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Review Article

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Exploring novel horizons - Bacterial phytases and their potential applications

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Abstract

The aims of this review are to focus on updating the current knowledge regarding the diversity of bacterial phytases, their importance in increasing the availability of phosphorus and other nutrients necessary for the growth and development of various plants and animals, and their roles in maintaining environmental sustainability. Phytases, enzymes that catalyze the hydrolysis of phytic acid, play a major role in various biotechnological processes; especially in agriculture. Among the diverse sources of phytases as prokaryotic and eukaryotic organisms, bacterial phytases have gained considerable attention due to their specific characteristics, potentials for genetic manipulation, and various biotechnological and industrial applications. Aerobic and anaerobic bacteria with phytase activity have been isolated from diverse ecological niches, including soils, fermented foods, plant rhizospheres, and manure. Additionally, probiotic bacteria, essential for maintaining a healthy microbiota, have been shown to produce phytase, suggesting their potential applications in animal and plant growth, human nutrition, and food and feed industry.

Keywords: Phytate, Phytase, Bacteria, Plant growth, Animal nutrition

1. Introduction

Phytate (inositol hexakisphosphate, IP6) is a salt of magnesium, calcium, or potassium, and/or an ester of phytic acid, composed of an inositol ring and 6 ester phosphate groups. Due to its pronounced negative charge, phytic acid forms chelates and complexes with divalent or trivalent metal cations and with proteins and enzymes, disrupting their activity. Because of these characteristics, phytic acid is considered as an antinutrient (López-Moreno *et al*., 2022). The primary phosphorus repository in plant-based foods is phytic acid. Oilseeds, legumes, and grains contain approximately 1-5 % of their weight as phytic acid, thereby impacting their nutritional value (Singh *et al*., 2020). Phytate is a vital dietary ingredient present in a range of edible plant-based foods, including seeds, legumes, nuts, and whole grains, contributing significantly to our overall nutrition and well-being. It commonly exists in these foods as calcium or magnesium salt with major sources containing 0.5 % to 3 % of dry weight as phytate. Other types of inositol phosphates such as inositol pentaphosphates and inositol tetraphosphates are present in smaller amounts, making up fewer than 15 % of all inositol phosphates in plant-based foods (Widderich *et al*., 2024). Early physiological studies labeled phytate as an anti-nutrient due to its hindrance of trace element absorption; specifically zinc and iron (III), by forming insoluble compounds with them. This inhibitory impact is most noticeable when phytate is consumed in significant quantities alongside with imbalanced diets (Feizollahi *et al*., 2021). The objectives of this review are to focus on biology of bacterial phytases, their importance in increasing the phosphorus availability and other nutrients, and their potential applications.

2. Phytases

Phytases are a large group of enzymes that play a crucial role in the hydrolysis of phytic acid. Phytases catalyze the removal of phosphorus of phytic acid, releasing inorganic phosphate and myo-inositol. This process is often referred to as phytate hydrolysis or phytate degradation. The liberated inorganic phosphate becomes available for absorption, while myoinositol can be utilized by the various organisms. Phytases can be categorized based on different criteria (Fig. 1); mainly the first dephosphorylated carbon in the myoinositol ring, comprising carbon 3, 5, and 6, resulting in three subgroups known as 3-phytases, 5-phytases, and 6-phytases, respectively. Furthermore, classification can be based on catalytic mechanisms, with four subgroups identified as Histidine acid phytases (HAP), purple acid phosphatase (PAP), β-propeller phosphatase (BPP), and protein tyrosine phosphatase (PTP). Another criterion for classification is the optimal pH of activity, dividing the above mentioned subgroups into acid phytases (*i.e*., HAP, PAP, and PTP) and alkaline phytases (BPP) (Joshi and Satyanarayana, 2014).

