subsp. *Shermanii*
5. Saccharomyces: *Saccharomyces boulardii*

Many probiotic products on the market contain Lactic Acid Bacteria, the population of which tend to be a relatively small proportion of the total gut microflora. There are more than 400 bacterial species in human flora, but only ten species cohabit at the highest population levels. *Bifidobacterium* sp., is the most popular probiotic [14]. These are strict anaerobic and most of them are very sensitive to contact with atmospheric oxygen. The most abundant population belong to the gram-negative Bacteriodes genus (30% of the dominant human flora) and to the Gram-positive Eubacteria, Bifidobacterium, Ruminococcus and the different Clostridia species. Bifidobacteria (**Figure 3**), are accounting for approximately 1% of total microflora.

Bifidobacterias as a single culture cannot grow easily in dairy milk without supplementations despite it grows well in soymilk [14]. The yoghurt bacteria *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are not mentioned as probiotic culture (false claimed) because they are not typical forming the intestinal flora of man. Despite it, yoghurt that made from dairy milk or yoghurt-like product from vegetable extract are considered as the best-known food vehicle for probiotics because, beyond its own physicochemical and functional characteristics [2, 14].

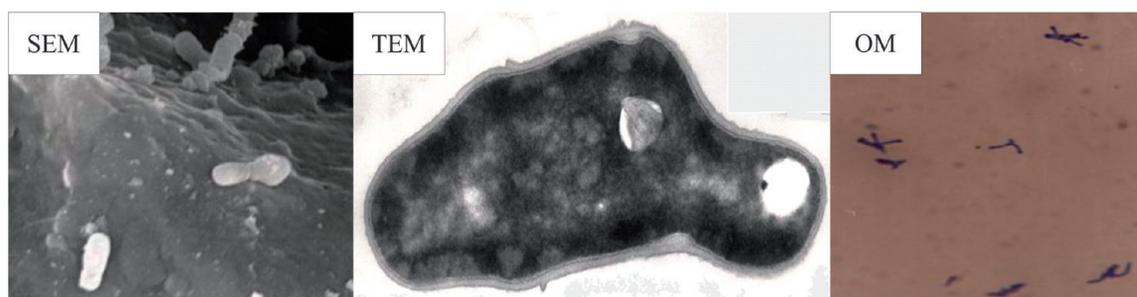


Figure 3. *Bifidobacterium pseudolongum* viewed at scanning electron microscopy (SEM), transmission electron microscopy (TEM) and ordinary microscope (OM).

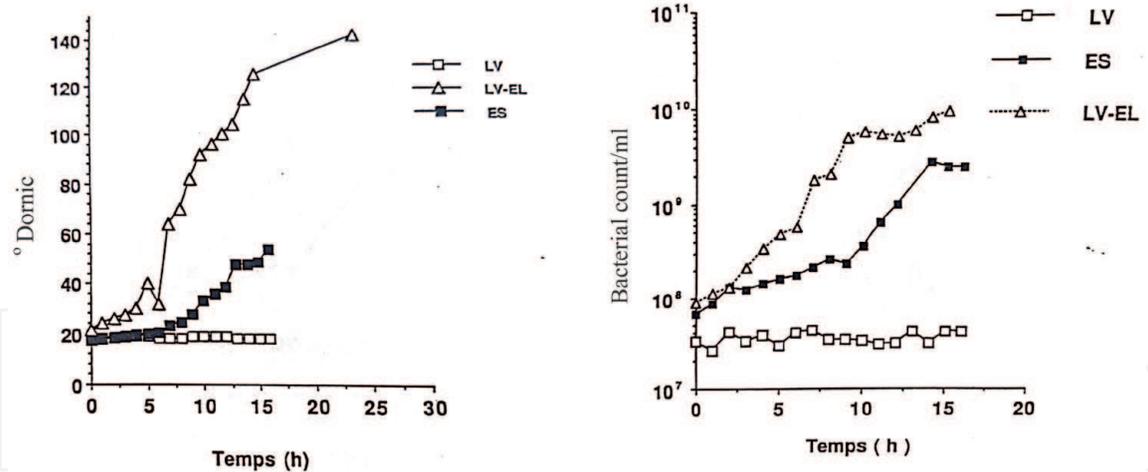


Figure 4. Acidity (left) and bacterial counts (right) of *Bifidobacterium sp.* in yoghurt (CM), CM+ yeast extract and Soyoghurt (SE).

