



Fermentation of Milk Using Folate-Producing Lactic Acid Bacteria to Increase Natural Folate Content: A Review

Fenny Amilia Mahara¹, Lilis Nuraida^{1,2*}, Hanifah Nuryani Lioe¹

¹Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, IPB University (Bogor Agricultural University), Darmaga, Bogor 16680, Indonesia

²Southeast Asian Food and Agricultural Science and Technology (SEAFST) Center, IPB University (Bogor Agricultural University), Bogor 16680, Indonesia

Corresponding Author: Lilis Nuraida, PhD, Professor, Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, IPB University (Bogor Agricultural University), Darmaga Bogor 16680, Indonesia. Tel/Fax: +62251-8626725, Email: lnuraida@gmail.com

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Abstract

Folate, also known as vitamin B9, is essential in cell metabolism and very important especially for pregnant women and lactating mothers. Natural folate is available in food but it is very unstable. Synthetic folate is generally used as an alternative to meet daily needs due to its stability, even though it has a negative effect causing a variety of metabolic disorders. Some lactic acid bacteria have been reported as being able to synthesize natural folate during the fermentation process. Lactic acid bacteria are the main microorganisms for lactic fermentation such as fermented milk, fruits, and vegetables. Milk is the most nutritious food and contains folate-binding protein, hence it is considered the ideal fermentation medium to increase folate stability during storage. Fermentation of milk with folate-producing lactic acid bacteria can be used as a technique to produce natural folate-rich fermented foods as an attempt to prevent folate deficiency without side effects to the consumers.

Keywords: Lactic Acid Bacteria, Fermentation, Fermented Milk, Folate, Folate-Rich Fermented Food

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Introduction

Folate or vitamin B9 is a water-soluble vitamin composed of several conjugated molecules namely pteridine ring, para-aminobenzoic acid (PABA), and glutamic acid.^{1,2} Folate is required for normal cell fission and growth. It functions as a cofactor involved in various metabolic reactions in the body, such as synthesis, repair, DNA methylation reactions; nucleotide synthesis; and amino acid metabolism.^{3,4} Folate is needed in a certain amount, especially during pregnancy and lactating period. The daily recommended intake of folate are 65 µg/d of dietary folate equivalents (DFEs) for 0-6 month infants as adequate intake (AI), 80 µg DFE for 7-12 month infants (AI), 150 µg DFE for 1-3 year old children as recommended dietary allowance (RDA), 200 µg DFE for 4-8 year old children (RDA), 300 µg DFE for 9-13 year old children (RDA), 400 µg DFE for 14-18 year old teens (RDA), 400 µg DFE for adults 19 years old and older (RDA), 500 µg DFE for lactating women (RDA), and 600 µg DFE for pregnant women (RDA).⁵ If folate requirement is not sufficiently met, the body will experience folate deficiency and trigger various diseases such as anemia, neural tube defects, homocysteinemia, cardiovascular disease, and cancer.^{4,6-9}

Folate cannot be produced in the body, therefore, it must be obtained from food intake. It is naturally present in various

types of food, such as cereals, fruits, vegetables, spices, nuts, eggs, and cheese.¹⁰ However, natural folate has unstable properties, and its content is easily reduced during washing and processing.^{11,12} Alternatively, folic acid, a synthetic form of folate, is generally chosen as the main source in fulfilling the daily needs as a food fortificant and food supplement due to its stability.^{13,14} However, the metabolic process of folic acid in the body is relatively slow hence the body is not able to completely convert folic acid in large quantities. This results in a substantial accumulation of unmetabolized folic acid in cells. High levels of unmetabolized folic acid in the blood will cause a variety of metabolic disorders, such as masking symptoms of vitamin B12 deficiency, cognitive impairment, reducing the immune system, and cancer.^{15,16} The emergence of health problems related to the use of synthetic folate has prompted many researchers to look for other, more stable, safer and more efficient sources of natural folate.

