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Phosphorus Nutrition and Health: Utilization of Phytase-producing Bifidobacteria in Food Industry

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Abstract

Phosphorus plays vital roles in human health and nutrition. In nature, phosphorus exists as phosphate, either inorganic or organic. The major form of phosphate in plant-derived diets is phytate that cannot be degraded by monogastric animal, as well as humans. Initially, this chapter reviews current research of phosphorus in human nutrition and health. Subsequently, problems of phytate degradation and phosphorus utilization in plant-derived diet are outlined. Next, as the main part, the enzymes of phytase, which catalyze the release of inorganic phosphorus from phytate, are compared, especially those produced by gut microbiota. Meanwhile, how probiotic bifidobacteria can be used for producing phytase and therefore enhance their beneficial effects are discussed. Phytase-producing bifidobacteria can be either isolated rarely in nature or constructed by genetic cloning of phytase genes from other well-characterized enzymes. The combination of bifidobacteria and highly active phytase may improve human health and nutrition especially as supplementary probiotic foods. Therefore, potential application is prospected. Finally, other considerations related to industrial production and usage of phosphorus-enriched additives are remarked. In conclusion, improving and maintaining the phosphorus balance in food by bifidobacteria may be promising for a healthier life.

Keywords: bifidobacteria, phytate, phytase, phosphorus, nutrition

1. Introduction

Phosphorus is an essential nutrient for the body and is routinely consumed through food. After consumption, phosphorus is usually bound with oxygen and exists as phosphate in the body. Both organic and inorganic forms of phosphate are present in regularly consumed foods. The

amount of total phosphate ingestion can be significantly influenced by processed food. Following a meal, inorganic phosphate can be rapidly absorbed across the small intestine and enter the bloodstream causing an elevation in serum phosphate levels. An increase in serum levels of inorganic phosphate usually reduces serum levels of ionic calcium by forming a calcium phosphate complex. High ratio of phosphate to calcium usually leads to hypophosphatemia. In contrast, dietary phosphate deficiency, mostly due to malnutrition, can also impair the bone mineralization process and eventually lead to the development of rickets [1]. Nevertheless, phosphorus homeostasis is important for versatile functions, especially skeletal growth, development, and maintenance [2].

Despite the essential role of phosphate in living cells and wide application of phosphate additives in kinds of food, humans cannot efficiently digest plant-derived phosphate, namely, phytate that is the main form in both cereals and vegetables [3]. Degradation of phytate is catalyzed by phytases, which are predominately presented on bacteria and fungi. Bifidobacteria are the most frequently used microbial supplements in functional foods and probiotic formulations [4]. Probiotics have many beneficial effects in human intestine [5]. Phytase activity was detected in a few species of bifidobacteria [6,7]. Furthermore, heterologous secretion of phytases cloned from other bacteria was reported in bifidobacteria as well [8]. These strains can be used in fermented foods for conversion of poorly digestible phytate enriched in plant materials, which serves an alternative approach for dietary phosphorus supplementation in humans, especially those health-compromised individuals.

2. Role of phosphorus in human nutrition and health

2.1. General biochemistry and distribution of phosphorus

In biological systems, phosphorus involves in many important reactions, including forming cell membrane and nucleic acids, generation of ATP, cell signaling through protein phosphorylation or dephosphorylation, urinary buffering, and bone mineralization. In addition, phosphorus widely takes part in biochemical reactions, e.g., glucose and triacylglycerol (triglyceride) utilize phosphate to synthesize glucose 6-phosphate and glycerol 3-phosphate respectively. Phosphorus is the sixth abundant element in the human body and comprises approximately 1% of total body weight [9]. In mammals, phosphorus is presented as phosphate, which is a predominantly intracellular anion. There is 85% phosphate in bone and teeth, 14% in other tissues, and 1% in extracellular fluid.

Under steady state, a regular Western diet provides approximately 20 mg/kg/day of phosphorus [10]. Around 16 mg/kg/day is absorbed in the proximal intestine, mainly in the jejunum. The normal range of serum phosphate concentration is 4.5–8.3 mg/dl and higher in infants who require more of the mineral for bone growth and soft tissue buildup [11]. At zero metabolic balance, about 13 mg/kg/day phosphorus is excreted in the urine in adults. Thus, under phosphate equilibrium state and normal renal function, the amount of urine phosphorus can be an indicator of the amount absorbed in the intestine [12]. Reasonably, phosphate absorp-

tion and reabsorption decline along human aging, respectively, in both the intestinal tract and kidney. Meanwhile, expression of sodium-phosphate co-transporters decreases [13].