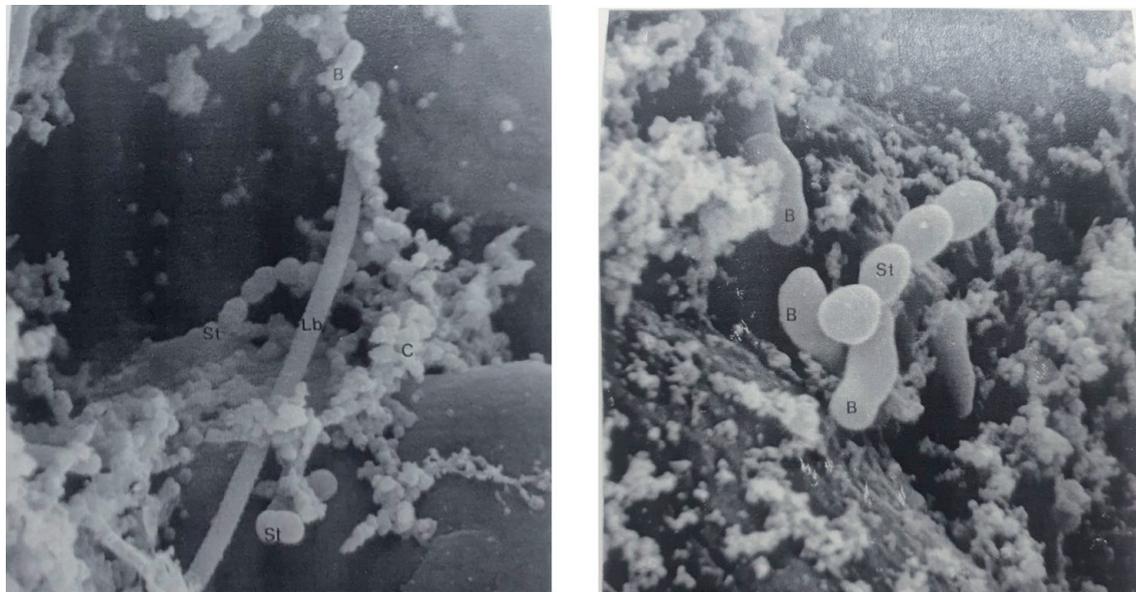


Figure 5. *B. pseudolongum* in yoghurt (left) or soyoghurt (right).

Role of probiotic cultures to give positive impact for human health depends on if they are life or not in media, as in **Figures 4 and 5** above [14]. These probiotics microbes require a great number of bacteria that genetically stable since the manufacturing, during storage and expressing their health benefit after consumption especially during transit gastric. Consumers will accept the probiotic products that have a pleasant aroma and taste. It should bear in mind that the development of these bacteria either indirectly to fermented milk or directly to human gut also depends on the type of food ingested by humans. Human, included infant live under rather unnatural conditions lead to unfavorable condition causing illness. We eat a great deal of processed antibacterial substances ranging from vinegar to antibiotics, and in many cases sterile food which many affects our access to and colonization by certain type of bacteria. The healthful effect of probiotic -containing fermented milk is presented supplemented or not by prebiotic substances are in (**Table 3**).

The food in Indonesia is quite different from those in Western countries or Japan. The predominant food ingested by the Indonesians are food from vegetable origins, while in Western countries are from animal origins (meat, eggs, and milk)

Health benefit of Fermented Probiotic Milk	
• Intestinal microbial balance	• Antidiarrhea
• Improved absorbability of certain nutrients	• Anticancer effect
• Alleviation of lactose intolerance symptom	• Antagonist environment. For pathogens
• Metabolism of some drugs	• Blocking adhesion sites from pathogens
• Improvement of intestinal motility	• Inactivating enterotoxins
• Stimulation of immune system	• Alleviating constipation
• Serum cholesterol reduction	• Relieving vaginitis
• Resistant to enteric pathogen	• Small bowel bacterial overgrowth

[1, 26, 27].