Lactic acid bacteria (LAB) are known to produce folate both intracellularly and extracellularly.^{12,17-19} Intracellular folate is in the intact cells, while extracellular folate is secreted to the growth medium. Extracellular folate is in the form of monoglutamate, thus it has a higher bioavailability compared to intracellular folate which is in the form of polyglutamate. Polyglutamate requires an enzymatic conjugation process

before being absorbed by the body.^{12,20,21} Therefore, extracellular folate secreted to the media can be effectively and readily used as an alternative source of natural folate.

As a fermentation medium, milk is an ideal matrix because it contains nutrients that are suitable for the growth of LAB and contains folate-binding proteins that can increase the stability of folate synthesized during fermentation.²²⁻²⁴ However, during fermentation, most LAB use folate for their growth, thereby reduce the amount of folate in the product.²⁴ The ability of LAB to produce folate depends on fermentation conditions, medium types, the presence of folate precursors, and several other factors.^{17,20} In this review, the utilization of LAB in increasing natural folate content in fermented milk products that could be used to prevent folate deficiency will be discussed.

Folates: Types, Sources, and Stability

Folate is the generic term of conjugated compounds formed by a pteridine ring linked to PABA and one or more L-glutamates. In nature, folate is in the form of 5-methyltetrahydrofolate (5-MTHF), 5-formyltetrahydrofolate (5-FTHF), 10-formyltetrahydrofolate (10-FTHF), 5,10-methylenetetrahydrofolate, 5,10-methenyltetrahydrofolate (5,10-methenyl-THF), 5-formimino tetrahydrofolate, 5,6,7,8-tetrahydrofolate (THF) and dihydrofolate (DHF). The forms of THF and MTHF are the two most important folates for human body. Folate in THF form has a significant role as a cofactor in carbon-1 transfer for DNA synthesis, while the MTHF form is the main form transported and stored in the human body.^{1,10,25}

Natural folate is found in various types of plants, animal organs, bacteria, and yeast, and is generally detected in heterogeneous forms, characterized by one unit of carbon atom (C) connected to the positions of N5 (R_1) and/or N10 (R_2) (Figure 1a), such as methyl (5-CH₃), methylene (5,10-CH₂), formimino (5-CH=NH), formyl (5- or 10-CHO), and methenyl (5,10-CH). In flowering plants or angiosperms, folate is mostly in the forms of methyl (45%-65%) and formyl (30-55%), whereas in vegetables and fruits, it is mostly in the form of 5-MTHF, and a few of 5- and 10-FTHF. In animal organs (liver and kidney), about 40% of folate is found in the form of methyl derivatives.^{10,26} Meanwhile, the most abundant folate forms in kefir yeast strains are 5-MTHF (43%-59%), 5-FTHF (23-38%), and THF 19%-23%.²⁷

Among bacteria, LAB and bifidobacteria such as *Streptococcus thermophilus*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactococcus lactis*, *Bifidobacterium longum*, *Pediococcus acidilactici*, and *Weissella confusa*, can produce folates naturally in numerous fermented food.^{12,29-31}

The dominant forms of folate synthesized by LAB in milk are THF, 5-MTHF, and 5-FTHF (Table 1), while in liquid media, *S. thermophilus* and *L. lactis* produce 5-FTHF and 5,10-methenyl-THF.³² However, the variety of synthesized folate forms depends on each strain and species of bacteria.^{1,10,19,25,33-35}

In addition to the substituent group connected to N5 and/or N10 of the pteridine ring, natural folate forms also vary in the amount of glutamic acid residue conjugated to pteric acid, namely monoglutamate or polyglutamate. Most of the folates

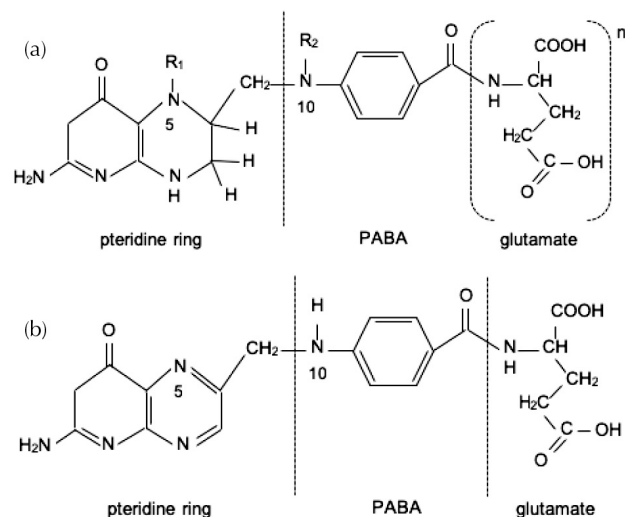


Figure 1. Folate Structure. (a) The main structure of folate with different substituents at R_1 and R_2 .²⁸ (b) The structure of folic acid.¹⁰