Phytases exist in both eukaryotic and prokaryotic organisms. Sources of these enzymes have been identified in animals, distinguishing between phytase produced in the small intestine and microfloral phytase associated with the intestines of ruminants, and in blood of some birds and reptiles (Kumar and Sinha, 2018). Phytases have also been found in plants and microorganisms. Microorganisms are the greatest potential sources of phytases followed by plants. Among microorganisms, bacteria, yeasts, and mold fungi are well known as phytase producers. Phytase producing bacteria, which are capable of both aerobic and anaerobic metabolism, have been isolated from a variety of ecological environments, including soils, fermented foods, plant rhizospheres, and animal manure (Singh *et al*., 2020).

2.1. Phytases from different bacterial species

Because of their wide pH profile, resistance to proteolysis, high temperature stability, and specificity for phytate substrates, bacterial phytases have significant advantages over fungal phytases (Demir *et al*., 2017). Phytases have been found in many aerobic and anaerobic bacterial genera isolated from various natural habitats such as soils, plants rhizosphere, gastrointestinal tracts of monogastric animals, plants, and saline or freshwater bassins. As shown in Table (1), phytases are widespread in various bacterial genera and increasingly attract the attention of scientists. The search for probiotic bacteria that produce phytase is important because these microorganisms could enhance the availability and absorption of essential minerals in the digestive system, including phosphorus, calcium, and iron. In addition, phytase is crucial for phosphate utilization in monogastric animals and humans. Furthermore, these bacteria have a wide application for treatment of different diseases such as diarrhea, obesity, and urinary tract infections.

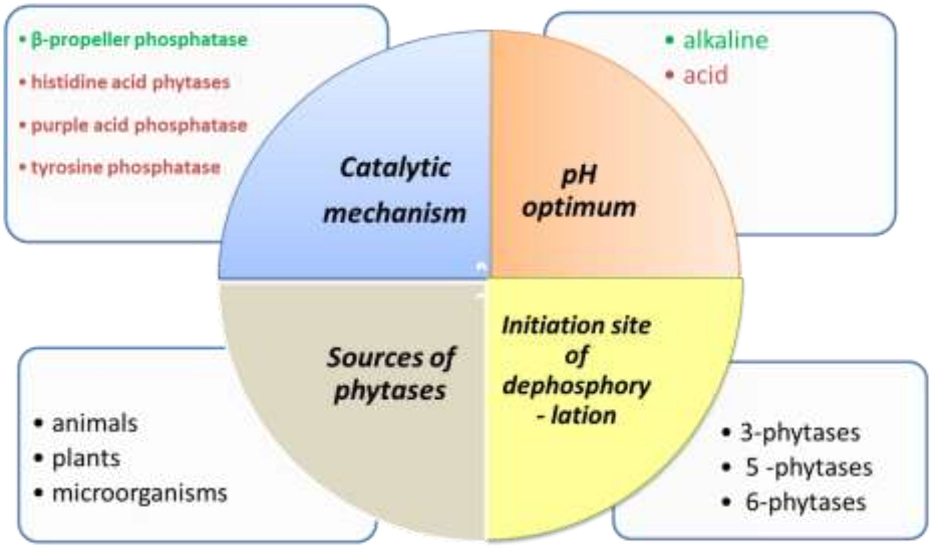

Fig. 1: Phytase classification based on different criteria

Table 1: Various genera of phytase-producing bacteria

Genus	References	Genus	References
Aeromonas sp.	Myung-JI *et al*., (2005)	*Micrococcus* sp.	Patki *et al*., (2015)
Bacillus sp.	Kumar *et al*., (2013); Khianngam *et al*., (2017); Trivedi *et al*., (2022)	*Mitsuokella* sp.	D'Silva *et al*., (2000); Tan *et al*., (2015)
Bifidobacterium sp.	Haros *et al*., (2005); García-Mantrana *et al*., (2014)	*Serratia* sp.	Kalsi *et al*., (2016)
Burkholderia sp.	Luang-In *et al*., (2021)	*Shigella* sp.	Roy *et al*., (2012)
Cyanobacteria sp.	Brasil *et al*., (2017)	*Sinomonas* sp.	Konietzny and Greiner, (2004)
Citrobacter sp.	Ebrahimian *et al*., (2017)	*Tetrathiobacter* sp.	Kumar *et al*., (2013)