Table 3.
Health effect of fermented milk's probiotics.

and in Japan which are from sea food origins. Some of the health and nutritional benefit of probiotics cultures containing LAB or others for human are generally are:

6.1 Enhanced lactose digestion

Lactose, the main carbohydrate in mammalian milk is fermentable compound, and digested by the intestinal enzyme, lactase, present especially in infants and lactose persistent adults. However, in large percent of the world population (70%) who are lactose non-persistent, lactose escapes digestion in the small bowel and is fermented in the colon with the evolution of gases (CO₂, H₂, CH₄) and the production of osmotically simple sugars. The gas, especially H₂ will be Trans located to lungs and can be detected as hydrogen exhalations. Improvement of lactose digestion and avoidance of symptoms of intolerance in lactose mal-absorbers are the most profoundly studied health—relevant effect of fermented milk products, despite are not specifically probiotics effects. The word lactose intolerance is well accepted rather than lactose mal-absorbers, due to no absorption in the proper term of lactose in small intestine. The symptom released due to the absence of or insufficient amount of *β-galactosidase (lactase)* in the human small intestine to digest the milk sugar, lactose. The prevalence of primary lactose intolerance is 3–5% in Scandinavia, 17% in Finland and Northern France, 65% in southern France, 20–70% in Italy, 55% in Balkans region, 70–80% in Africa, 80% in Central Africa, 90–100% in Eastern Asia, 30% in Northern India, 70% in southern India and 80% in black American [28]. While secondary form of lactose intolerance may be due to inflammation or functional loss of the mucus of small intestine (enteritis, bacterial or parasitic infection or small bowel syndrome) and by protein-energy malnutrition. It is generally accepted that lactose intolerance symptoms will appear after consuming more than 250 mL a day of whole milk [2]. Fermented milk contains less lactose (**Table 4**).

Lactase deficiency is due to low activity of intestinal lactase (lactase phlorizine hydrolase's in enterocyte of villi). The symptoms associated with lactose intolerance included: bloating, belching, flatulence, cramping and watery stools. Individual with this condition experience gastric distress when they consume fresh, unfermented dairy products. Fermented milk products can especially improve lactose digestion in individual lactose intolerance (**Table 5**).

The explanation will be given that microbial *β-galactosidase* auto digest this lactose and reduce the content of lactose present in dairy products between 20–30% of original lactose [33]. Some developed fermented milk has reduced lactose content around 30% with the range from 14 to 74% in fermented milks. The reduction of lactose

Fermented milks	Lactose contents (%), at		Reduction (%)
	0 h	8 h	
1. Cow milk yoghurt	4.31.	3.72	13.7
1. Cow milk yoghurt	4.4	1.9	74
1. Goat milk yoghurt	4.55	3.6	19.5
1. Goat milk yoghurt	4.4	3.0	31
1. 4+ Soy Growth Factors	4.9	3.85	21.4
1. Bifidus cow milk	4.2	3.28	21.9
1. Bifidus cow milk	4.7	3.5	30
1. 6+ Soy GF	4.7	4	14.8
1. 1+ 8	4.31	1.9	56
1. Bifidus goat milk	4.2	2.76	34.3
1. Acidophilus cow milk ^b	5	3	40
1. Cow milk ^b	5	3	40
1. Lactococci fermented milk ^c	4.5	3.4	24.5
1. Citrat-Lactococci fermented milk ^c	5.5	3.65	33.4
1. Leuconostoc fermented milk ^c	6.5	3.68	43.3
1. 13 + Bifidobacteria	3.4	2.58–3.29	> 25
1. 14 + Bifidobacteria	3.65	2.51–3.55	> 32
1. 15 + Bifidobacteria	3.68	2.92–3.43	21

^aSugars were detected by HPLC using Column Aminex HPX-87 H at flow rate 0.4 ml/min and mobile phase acetonitrile 30% in 0.005 M H₂SO₄ (Bio-rad), temp 65 C [2, 28–31].

Table 4.
 Reduction of lactose content of some fermented milks^a.

Fermented milks	% lactose	No cell/ g prod exceptb.	Lactase (mg/h/g prod)
Yoghurt	4	3×10^8	0.64
Pasteurized yoghurt	4	3.4×10^6	0.07
Cultured milks	4.3	2.8×10^6	0.02
Sweet acidophilus milk	4.8	1.1×10^7	0
Pasteurized milk	4.9	Not measured	0
<i>Bifidobacterium breve</i> ^b	3.4	8.7 log CFU/mL	NA
<i>Bifidobacterium longum</i> ^b	3.65	8.7 log CFU/mL	NA
<i>Bifidobacterium bifidum</i> ^b	3.68	8.7 log CFU/mL	NA

[29, 32].

Table 5.
 Percent lactose, cell counts and lactase activity of fermented milk fed to lactase deficient subjects.

varies either with raw milk, material, bacterial species used and its combination or the present of growth factors. It is therefore, Fermented milk reduce lactose content and consumers will adapt to different levels of lactose in any commercial fermented milks.