in plants, animals, and microbes are in the reduced form of THF-polyglutamate, where two double bonds of the pteridine ring are reduced, and few are found in the form of free folate (monoglutamate). Monoglutamate folate can be found in milk (60%), soybeans (50%), and orange juice (>30%), whereas polyglutamate forms can be found in cabbage (hexane and heptaglutamate), orange juice (pentaglutamate), liver and kidney (pentaglutamate).²

In bacteria, there are two types of synthesized folate, namely intracellular and extracellular folate. Intracellular folate is mostly present in the form of pteroilpentaglutamate (5 glutamate residues), whereas extracellular folate tends to be in

Table 1. Forms of Folate Synthesized by LAB in Fermented Milk

Bacterial Species	Forms of Synthesized Folate				Ref.
	THF ^a	5-MTHF ^b	5-FTHF ^c	5,10-methenyl-THF ^d	
<i>S. thermophilus</i>	+ ^e	+	+	- ^f	25
<i>Lactobacillus acidophilus</i>	+	+	+	-	25
<i>Bifidobacterium longum</i>	+	+	+	-	25
<i>Lactobacillus bulgaricus</i>	+	+	+	-	25
<i>S. thermophilus</i>	-	+	-	-	33
<i>S. thermophilus</i>	+	+	+	-	36
<i>Lactobacillus bulgaricus</i>	+	+	+	-	36
<i>Lactobacillus lactis</i>	+	+	+	-	36
<i>Lactobacillus helveticus</i>	+	+	+	-	36
<i>Lactobacillus lactis</i> ssp. <i>cremoris</i>	+	-	-	-	34
<i>Lactobacillus lactis</i> ssp. <i>lactis</i>	+	+	-	-	34
<i>Lactobacillus plantarum</i>	-	+	-	-	35
<i>Lactobacillus delbrueckii</i>	-	+	-	-	35
<i>Bifidobacterium adolescentis</i>	+	-	-	-	35
<i>Bifidobacterium catenulatum</i>	+	-	-	-	35
<i>S. thermophilus</i>	+	+	-	-	19

^a 5,6,7,8-tetrahydrofolate; ^b 5-methyltetrahydrofolate; ^c 10-formyltetrahydrofolate; ^d 5,10-methenyltetrahydrofolate; ^e + means detected; ^f - means not detected or not analysed.

the form of pteroilmonoglutamate (1 glutamate residue). The form of synthesized folate by LAB also depends on the species. In *Lactococcus lactis* species, intracellular folate has the form of tetra-, penta-, to hexaglutamate, and extracellular folate in the form of mono-, di-, to triglutamate. In *S. thermophilus* species, both intracellular folate and extracellular folate, have mono-, di-, and triglutamate forms. Most of the folate produced by species *L. lactis*, and other species such as *Leuconostoc* spp., *Propionibacteria* spp., and *Bifidobacteria* spp., are intracellular folates and are not secreted to the media, while *S. thermophilus* species produce extracellular folate which is higher than intracellular folate.^{21,32,37}

Based on its variations, natural folate has different stability. The stability order is 5-FTHF > 5-MTHF > THF, studied during fruit and vegetable processing. These folate forms become rapidly unstable due to the longterm contact with water, the presence of oxygen, the low temperature, and the acidic condition. Leaching into the water and the oxidative degradation become two main mechanisms of folate losses.²⁶ Furthermore, the oxidation will reduce folate bioavailability due to the conversion of folate into an inactive form, p-aminobenzoyl glutamate, significantly reducing its activity.²⁰