2.2. Phosphorus for nutrition and health

Phosphorus can be supplied in two forms, namely, organic phosphate and inorganic phosphate. Inorganic phosphate additives have greater bioavailability than organic sources of phosphorus that are the main form of phosphate in plant-derived foods. Phosphorus serves vital roles in the human body and is essential component of nutrient. It is crucial for bone growth and mineralization. Both bench and clinical researches show that phosphate is one of the major factors in the maintenance of bone health, and its deficiency results in bone pathology and clinical illness, such as rickets and osteomalacia [14]. Inorganic phosphorus is one of the two main ionic components required for hydroxyapatite formation during the mineralization of the extracellular matrix [15].

Roughly, 80–90% of the mineral content of bone is calcium and phosphorus, and 85% of the phosphorus is in the skeleton. Adequate phosphorus intake is essential for skeletal mineralization. Although calcium plays an important role in regulating chondrocyte maturation, apoptosis of hypertrophic chondrocytes is dependent upon circulating phosphate at normal levels [16]. Diets high in phosphorus often lead to diminished intestinal calcium absorption, reducing serum calcium concentration, and stimulating parathyroid hormone (PTH) secretion [17]. Phosphorus also directly regulates the production of 1,25(OH)₂D by kidney cells. Furthermore, phosphorus is considered to be a major dietary source of acid [18].

2.3. Phosphate homeostasis and health

As more than 2000 chemical reactions in living cells use phosphate, optimal phosphate balance is essential for effective regulation. Generally, phosphate homeostasis is determined by both intestinal absorption from consumed food and renal excretion of the serum phosphate. Sodium-dependent phosphate (NaPi) transporters actively regulate the intestinal phosphate absorption and partially mediate renal phosphate excretion and reabsorption as well. Parathyroid hormone (PTH) facilitates urinary phosphate excretion because of strong inhibition of NaPi transporters function [10]. Dietary phosphate restriction induces an adaptive increase of intestinal phosphate uptake, and prolonged restriction increases NaPi-2a activity, thereby attempting to restore the balance by increasing kidney phosphate reabsorption [19].

The maintenance of optimal phosphate balance is managed by complex interactions between the gut, kidney, and bone, as well as “phosphatonins” involving multiple regulators. More precisely, the duodenum and jejunum are responsible for phosphorus absorption in the diet via both passive diffusion and active sodium-dependent transportation [20]. The kidney is the major organ involved in the regulation of phosphate homeostasis. A variety of factors along the proximal convoluted and straight tubule of the kidney, including serum PTH, calcium, 1,25(OH)₂D₃, and bicarbonate concentrations, take part in the regulation of phosphate. In animals with intact parathyroid glands, the phosphate concentration in the proxi-

mal tubules is 70% of the plasma level. There is little phosphate reabsorption in the proximal straight tubule in the presence of PTH. However, in the absence of PTH, phosphate is avidly reabsorbed along the proximal straight tubule. As previously reported, phosphate renal losses were enhanced by increasing fibroblast growth factor 23 (FGF-23).

2.4. Phosphate toxicity

Excessive retention of phosphate in the body is toxic to humans and can cause a wide range of cellular and tissue injuries; partial toxicities are shown in **Figure 1** [21]. Common toxicity of phosphate in humans includes impaired renal function, rhabdomyolysis, and tumor lysis syndrome [22]. Occasionally, exogenous phosphate toxicity is also documented in patients when exposed to hypertonic phosphate enemas [23]. Horribly, excessive exogenous phosphate administration can be fatal though the lethal dose in humans is unknown [24]. Overall, it is convincingly demonstrated that phosphate accelerates various pathologies. Acute toxicity can provoke hypocalcemia and associated symptoms including tetany, hypotension, and tachycardia. Moderate toxicity leads to deposition of calcium phosphate crystals, including often fatal cardiovascular calcification that usually irreversible. For instance, phosphate toxicity has been implicated as independent risk factor for high mortality in chronic kidney disease patients [25]. In an animal study, a 7–20-fold higher commercial phosphate-containing enema induced 100% mortality [26]. In another study, the *NaPi2a/klotho* double-knock-out mice lost their fertility when fed with a high-phosphate diet [27]. Phosphate toxicity can induce an increased rate of apoptosis in various tissues. It has been found that phosphate toxicity accelerates the mammalian aging process by inflicting tissue damage and reducing survival as well [28]. Meanwhile, several studies reported links between high dietary inorganic phosphate intake and cancer development [17,29], as well as bone health problems [10, 30].