Enterobacter sp.	Kalsi *et al*., (2016)	*Weissella* sp.	Demir *et al*., (2017); Mohammadi-Kouchesfahani *et al*., (2019)
Enterococcus sp.	Daodu *et al*., (2020)	*Selenomonas* sp.	D'Silva *et al*., (2000)
Erwinia sp.	Huang *et al*., (2009)	*Paenibacillus* sp.	Khianngam *et al*., (2017)
Esherichia sp.	Greiner and Farouk, (2007)	*Pantoea* sp.	Suleimanova *et al*., (2023)
Geobacillus sp.	Parhamfar *et al*., (2015); Dokuzparmak *et al*., (2017)	*Pseudomonas* sp.	Lin *et al*., (2023)
Kushneria sp.	Alori *et al*., (2017)	*Rhodococcus* sp.	Khan *et al*., (2011)
Klebsiella sp.	Greiner and Carlsson, (2006)	*Yersinia* sp.	Tan *et al*., (2015)
Lactobacillus sp.	Dikbaş *et al*., (2023)	*Streptococcus* sp.	Priyodip and Balaji, (2020)
Lactoccocos sp.	Sharma *et al*., (2020a)	*Raoultella* sp.	Konietzny and Greiner, (2004)

In a previous study, Priyodip *et al*., (2017) reported that fermented foods were sources of probiotics, and several bacterial spp. such as *Lactobacillus brevis* and *Bacillus subtilis* were capable of producing higher amounts of phytase. Intestinal bacterial isolates and probiotic species belonging to the genus *Bifidobacterium* (*i.e.*, *B. adolescentis*, *B. angulatum*, *B. globosum*, *B. longum*, and *B. pseudocatenulatum*) exhibit phytase activities with diverse catalytic and regulatory features. These bacterial species may play a role in breaking down phytic acid during both food processing and passage through the gastrointestinal tract. The presence of prebiotics such as fructooligosaccharides promotes and enhances the phytase activity of *B. pseudocatenulatum* and degradation of phytic acid (Haros *et al*., 2005). According to Yanke *et al*., (1998), phytase activity had been recorded by *Selenomonas ruminantium*, *Megasphaera elsdenii*, *Prevotella ruminicola*, *Mitsuokella multiacidus*, and *Treponema* sp. and other anaerobic ruminant bacteria. It is interesting to note that some strains of *Escherichia coli* produce phytases with industrially useful properties such as low optimum pH that is close to natural acidity of the animal stomach. However, distribution of phytase among the different strains of *Escherichia coli* may vary (Bandari *et al*., 2024).

It has been proven that some phytases are active at low temperatures, including those from *Erwinia carotovora var. carotovota* ACCC 10276, which is Gram-negative plant-specific bacterial pathogen (Huang *et al*., 2009). Other phytate-degrading enzymes retain their activity in the presence of different salt concentrations. In food production processes, thermostable and halotolerant phytases are preferred and can find wide applications. Enzymes with similar desirable characteristics have been detected in *Bacillus amyloliquefaciens* US573, retaining their activity in the presence of high NaCl and lithium chloride (LiCl) concentrations (Boukhris *et al*., 2015), and in the lactic acid bacterium *Weissella halotolerans* (Demir *et al*., 2017). An acidic and thermostable phytase has been produced by *Geobacillus stearothermophilus* strain DM12, which is stable at temperatures below 60 °C (Parhamfar *et al*., 2015). Later a highly thermostable pytase with temperature optimum 85 °C and pH optimum 4.0, has been isolated from thermophilic *Geobacillus* sp. TF16 (Dokuzparmak *et al*., 2017). In addition, phytase isolated from *Streptococcus thermophilus* 2412 has shown activity at higher temperatures up to 90 °C (Priyodip and Balaji, 2020).