Compared to natural folate present in the reduced form, folic acid is in the fully oxidized monoglutamate form. Shown in Figure 1b, the structure of folic acid is similar to the natural folate form but has fewer hydrogen atoms. This, chemically leads to a more stable form, hence produced synthetically and found in dietary supplements or fortified foods. However, although not naturally found in foodstuffs, some vegetables such as spinach, chickpeas, tomatoes, green beans, and cabbages were reported to have folic acid content in small amounts. The folate degradation during analytical procedures become the likely reason for folic acid being present in plant matrixes.^{10,26,38}

Due to the unstable characteristics, natural folate contained in food ingredients is easily damaged or reduced due to the harvesting process, storing, distributing, and processing.^{12,39-41} Therefore, usually synthetic folate in the form of folic acid is chosen as an alternative to meet the demands through fortification or food supplement programs because of its stable characteristic and due to the fact, that it is not easily degraded.^{6,13}

Folate-Producing Lactic Acid Bacteria

Lactic acid bacteria are commonly present or used in the fermentation process of various types of food, such as fermented dairy products and its derivatives including yogurt, cheese, kefir, and others; fermented fruits and fermented vegetables. LAB used for fermentation are usually the genus of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. The LAB in milk fermentation can be directly added as a starter culture, or naturally available because milk is a natural habitat of LAB.⁴²

During fermentation, LAB convert lactose into lactic acid as the main end product, which increases the acidity and suppresses the growth of pathogenic bacteria, thereby increasing product safety. These bacteria also have a proteolytic

activity that can degrade milk protein into components that contribute to the texture and the organoleptic properties of the product.⁴³ Furthermore, LAB are also known to have the ability to synthesize various groups of vitamin B such as folate (B9), riboflavin (B2), and cobalamin (B12), which can increase the nutritional value of the products.⁴⁴

The group of LAB known to be able to synthesize folate, consist of various genus, species, and strains (Table 2). These bacteria primarily synthesize folate to meet their own needs. LAB and most of the other organisms (prokaryotic and eukaryotic) require folate cofactors in a reduced form as acceptors or donors of carbon units. This cofactor is involved in various biosynthetic processes such as the formation of methionine, purine, thymine, and various degradation reactions.⁷

Folate Biosynthesis and Secretion by LAB

The LAB have different ways of fulfilling their folate needs. Some LAB, especially from *S. thermophilus*, *Lactobacillus plantarum*, and *L. lactis* species, can synthesize folate through de novo pathway for folate biosynthesis. Other LAB tend to consume folate in the media hence their growth depends on the presence of folate in the media. In fact, the behavior of LAB to meet their folate needs is very different between strains. Several strains of bifidobacteria such as *B. adolescentis* MB 114, *B. adolescentis* MB 115, and *B. pseudocatenulatum* MB 116, do not produce folate when folate is available in the media. Several other strains such as *B. adolescentis* MB 227, *B. adolescentis* MB 239, and *B. pseudocatenulatum* MB 237,

Table 2. Species of Folate-Producing LAB

Species	Literature
<i>Streptococcus thermophilus</i>	17-19,25,31-33,35,37,45,46
<i>S. lutetiensis</i> , <i>S. infantarius</i>	31
<i>Lactobacillus fermentum</i>	12,18
<i>L. acidophilus</i>	18,25,47
<i>L. rhamnosus</i> , <i>L. reuteri</i>	18
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i>	17,25,32,35,45
<i>L. plantarum</i>	12,30,32,35,46-50
<i>L. helveticus</i>	31,51
<i>L. paraplantarum</i>	12,50
<i>L. salivarius</i>	50
<i>L. sakei</i> , <i>L. coryniformis</i>	30
<i>L. casei</i>	47
<i>L. johnsonii</i>	52
<i>Leuconostoc lactis</i> , <i>Leu. paramesenteroides</i>	32
<i>Lactococcus lactis</i>	20,31,32,34,48,53
<i>Bifidobacterium lactis</i>	45
<i>B. animalis</i> , <i>B. breve</i>	29,54
<i>B. infantis</i>	45,54
<i>B. dentium</i>	54
<i>B. bifidum</i>	29,54
<i>B. longum</i>	25,29,33,54
<i>B. adolescentis</i> , <i>B. catenulatum</i>	29,35,54
<i>B. pseudocatenulatum</i>	29,54
<i>Pediococcus pentosaceus</i>	50
<i>P. acidilactici</i>	12,50
<i>Weissella cibaria</i>	55
<i>W. paramesenteroides</i>	31
<i>W. confusa</i>	30,55

continued to produce folate even though folate was available in the media.⁵⁴ This shows that the ability of LAB to synthesize folate is dependent on the strains.^{12,24}