Figure 1. Partial list of pathological events related to phosphate toxicity composed by Razzaque [21] from related literatures in both human and animal studies.

3. Phytate in plant-derived diets

Phytate, the salt form of phytic acid, represents 60–80% of total phosphorus in plant seeds that can be hydrolyzed by phytase. Even milk and its related products are the richest phosphate sources in human diet; the major sources of phosphate in all natural foods are protein-rich foods and cereal grains. However, humans do not encode genes for phytase and hence can poorly digest phytate in plant-derived diets. Lacking of phytase causes three major problems in simple-stomached animals as well as humans: (1) environmental pollution from manure phosphorus, (2) dietary addition of inorganic phosphorus, and (3) depletion of rock phosphorus deposits. For instance, in a crossover trial with chronic kidney disease-suffering patients, the fasting serum phosphorus concentration was lower after the vegetarian diet than after the meat diet that contained identical phosphorus. More notably, secretion of plasma FGF-23 was about 40% lower in subjects treated with vegetarian diet after one week [31].

Phosphate interacts with several dietary minerals, such as calcium, sodium, and magnesium. Therefore, deficiency of these minerals is more common than deficiency of phosphate when its bioavailability is low. It was noted that the phosphorus bioavailability of natural foods is variable. Particularly, the bioavailability of phosphorus in phosphate-rich plant foods such as whole grains, legumes, peas, nuts, and seeds is relatively low, because a high proportion of it is tied up in poorly absorbed phytates. Considering many studies link high phosphorus intakes that are mainly supplied by inorganic phosphate additives to increased morbidity and mortality, natural plant foods may favorite health outcomes as their relatively low bioavailability of phosphorus.

4. Phytases and gut microbiota

Phytases (myoinositol hexakisphosphate phosphohydrolase) are enzymes that catalyze the stepwise removal of phosphates from phytic acid (myoinositol hexakisphosphate) or its salt phytate. Until now, a plenty of phytases were discovered and they show different catalytic mechanisms. The first and most extensively studied group of phytases, such as *Escherichia coli* AppA, belongs to the class of histidine acid phosphatases (HAPs) [32]. The other three groups of phytase are classified as b-propeller phosphatase (BPP; also referred to as alkaline phytase, exemplified by *Bacillus amyloliquefaciens* phytase) [33], protein tyrosine phosphatase (PTP; also referred to as dual-specificity phosphatase) [34], and purple acid phosphatase (PAP; metalloenzymes) [35]. Corresponding three-dimensional structures and catalytic sites of these phytases are created using protein sequences. As shown in **Figure 2**, they have different secondary structures together with different active sites [36]. The currently known distribution of different types of phytases had been summarized in a previous review [3]. Among them,

the majority are encoded by bacteria and fungi even a few species of animal and plant possess phytase activity as well.

Figure 2. Secondary structure structures of representatives of each of the four structural classes of phytases. (A) Histidine acid phytase (HAPhy), (B) b-propeller phytase (BPPhy), (C) protein tyrosine phytase (PTPhy), and (D) purple acid phytase (PAPhy). Pictures are adapted from the work done by Lei et al. [3].

Phylum	Number	Class
Proteobacteria	124	a-Proteobacteria (57) b-Proteobacteria (4) g-Proteobacteria (61)
Actinobacteria	39	Streptomycetales (20) Micromonosporales (11) Corynebacteriales (4) Pseudonocardiales (3) Bifidobacteriales (1)
Firmicutes	18	Bacillales (9) Clostridiales (8) Lactobacillales (1)
CFB group bacteria	7	Flavobacteriales (5)
Cyanobacteria	6	Gloeobacterales (4)
GNS bacteria	2	
Dictyoglomales	2	

BBPR_1292, which is annotated as lipoprotein in *Bifidobacterium bifidum* PRL2010, has two conserved domains that are phytase-like or esterase-like (pfam13449) and NHL repeat unit of beta-propeller proteins (cl18310).

Table 1. Existence of phytase genes in bacteria.