These still alive microbes will implant in the small intestine of humans and continue to digest of lactose lead to better adaptability to the present of such compound or giving improve to lactose tolerance. Yoghurt bacteria, *L. acidophilus*,

and *Bifidobacterium* sp. are now well known can survive during gastric transit alive, numerous, and active in the human intestine despite it is not natural inhabitants. These bacteria then deliver their lactose-metabolizing enzyme to its site of action over an extended period. Therefore, probiotic-containing fermented milk could partially explain the better lactose digestion after ingestion of milk.

6.2 Colonization and role anti-pathogenic bacteria

The antimicrobial or antagonistic activity of probiotic cultures is an important property that people need to understand for using it as health benefit microbes for human beings. Breast-fed infants thrive better than bottle-fed or weaned babies do. This, in part can be associated with a predominant bifid gut flora that inhibits growth of coliforms, enterococci, and clostridia [34]. *Bifidobacterium* sp. account for 92% of the intestinal flora of breastfed infants, but only 20% of bottle-fed or weaned infants. The production of acetic and lactic acid has caused lower pH to inhibit pathogenic bacteria lead for children with high numbers of bifidobacteria to resist some enteric infection very effectively. Regularly supplementation of the infant diet with bifidobacteria can maintain normal intestinal condition. When *B. longum* administered orally to germ-free mice, the bacteria colonize the intestinal tract and reach a concentration of 10^9 – 10^{10} /g intestinal content in 2–3 days. Ability to transit gastric of living probiotic bacteria is important criteria to be used as human health promoting bacteria [35–37]. Translocation of the colonized *B. longum* to the mesenteric lymph node (MLN), liver and kidney occurs between 1–2 weeks after the association caused neither infection nor harmful effect. When *E. coli* O: 111 or O: 157 was administered orally to germ-free mice, they will be translocated to various organs occurred and the mice died by endotoxin shock or organ failure. When *B. longum*—mono associated mice challenged *E. coli* O: 157, the intestinal count of strain O: 157 suppressed at a low concentration and no death after 5 weeks. When with *s* O: 111 at a lethal dose, death was avoided [38, 39]. Treatment and prevention of infectious diarrhea are probably the most widely accepted health benefits of probiotic microorganisms. Rotavirus is the most common cause of acute infantile diarrhea in the world. Well-controlled clinical studies have shown that probiotics such as *L. rhamnosus* GG, *L. reuteri*, *L. casei*, strain Shirota, and *B. animalis* Bb12 can shorten the duration of acute rotavirus diarrhea with the strongest evidence pointing to the effectiveness of *L. rhamnosus* GG and *B. animalis* Bb12 [40].

6.3 Role anti-cancer, anti-hypercholesteremic and anti-virus

In order to permanently establish the bacterial strain in the host's intestine, the organism must be able to attach to intestinal mucosal cells. The intestinal microflora within a given individual are remarkably stable, although major differences may exist among different persons.

Nevertheless, administration of especially probiotic cultures to either newborn or adult results in certain change in the microbial profiles and metabolic activities of feces. The Fermented Milks and Lactic Acid Beverages Association in Japan have introduced a standard of a minimum of $>1 \times 10^7$ CFU/ml or CFU/g viable probiotic cells for fresh dairy products [38]. It has suggested a minimum viable number of 10^6 CFU/ml or grams but recommended 10^8 CFU/g to compensate the reduction through passage through the gut.

In contrary to the general definition of probiotics, dead probiotics or bacterial cell components can still combat cancer effectively [41]. In fact, it has been proposed that inactivated or non-viable probiotic cells, as well as their metabolic byproducts (herein referred as “postbiotics” and “paraprobiotics”), also have the ability to provide benefits to the host's health [19].

Probiotics are able to conjugate bile acids and to assimilate cholesterol. The feeding of milk formula supplemented with *L. acidophilus* to infants was shown to result in lower level of serum cholesterol [42]. While culture of *S. thermophilus* assimilated less cholesterol than *L. delbrueckii subs. Bulgaricus*. *L. acidophilus* assimilated more cholesterol than those *S. thermophiles* and a commercial yoghurt. Bifidobacteria were also actively assimilated cholesterol. [43] have studied the influence of probiotic yoghurt on serum lipids in women, found that the concentration of total cholesterol, LDL Cholesterol and triacyl glycerol on serum decreased after consuming the standard and this reduction did not depend on the cholesteroeimi status (Table 5).