The folate biosynthesis pathway in microorganisms consists of several parts based on the three main constituents of folate structure namely pteridine, PABA, and glutamate. The pteridine is made from guanosine triphosphate (GTP), synthesized in the purine biosynthetic pathway, whereas PABA comes from chorismate, involved in the same pathway as the aromatic amino acid pathway, glycolysis, pentose phosphate, and the shikimate pathway.²¹ Basically, many microorganisms can synthesize glutamate by the conversion of α -ketoglutarate from glycolytic intermediates. However, most LAB except *L. lactis* ssp. *lactis* are reported to be not able to produce glutamate due to the lack of isocitrate dehydrogenase and glutamate dehydrogenase activities, shown to be the main enzymes in the formation of glutamate.⁵⁶ Therefore, the ability of LAB in synthesizing folate is also highly dependent on the availability of glutamate in the media.

In overview, the folate biosynthesis pathway of LAB is catalyzed by nine enzymes, converting GTP to THF-polyglutamate (Figure 2). The biosynthetic process starts with the conversion of GTP to dihydroneopterin, involving the activities of three enzymes, namely GTP cyclohydrolase I, catalyzing the reaction of GTP into dihydroneopterin triphosphate; dihydroneopterin triphosphate pyrophosphohydrolase, converting dihydroneopterin triphosphate to dihydroneopterin monophosphate; and α -specific phosphatase, producing dihydroneopterin. Furthermore, dihydroneopterin is converted to 6-hydroxymethyl-7,8-dihydropterin by dihydroneopterin aldolase, and is further changed to 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPPP) by hydroxymethyl

dihydropterin pyrophosphokinase. Afterwards, DHPPP fuses with PABA by dihydropteroate synthase enzyme. Incorporating C-N bonds from DHPPP to PABA forms dihydropteroate, and is further conjugated with glutamate by dihydrofolate synthase, producing DHF. Moreover, DHF will be reduced by dihydrofolate reductase to the active form of THF. In the end, glutamate residues are added in large amounts by the folylpolyglutamate synthase enzyme to form THF-polyglutamate.^{21,57}

The distribution of intracellular and extracellular folate synthesized by LAB depends on the degree of polyglutamylation or the pH of growth medium. In *L. lactis* species, intracellular and extracellular folate distribution only depends on the degree of polyglutamylation and is not influenced by pH. Intracellular folate has a long polyglutamic tail that is more than three glutamate residues (4, 5, and 6 glutamate residues), whereas extracellular folate has shorter polyglutamate tails namely mono-, di-, to tri-glutamate. Long polyglutamic tails (more than 3 glutamate residues) cannot be transported through cell membranes, so it tends to remain inside cells. Longer glutamate tail increases folate retention in the cell. This is because glutamate has a negatively charged carboxyl group (pKa of 4.6) so that the more glutamate residue is bound to the polyglutamic tail, the more negative the folate content is and as a result, increases the folate retention in the cell.³² Therefore, the intracellular folate release is highly dependent on cell destruction as it passes through the digestive tract.⁵⁸

In *S. thermophilus* species, intracellular folate and extracellular folate have a short polyglutamic tail, which is not more than three glutamate residues. Thus, the folate distribution does not depend on the degree of polyglutamylation but is dependant on the pH of the media.