The gut, especially the jejunum, is the most active site, responsible for the absorption of two thirds of phosphate intake in humans. However, as mentioned above, gut cannot absorb

organic phosphorus presented as phytate in plant-derived diets. We know that human gut consists of a complex community of microorganisms, namely, gut microbiota. One main role of gut microorganisms is they benefit the host by fermentation of human readily undigested substrates to absorbable nutrients. Some gut microorganisms produce kinds of enzymes and many of these enzymes are deficient in host, thereby symbiotic relationship is developed. Such a case is phytase producing microbe, like *Escherichia coli*, *Streptomyces coelicolor*, *Clostridium* spp., and so on. Search of “phytase” in the NCBI gene database yielded 198 genes annotated as bacterial phytase. A detailed presence of phytase genes in bacteria is demonstrated in **Table 1**. Among them, Proteobacteria and Actinobacteria are the most predominating groups that are also natural habitants of human gut.

5. Phytate degradation by bifidobacteria

5.1. Phytase-encoding genes

As shown in **Table 1**, there is only one gene in *B. bifidum* PRL2010 that was annotated as possible phytase-encoding gene. Meanwhile, two enzymes in bifidobacteria, exactly *B. pseudocatenulatum* ATCC 27919 and *B. longum* subsp. *infantis* ATCC 15697 with phytase activity, have been characterized [37]. Therefore, protein sequences of these three enzymes (BBPR_1292, BIFPSEUDO_03792, and BLON_0263) were used for searching homologues in *Bifidobacterium* (taxid: 1678).

Organism	Protein name	Accession	Locus_tag	Length (aa)
<i>B. dentium</i> ATCC 27678	Histidine acid phosphatase	WP_003838654	BIFDEN_01159	637
<i>B. dentium</i> ATCC 27679	Histidine acid phosphatase	WP_003843340	HMPREF0168_2166	631
<i>B. dentium</i> Bd1	Histidine acid phosphatase	WP_012902513	BDP_1985	643
<i>B. longum</i> DJO10A	Histidine acid phosphatase	WP_010081042	Blon03000750	617
<i>B. longum</i> DJO10A	Histidine acid phosphatase	WP_012472023	BLD_1202	622
<i>B. longum</i> NCC2705	Histidine acid phosphatase	WP_011068470	BL0400	606
<i>B. longum</i> subsp. <i>infantis</i> 157F	Histidine acid phosphatase	WP_015713264	BLIF_0216	622
<i>B. longum</i> subsp. <i>infantis</i> ATCC 15697=JCM 1222	Histidine acid phosphatase	WP_012576702	Blon_0263	623

Organism	Protein name	Accession	Locus_tag	Length (aa)
<i>B. longum</i> subsp. <i>infantis</i> ATCC 15697=JCM 1222	Histidine acid phosphatase	WP_014484530	BLIJ_0267	618
<i>B. longum</i> subsp. <i>infantis</i> CCUG 52486	Histidine acid phosphatase	WP_007051528	BLIG_00414	617
<i>B. longum</i> subsp. <i>longum</i> 35B	Histidine acid phosphatase	WP_007057720	HMPREF1314_0451	572
<i>B. longum</i> subsp. <i>longum</i> 44B	Histidine acid phosphatase	WP_007056476	HMPREF1312_1349	617
<i>B. longum</i> subsp. <i>longum</i> BBMN68	Histidine acid phosphatase	WP_013410389	BBMN68_1139	622
<i>B. longum</i> subsp. <i>longum</i> F8	Histidine acid phosphatase	WP_015512490	BIL_17170	617
<i>B. longum</i> subsp. <i>longum</i> JCM 1217	Histidine acid phosphatase	WP_007054753	BLLJ_0234	617
<i>B. longum</i> subsp. <i>longum</i> KACC 91563	Histidine acid phosphatase	WP_014485906	BLNIAS_02473	617
<i>B. pseudocatenulatum</i> DSM 20438=JCM 1200	Histidine acid phosphatase	WP_004222312	BIFPSEUDO_03792	639
<i>B. sp.</i> 12_1_47BFAA	Histidine acid phosphatase	WP_008783259	HMPREF0177_01170	561

Table 2. Protein list of histidine acid phosphatase in *Bifidobacterium* sp.*.

Available from http://www.ncbi.nlm.nih.gov/proteinclusters/?term=BIFPSEUDO_03792.