The production and functional properties of bacterial phytase enzyme is affected by different metal ions and enzyme modulators, including ascorbic acid, ethylenediamine-tetraacetic acid, β-mercaptoethanol, dithiothreitol, and urea (Kumar and Sinha, 2018). In Thailand, intracellular phytase activity has been detected in four species of thermotolerant cyanobacteria, including *Synechococcus lividus* SKP50, *S. lividus* DSK74, *S. bigranulatus Skuja*, and *Chroococcidiopsis thermalis* (Shoarnaghavi *et al*., 2022).

2.2. Bacteria as microbial hosts for expression of phytases

Genetic engineering techniques are used to enhance the production of phytases in microorganisms, tailoring their properties for specific applications. As the field of enzyme technology continues to advance, ongoing researches aim to identify and optimize microorganisms with superior phytase characteristics, contributing to the development of sustainable and efficient biotechnological processes. Recombinant expression of phytase in different host microorganisms offers a versatile approach to meet the demands of various biotechnological applications. Each host system presents unique advantages and challenges; influencing the choice, which depends on various factors, including desired expression levels, post-translational modifications, and downstream processing requirements for specific application of the phytase. Common host bacteria utilized for expression of phytase through genetic engineering are summarized on Table (2).

Escherichia coli, a commonly used prokaryotic bacterium in biotechnology has been employed for recombinant phytase expression due to its rapid growth, well-established genetic tools, and cost-effectiveness. In a specific application, *E. coli* BL21 is a host microorganism for expression *of Selenomonas ruminantium*/ phyA-7 in a big volume bioreactor. Optimal expression of mutant phytase has been obtained on cultivation at a temperature of 30 °C by adding extra yeast in the induction phase (Lan *et al*., 2014). Due to its favorable characteristics such as high specific activity, pH stability, and thermostability, a new phytase from *Yersinia intermedia* has a good potential to be produced commercially. It was expressed in *E. coil* by Mirzaei *et al*., (2016) and proved to have high activity under optimal conditions (pH 5, 55 ^{0}C), pH stability (3-6), and thermostability (80 °C for 15 min.).

Mitsuokella jalaludinii has been reported to have a high phytase activity (Lan *et al*., 2010). This strain of ruminant bacteria requires strictly anaerobic conditions, making it difficult and expensive to cultivate. This challenge has been solved by cloning and expressing the phytase gene from *M. jalaludinii* in *E. coli*, allowing the enzyme to be obtained in larger quantities, purified, and characterized. The study showed that recombinant phytase has reduced pH stability and is resistant to trypsin proteolysis, but susceptible to pepsin proteolysis. Some ions may have negative impacts (K^+); however, Ca^{2+}, K^+, and Mg^{2+} have significant positive effects (Tan *et al*., 2015).

As recombinant proteins, the bacterial phytases could be directly expressed in plants. It is important to evaluate the characteristics of the recombinant phytase in advance because this process of gene expression may lead to undesirable characteristics of this recombinant enzyme. The phytase gene from *Pantoea agglomerans*; a bacterium associated with plants, has been successfully expressed in *E. coli* (Khabipova *et al*., 2016).

Table 2: Diverse host bacteria used for recombinant phytase expression

Host strain	Gene source/ Gene	Yield/ Activity	Reference
E. coli BL21	*Selenomonas ruminantium*/ phyA-7	107.0 U/ ml	Lan *et al*., (2014)
E. coli Rosseta gami	*M. jalaludinii/ PHY7*	303.24 U/ ml	Tan *et al*., (2015)
E. coli Bl21 (DE3)	*Yersinia intermedia/appA*	3849 U/ ml	Mirzaei *et al*., (2016)
E. coli BL21 pLysS	*Pantoea agglomerans/PaPhyC*	140 U/ ml	Khabipova *et al*., (2016)
E. coli BL21 (DE3)	*Bacillus subtilis KM-BS*	PhyC-37 3.73 U/ ml PhyC-55 2.51 U/ ml	Ho *et al*., (2023)
Bacillus subtilis BD170	*phyC* gene	48 U/ ml	Vuolanto *et al*., (2001)
B. subtilis	*phyC* gene	28.7 U/ ml	Kerovuo *et al*., (2000)
Lactococcus lactis	*B. subtilis GYPB04/ phyC*	42.12 U/ ml	Miao *et al*., (2013)
L. lactis	*E. coli/ appA*	19 U/ ml	Pakbaten *et al*., (2019)
Lactobacillus plantarum 755	*B. subtilis* VTT E-68013/ *phyC*	na	Peirotén and Landete, (2020)
Lactobacillus casei BL23	*B. pseudocatenulatum* and *B. longum* spp.	na	García-Mantrana *et al*., (2014)
Bifidobacterium longum JCM 1217	Phytase *appA*	na	Sun *et al*., (2019)