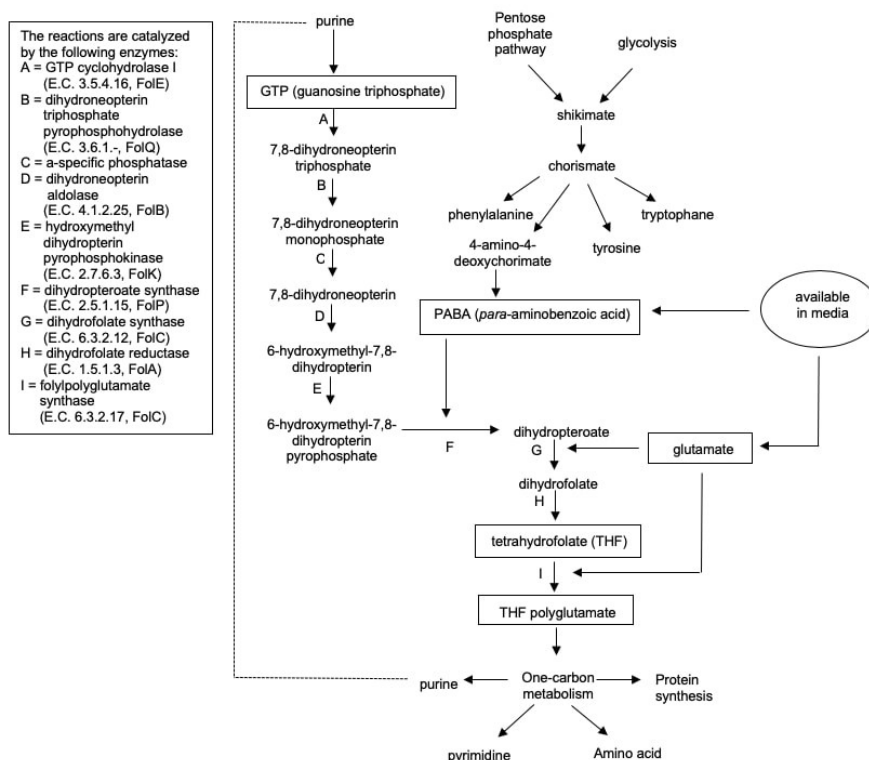


Figure 2. Folate Biosynthesis Pathway in LAB.^{21,57}

Cells that grow in low pH will produce higher extracellular folate than cells that grow in high pH. When the media pH is low, intracellular pH becomes low too. This condition will cause higher intracellular folate to be protonated and become neutral so that it is easily transported through the cell membrane. In *L. lactis*, due to the long polyglutamate tail of the intracellular folate (more than three glutamate residues), a decrease in intracellular pH does not cause intracellular folate protonation so that it cannot be transported across the cell membrane.³²

Intracellular folate protonation due to low pH can explain that in certain species of LAB, such as *S. thermophilus*, the pH of the media greatly determines the ability of LAB to synthesize intracellular and extracellular folate. Low pH can increase the secretion of extracellular folate, and high pH can maintain intracellular folate retention. In addition to maintaining intracellular folate retention, high pH can also increase the ability of LAB to synthesize folate. This is because the enzymes involved in folate biosynthesis in various microorganisms have optimum activity at higher pH which is between 7.3 and 9.5. Thus, the activity of these enzymes in synthesizing folate can be increased by increasing external pH which can create more alkaline cell cytosolic conditions.^{32,35} However, even though low media pH can increase extracellular folate secretion in certain LAB species, this condition will lead to a decrease in extracellular folate concentration. This is due to the fact that extracellular folate has an unstable form so that acidic pH in the media will cause folate destruction reactions. This shows that the acidity of pH does not positively correlate with folate production by LAB.^{35,59}

The ability of LAB to synthesize folate is also associated with the role of 3 main genes namely *folB*, *folK*, and *folP*. These genes can encode the formation of dihydroneopterin aldolase (DHNA), hydroxymethylpterin pyrophosphokinase (HPPK), and dihydropteroyl synthase (DHPS), which are enzymes involved in the formation of THF molecules in de novo folate biosynthesis.¹⁹ Almost all bacteria with *folK* and *folP* genes are assumed to have an ability in synthesizing folate. However, the detection of these genes is not sufficient to estimate its capacity in folate production.¹²