BLAST searches revealed that (1) BBPR_1292-like proteins are exclusively presented on the genomes of all *B. bifidum* strains with at least 99% identity and (2) BIFPSEUDO_03792 and BLON_0263 are presented in a few strains of *Bifidobacterium*. Though there is the presence of BBPR_1292-like proteins, there is no specific phytase activity that had been detected in *B. bifidum*. Based on sequence comparisons, these two characterized enzymes are more close to the phytases of plants, fungi, and vertebrates. However, in the protein clusters database of histidine acid phosphatase (PCLA_3557679), there are 18 proteins that belong to 16 *bifidobacteria* strains as listed in **Table 2**. Notably, all these predicted phytases belong to *B. dentium*, *B. longum*, and *B. pseudocatenulatum*. Nevertheless, phytase activity has been detected in some *Bifidobacterium* sp. even it is not a common metabolic feature.

5.2. Phytase enzyme activities

Initially, it was believed that bifidobacteria are phytase negative, as very low level activity may be because of unspecific release by phosphatase, except *B. pseudocatenulatum* ATCC 27919 [6].

To further evaluate the enzyme activities, five strains of different bifidobacterial species, i.e., *B. animalis*, *B. bifidum*, *B. infantis*, *B. longum*, and *B. pseudolongum*, were inoculated to degrade myoinositol hexaphosphate (InsP(6)). In a complex medium in which phytic acid was the only source of phosphorus, *B. infantis* ATCC 15697 showed the highest level of phytate-degrading activity. The optimal condition is at slight acid pH (6.0–6.5) and higher temperature (50°C). Maximum activity appears at the stationary phase of growth and when 1% lactose was used as carbon source [7]. The same research team compared phosphatase and phytase activities of 23 bifidobacterial strains (13 from infants and 10 from adults) belonging to three different species (*B. longum*, *B. breve*, and *B. catenulatum*). The highest phytate-degrading activity is displayed in *B. longum* BIF307, similar to previous comparison in which is *B. infantis*, a subspecies of *B. longum* has the highest phytase activity.

Although two novel phytases from *B. pseudocatenulatum* and *B. longum* subsp. *infantis* had been characterized, parallel comparison of bifidobacterial phytase activities to *E. coli* AppA is difficult, as they were expressed as relative percentage activity. Nevertheless, the enzymes that belong to a new subclass are highly specific for the hydrolysis of phytate and render myoinositol triphosphate as the final hydrolysis product [37]. From our experience, native phytase activity in bifidobacteria is extremely lower than commercial enzymes. Therefore, we constructed a series of recombinant *B. bifidum* S17 that can secrete heterologous AppA within high specific activity to phytate; even our primary aim is using appA as a suitable secretion reporter [8]. Among these constructs, *B. bifidum* S17/pMgapS6P using the GAP promoter and BBIF_1761 signal direct the most efficient phytase secretion (**Figure 3**).

Figure 3. Phytase activity in spent cell-free medium of recombinant *B. bifidum* S17 strains. *B. bifidum* S17-harboring pMgapP-derived plasmids containing different SPs (S0–S6) were grown in 5 ml reinforced clostridia medium under anaerobic condition. The control plasmid pMgapP (–) contains no SP and serves as a background control for expression of a nonsecreted phytase. Values are relative phytase units (RPU) per ml supernatant and are mean $\pm$ standard deviation of three independent cultures measured in technical triplicates. The figure is adapted from Osswald et al. [8].

6. Potential application of phytase-producing bifidobacteria in foods

Despite phytase's main application in animal feeds, its applications in human foods can be equally important, if not exceed. To a large extent, using phytase in human foods is not primarily the target to improve phosphorus consumption, because depletion of phytate is more important as it chelates essential minerals, including iron, zinc, and calcium, contributing to deficiencies of these nutrients. It was estimated that there are approximately two to three billion people around the world suffering from mineral deficiency. The application of phytase in human health may be more exciting but need further in-depth study of potential adverse effects. Because certain inositol phosphates are beneficial to human health, phytase and phytase-producing cells can be immobilized as cost-effective bioreactors for large-scale production of these compounds [39]. There are many successful attempts to use phytase in brewing, baking, and dephytination of soy milk [25].

In fact, application of phytase or phytase-producing bacteria in food has already been illustrated. For example, *B. pseudocatenulatum* ATCC 27919 was tested as a starter in sourdough for the production of whole rye-wheat mixed bread [40]. In situ production of phytase during fermentation by probiotics results higher mineral availability in breads. The ability of *B. infantis* ATCC 15697 to degrade InsP(6) and accumulate InsP(3) could contribute to the reduction of the anti-nutritional properties of InsP(6) and generation of intermediate compounds with beneficial properties. *B. longum* BIF307, another phytase producer, was used in whole wheat bread making and decreased InsP(6) content. In another study, phytase-producing bifidobacterial strains significantly reduced the InsP(6) + InsP(5) concentrations compared to control samples during the bread-making process. Meanwhile, dialyzable Fe contents were increased from 2.3- to 5.6-folds. However, the effects appeared to be still insufficient to improve Fe bioavailability in Caco-2 cells [41]. Anyway, with similar technological and sensorial quality, levels of InsP(6) are significantly lower in bifidobacteria-fermented bread. Collectively, probiotic bifidobacteria are particularly suitable to reduce the content of InsP(6) in rich fiber products for human consumption [42].