However, the beneficial impact of probiotic is not limited to the gut-associated diseases alone, but also in different acute and chronic infectious diseases. This is because probiotics are able to modify the intestinal microbial ecosystem, enhance the gut barrier function, provide competitive adherence to the mucosa and epithelium, produce antimicrobial substances, and modulate the immune activity by enhancing the innate and adaptive immune response. Dairy-based matrix is suitable for proliferation of probiotics due to supplying a rich source of carbon and essential amino acids as a consequence of lactose hydrolyzing and proteolytic systems involved casein utilization [44]. Oral administration of *L. casei* SY13 also significantly enhanced the gut microbial diversity [45]. When, such change even minor applied to pathologic situation, they are sufficient to beneficially alter the course of disease, especially that are associated with the developments of colon cancer [46] as in Table 6.

It has explained that some research results using *L. acidophilus* (1×10^9 CFU/day) and *B. bifidum* (1×10^{10} CFU/day) during 3 weeks reduced fecal activity of

	Probiotic	Experiment	Major finding	Reference
<i>in vivo</i>	Yoghurt (unknown)	Human	Reduced total cholesterol and LDL	Agerhol Larsen <i>et al.</i> (2000)
	Fortified buffalo	Rat	Reduced total cholesterol, LDL-cholesterol, and triglyceride	Abd El-Gawad <i>et al.</i> (2005)
	<i>L. plantarum</i>	Mice	Reduced blood cholesterol Decreased triglycerides	Nguyen <i>et al.</i> (2007)
	<i>L. acidophilus</i> 145, <i>B. longum</i> 913	29 woman/21 week	Up HDL-cholesterol, down LDL/HDL ratio	Khani <i>et al.</i> , 2012
<i>in vitro</i>	<i>L. fermentum</i>	Culture media	BSH activity	Pereira <i>et al.</i> (2003)
	<i>L. acidophilus</i> <i>L. bulgaricus</i> <i>L. casei</i>	Culture media	Assimilation of cholesterol Attachment of cholesterol onto cell surface Disrupt the formation of cholesterol micelle Deconjugation of bile salt Exhibited bile salt hydrolase activity	Lye <i>et al.</i> (2010)
	<i>Lactobacillus plantarum</i> CGMCC,	Colorectal cancer patients	Probiotics decreased the serum zonulin concentration,	Liu <i>et al.</i> , 2013
	<i>L. rhamnosus</i> GG, <i>B. lactis</i> and inulin	37 colon cancer/12 week	Down colorectal proliferation & necrosis	Khani <i>et al.</i> , 2012

[47–49].

Table 6.
Beneficial role of probiotics cultures.

nitro reductase, azoreductase, and β -glucuronidase, which is considered implicated to cancer in colon activity after daily intake of fermented vegetables for several weeks [50]. The anti-carcinogenic effect of bifidobacteria may be the results of direct removal of pro-cancerigenes, indirect or activation of the body's immune system [34]. By directly removing pro-cancerigenes, bifidobacteria, indirectly, *B. longum* remove the source of pro-cancerigenes or the enzyme, which lead to the formation of liver tumor, developed in mice when an intestinal flora of *E. coli*, *Enterococcus faecalis* and *C. paraputrificum* was presence.

Probiotics mainly colonize in the gut, but it has a fundamental impact on the systemic immune response (Immune enhancer), and exert the immune responses at distance ucosal site, including the lung [51]. There is accumulating evidence that bidirectional communications exist between gut and lung, which is called the gut-lung axis, in which probiotics modulate mucosal immune function. *L. acidophilus* and *L. casei* have some positive role to inhibit viral replication [52]. This bidirectional crosstalk is involved in the support of immune homeostasis. It is believed that the gastrointestinal inflammation results in lung inflammation through this connection [23].

It was found that COVID-19 patients with GI symptoms such as diarrhea experienced more severe respiratory disorders than those without GI symptoms. Probiotic consumption improves the level of type I interferons, antigen-presenting cells, NK cells, and T and B cells in the lungs' immune system. Probiotic administration can also improve the pro- and anti-inflammatory cytokines, helping to clear the viral infection by minimizing the cell damage in the lungs [51]. Antunes et al. [53] explained that some research about the role of fermented milk to fight COVID-19. They have been conducting in US as for the moment, a clinical trial is being performed on the effect of *L. rhamnosus* GG on the microbiome of household contacts exposed to COVID-19 (U.S. National Library of Medicine, Clinical Trial gov Identifier NCT04399252, 2020).