Effect of Precursors on Folate Production by LAB

In addition to having certain genes, the ability of LAB to produce folate is associated with their ability to synthesize de novo PABA, formed through the shikimate pathway and the conversion of chorismate to PABA. In general, *Lactobacillus* (except *Lactobacillus plantarum*) strain can not synthesize de novo PABA hence its ability in producing folate is highly dependent on the availability of PABA in the media,⁷ or instead will consume folate available in the media for its growth.³² The *L. lactis* and *S. thermophilus* are reported to be able to produce folate, accumulate folate in cells, and secrete it into the media. Both strains can produce folate without PABA supplementation because they have all the genes needed in the shikimate pathway and the conversion of chorismate into PABA.^{7,20,21,57}

The production of extracellular folate by LAB such as *L. lactis* can be increased by adding PABA as a folate precursor. The high

amount of PABA can inactivate folypolyglutamate synthetase, which functions in the extension of the polyglutamic tail in folate molecules. When the amount of PABA in the media is increased, the extension of the polyglutamic tail on the folate molecule in the cell becomes inhibited. As a result, monoglutamate folate production elevates. Monoglutamate folate is known to have a lower affinity for most folate-dependent enzymes, compared with polyglutamate folate, therefore the retention of monoglutamate folate in cells will decrease. Low folate retention in cells will raise the amount of folate secreted to the media. In other words, adding PABA in the media will promote extracellular folate production, being higher than intracellular folate.^{20,32,57,60}

The inactivation enzyme mechanism of folypolyglutamate synthetase is also supported by the results of a study,⁵⁷ where metabolic engineering of overproduction of PABA in folate-producing strain of *L. lactis* does not result in an increase in total folate synthesized but causes changes in the distribution of folate across the cytoplasmic membrane. The intracellular folate concentration is measured to be relatively lower than extracellular folate secreted to the media.

In addition to PABA, in folate biosynthesis, glutamate as a folate precursor is also needed along with dihydropteroyl for the synthesis of DHF, further converted to THF. However, most LAB are reported to be auxotrophic of glutamate, hence its requirement has to be fulfilled by its availability in the media. The availability of glutamate residue in the media also plays a role in the extension of the polyglutamate tail (THF-polyglutamate) which has high intracellular retention. Therefore, the more glutamate available, the higher intracellular folate is produced and the lower extracellular folate secreted.⁵⁷ However, by the addition of glutamate in a certain concentration (75 $\mu\text{mol/L}$) in the media, extracellular folate produced by *L. lactis* becomes doubled when fermented for 8 hours incubation at 37°C in milk media.²⁰ No literature has reported the mechanism of glutamate in increasing extracellular folate production. Unlike PABA and glutamate, the addition of purine bases in the media (i.e. adenine, guanine, and xanthine), needed for the synthesis of GTP as a folate precursor, can support the growth of LAB but does not affect the folate biosynthesis. Essentially, folate biosynthesis is carried out to meet the growth needs of the bacteria itself. However, the availability of purines in the media can directly replace the role of folate for growth, thus bacteria automatically do not synthesize folate in their cells.⁵⁷

Folate Production in Fermented Milk Products

Milk is an ideal source of essential nutrients for humans, both children, and adults, for the body's development and maintenance. Milk contains enzymes, proteins (casein, serum proteins, and vitamin-carrying proteins), various minerals (calcium, nickel, selenium, zinc, and iron), various vitamins (vitamin A, riboflavin, niacin, folate, and vitamin C), immunomodulatory, and antimicrobial compounds such as immunoglobulins, lactoperoxidase, and lactotransferrin.⁶¹ Because of its complex nutritional content, milk has become an important part of daily nutritional intake throughout the globe.⁵¹ Cow's milk and goat's milk are the most popular