7. Advantages of using phytase-producing bifidobacteria

One of the most important advantages of using phytase-producing bifidobacteria is safety. As widely known, several species of bifidobacteria are generally regarded as safe (GRAS) or qualified presumption of safety (QPS). The GRAS status made these strains particularly attractive for application in both food and pharmaceutical industries. Currently, probiotic bifidobacteria are widely used as micro-ecological reagent in many countries. These micro-ecological reagents had been added into both foods and pharmaceuticals without additional toxicity test.

Secondly, as important as the safety issue, many beneficial effects of bifidobacteria made them promising in industry especially ameliorating gastrointestinal disorders (both bacterial- and viral-induced gastroenteritis), allergic diseases, antibiotic-associated diarrhea, lactose

intolerance, constipation, and irritable bowel disease. Let alone increasing iron accessibility, phytase-producing bifidobacteria has expanded nutrition profile [41,42]. In the gut of human eating plant-derived diets, phytase-producing bifidobacteria can degrade phytate-based components, therefore improving their adaptability or cross-feeding other symbiotic inhabitants in the same niche.

Thirdly, intake of phytase-producing bifidobacteria is superior to eating inorganic phosphate additives for human. In one aspect, phosphorus homeostasis can be easily disturbed after eating external inorganic phosphate additives. In another aspect, phytase-producing bifidobacteria can improve organic phytate-originated phosphorus. Thereby, supplementation of external inorganic phosphate additives becomes unnecessary. This is particularly significant for avoiding excessive phosphate, resulting in different kinds of toxicities that are largely caused by phosphate-containing additives in foods and drinks. For example, phosphorus-based food additives may pose high risk in people suffering chronic kidney disease, as this made dietary management of hyperphosphatemia practically difficult. In dialysis patients, managing hyperphosphatemia may require using phosphate binder other than restricting protein intake as this allows patients to eat more protein-rich foods [43]. In addition, a study evaluated 93 premature infants with a mean gestational age of 27.5 ± 2.0 weeks. The result demonstrated that elevated serum phosphorus was inversely correlated to the day of life of the infant after receiving human milk-derived fortifier though the incidence of hyperphosphatemia was mild and transient in this population [44]. For those health promised people, intake of phytase-producing bifidobacteria supplied a novel interventional approach.

Lastly but not least, bifidobacteria can produce phytase in human gut as microbial cell factories. They can be ingested as live cells and then colonized in the intestine to facilitate the degradation of plant-derived diets. The relatively constant replication of bifidobacteria in human intestine can either enlarge the bioavailability of organic phytate or downsize the toxicity of excessive phosphorus, hence maintaining the balance of phosphorus in a long term.

8. Final remarks

Phosphorus salts are added to foods as additives in many countries. Thus, dietary intake of phosphorus is higher than the recommended daily allowance in these countries and populations. For instance, phosphorus additives were particularly common in the categories of small goods, bakery goods, frozen meals, and biscuits in Australia [45]. In the United States, it has been estimated that phosphorus additives may add as much as 1 g of phosphorus to the diet [46]. However, high phosphorus intake has been shown to inhibit the increase in serum $1,25(\text{OH})_2\text{D}$ concentration in response to low dietary calcium intake [30].

Collectively, although long-term and large amount consumption of phosphorus additives, little is known about risk associated with dietary phosphorus intake. A prospective cohort study of healthy adults reveals that high dosage of dietary phosphorus intake is associated with increased mortality [47]. Considering that the deleterious effects of chronic ingestion of unrestricted amounts of phosphate in individuals are not clear, consumption of high phos-

phate-containing processed foods and soft drinks should be alarming, particularly for health-compromised individuals [48,49]. Under those circumstances as mentioned above, using phytase-producing bifidobacteria may be a new way for increasing bioavailability of phosphorus from plant-derived diets, therefore avoiding supplementation of inorganic additives.

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