Other ongoing studies using probiotics to prevent or treat COVID-19 are "Evaluation of the probiotic *Lactobacillus coryniformis* K8 on COVID-19 prevention in healthcare workers" (U.S. National Library of Medicine, Clinical Trial gov Identifier NCT04366180, 2020).

6.4 Development of flavor and enhance consumers' acceptance

The wholesome and goodness of food products included the flavor influence acceptability of consumers. While, Muslim's community, they also need halal status of food and beverage products. Flavor is defined as complexes' sensation of taste, odor, and some physical aspects as texture, and hardness. Odor relates the volatile components that released during mastication or as the results of bacterial activity [2].

Some odors have reduced the perception score of consumers lead to rejection from people, as beany flavor of soybean foods. Fermented milk can mask this beany flavor [14, 20, 54, 55] have studied the odor of some fermented milk (Tables 7 and 8) lead to more acceptability in diverse consumers.

Fermentation ability to produce differ flavor is depended also on genotypes of dairy animals. The genetic polymorphisms were related to the milk titrable acidity, alcohol stability, pH and bacterial count, as a possible effect of CN and LGB gen polymorphisms [56]. It also influenced on the physical properties of cultured milk as for example cheese syneresis, particle size distribution and odor production [57].

Preliminary data of our research on milk polymorphisms of Saanen Goat and Ettawa grade goat in Indonesia explained that there were at least 9 alleles of CSN1S1: AE (7.01%), AF (15,79%), AN (5,26%), A01 (1,75%), EE (8,77%), EF (47,37%), FF (10.53%), F01 (1,75%), NN (1,75%) for Saanen goats and 5 alleles of CSN1S1: AA

Species	Acetaldehyde	Acetone	Butanal	Butanol 2	Ethanol
<i>L. delbrueckii subsp. Bulgaricus</i>	3,1	1.4	0.1	0.17	0.5
<i>L. helveticus</i>	3.95	3.05	0.1	0.55	0.2
<i>L. acidophilus</i>	5.75	3.3	0.55	0.6	0.5
<i>L. casei</i>	3.2	4	0.1	0.8	0.4
<i>L. casei strain Shirota</i>	4.81	19.5	—	—	232
Average Lactobacillus	4.16	6.25	0.19	0.42	46.6

*Skim milk, [54].

Table 7.
 These bacteria have been developed in skim milk. * at Lactobacillus (Lactobacillus*).

Compounds	Yakult	Yoghurt 1	Kefir
Acetaldehyde	4.81 ± 4,3 ^a	14,82 ± 1,99 ^b	4.48 ± 1,41 ^a
Acetone	19.58 ± 13,7 ^d	39,92 ± 4.56 ^e	7,51 ± 3,38 ^e
Ethanol	232,1 ± 158 ^g	1,56 ± 1,32 ^f	214 ± 63 ^g
Diacetyly	14,63 ± 3,8 ⁱ	2,72 ± 1,49 ^h	80 ± 55 ^j

* ^{a-j} means the different letter in the same row was significantly different [55].

Table 8.
 Odor of fermented milk containing lactic acid bacteria cultures (ppm)*.

(67,65%), AE (2,94%), AF (14,71%), AN (11,76%), EF (2,94%) of Ettawa grade goat (Murti, 2020; unpublished data). These polymorphisms can influence physico-chemical and flavor of fermented milk.

7. Conclusion

Fermented milks especially which use intestinal microflora, known as probiotic cultures link to many aspects of host health. Dairy-based matrix is suitable for proliferation of probiotics due to supplying a rich source of carbon and essential amino acids as a consequence of lactose hydrolyzing and proteolytic systems involved casein utilization. However, we still have limited understanding within the mechanisms involved in these systems, particularly with regard to colonization resistance. Despite a lot of research to date, many questions still remain as to how the modulations of the microflora are able to offer benefits for the host in either good health or illness. Knowledge of its composition and activities are central to this, as is a better understanding of how influence the toleration of microflora. Colonization of the gut may not be an absolute necessity since the healthy host influence the toleration of the strain becoming tolerant, thereby removing its immunological capacity to be a probiotic. Since different probiotic strains are known to have different immunological and microbiological properties, molecular studies should enable specific components of bacterial phenotype, and so identify strains that can benefit the host with diarrhea or inflammatory diseases as well as their role to fight Covid-19.

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