and widely consumed milk. Cow's milk and goat's milk have different contents of protein, fat, and enzyme which affects the physical and sensory characteristics of the dairy products. Goat milk produces softer curds, higher amounts of small globular fat, and lower allergen properties. Regarding nutrition, cow's milk and goat's milk contain various nutritional components such as protein, calcium, niacin, pantothenic acid, phosphorus, potassium, riboflavin, thiamin, and vitamin A, which are sufficient for the human diet, even in different concentrations. In addition, both cow's milk and goat's milk are not a good source of iron, vitamin C and D, unless fortified. However, unlike cow's milk, goat's milk contains insufficient levels of vitamin B6, B12, and folate which are not suitable for the growth and development of infants and children. In fact, in 1970, megaloblastic anemia was reported in children exclusively consuming goat's milk, caused by a lack of folic acid and vitamin B12,⁶² therefore, folate fortification was widely applied to goat milk products.⁶³ Cow's milk contains a small amount of folate ranging from 20-60 $\mu\text{g L}^{-1}$,⁵⁸ while goat's milk contains smaller, which is 2-11 $\mu\text{g L}^{-1}$.⁶⁴ The folate content will be reduced or lost due to the UHT sterilization or heating process.^{25,36} In contrast, both types of milk are reported to contain folate-binding proteins which can increase the stability of folate during storage. Therefore, milk is considered as the most suitable and ideal food medium for folate fortification.^{22,51,65}

Milk and dairy products provide 10-15% of daily folate intake. Some fermented milk products contain a greater amount of folate as a result of its synthesis by LAB during the fermentation process.⁶ However, in some other fermented milk products, LAB could not synthesize folate hence their presence can actually reduce folate levels because they consume available folate for their growth. The ability of LAB to synthesize folate is strain-dependent.^{12,18,24,42} In addition, fermentation conditions such as temperature and incubation time could also affect the production of folate by LAB.²⁰ Moreover, *S. thermophilus* is reported to

produce maximum folate level at 40-42°C after 6 hours of fermentation,^{19,51} while the highest folate level synthesized by *Lactobacillus helveticus* was achieved at 37°C for 18 hours of fermentation.⁵¹ *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Bifidobacterium adolescentis*, and *Bifidobacterium catenulatum* produced folate at the highest amount at 37°C for 10 hours fermentation.³⁵ The maximum folate levels synthesized by different LAB in fermented milk products at various temperatures and incubation times are presented in Table 3.

Conclusions

Increasing folate concentration in fermented milk products using folate producing LAB can be an alternative in producing natural folate-rich products as an effort to prevent folate deficiency without prompting the side effects. Milk is considered as the best medium for folate production because of its complex nutritional content and the presence of folate binding proteins that can increase the stability of folate. Increasing folate content in fermented milk depends on the LAB used as a starter culture to produce extracellular folate which secreted to the media. The ability to synthesize folate is a special characteristic of the strains of LAB, hence the selection of folate-producing strains is critical to obtain LAB that are able to synthesize folate. Folate production by LAB is affected by temperature, incubation time, medium composition and the availability of folate precursors such as PABA and glutamate. Therefore, the technological approach to improve the nutritional value of fermented milk has to include optimizing conditions along with a substrate for fermentation.

Authors' Contributions

All authors equally contributed to the current study.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

Table 3. The Maximum Level of Folate Synthesized by Certain LAB in Fermented Milk Products at Various Temperatures and Incubation Times

Substrate	Bacterial Species	Incubation Temperature (°C)	Incubation Time (h)	Maximum Folate Level (ng mL ⁻¹)	Literature
Reconstituted non-fat dry milk	<i>S. thermophilus</i>	37	6	46.7-59.6	25
	<i>Lactobacillus acidophilus</i>			53.9-63.9	
	<i>Bifidobacterium longum</i>			75.8-99.2	
	<i>Lactobacillus bulgaricus</i>			62.8-68.5	
UHT milk with 1.5% fat	<i>S. thermophilus</i>	37	12	36.9	33
Skim milk (5%)	<i>Lactobacillus lactis</i> ssp. <i>cremoris</i>	37	7	12.5	34
	<i>Lactobacillus lactis</i> ssp. <i>lactis</i>			14.2	
Reconstituted non-fat-dry milk (12%)	<i>Lactobacillus plantarum</i>	37	10	25.3	35
	<i>Lactobacillus delbrueckii</i>			110.1	
	<i>Bifidobacterium adolescentis</i>			8.3	
	<i>Bifidobacterium catenulatum</i>			19.4	
Reconstituted non-fat milk (10%)	<i>S. thermophilus</i>	40	6	47	51
	<i>Lactobacillus helveticus</i>	37	18	42	
Skim milk	<i>S. thermophilus</i>	42	6	20-80	19

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