Several authors proved that lysate from *E. coli* BL21 pLysS cells has high phytase activity (140 U/ ml). Two recombinant PhyC-37 and PhyC-55 enzymes represent potential candidates for application in the animal feed industry under the optimal temperature of 55 °C. It has been shown that metal ions, including Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, and Zn^{2+} have stimulated PhyC-37 and PhyC-55 activity (Ho *et al*., 2023).

The Gram-positive bacterium *B. subtilis* has been explored as a host for phytase expression due to its secretion capability and lack of endotoxins, simplifying downstream purification processes. The use of *B. subtilis*, generally recognized as safe (GRAS) species, allows for the secretion of phytase directly into the culture medium, reducing the need for cell disruption steps (Wang *et al*., 2014). Phytase gene expression has been optimized for large-scale production by creating an efficient expression system in *B. subtilis* BD170. The strain carrying the *phyC* gene responsible for phytase activity has been grown in a medium containing peptone, which represented the necessary nitrogen source for the cells. Maximum extracellular enzyme activity of 48 U/ ml has been reached in a batch feed process (Vuolanto *et al*., 2001). Furthermore, the extracellular calcium dependent phytase from *B. subtilis* US417 (PHY US417) has been expressed in the GRAS *B. subtilis* 168, which is convenient for a cost-effective and high-volume production. This enzyme exhibits perfect stability at pH values ranging from 2.0 to 9.0 and high thermal stability (optimally active at pH 7.5 and 55 °C). Following optimization of the cultivation conditions, the phytase activity achieved is 73 times higher compared to the activity generated by the original *B. subtilis* US417 strain prior to optimization (Farhat-Khemakhem *et al*., 2012). These advancements in *B. subtilis* expression systems highlight its potential for efficient and scalable production of phytase with improved characteristics.

Recombinant phytase expression can be efficiently achieved using lactic acid bacteria, which offer multiple advantages such as safety, cost-effectiveness, and production of enzymes with high purity and stability. In recent years, many strains of lactic acid bacteria that possess probiotic capabilities have attracted considerable attention as promising candidates for phytase gene expression, due to their probiotic characteristics. In order to improve the efficiency of nutrition and maintain the overall health of humans and animals, genetically modified probiotics are used to produce and deliver natural or modified substances to mucosa of the digestive tract (Pakbaten *et al*., 2019). An interesting approach is the creation of transformed *Lactobacillus* spp. that possess both functions of phytase production and probiotics properties. Once the transformed *Lactobacillus* sp. is given to animals, it could survive in the animal gut to play both roles of secreting phytase and probiotics. Moreover, the transformed *Lactobacillus* spp. with phytase gene and probiotic activities decrease several digestive diseases and improve nutrient availability. Animal's production will be increased by using this transformed Lactobacillus spp. in their diets (Zuo *et al*., 2010).

The use of genetically engineered probiotics to express specific enzymes has been the subject of considerable attention in poultry industry, due to increased nutrient availability and reduced costs of enzymes supplementation. Phytase enzyme is commonly added to poultry feed to improve digestibility and availability of phosphorus from plant sources. Phytase gene (*appA2*) derived from *E. coli* has been successfully expressed in *L. lactis*, where phytase activity has been detected in the supernatant (19 U/ ml) and cells extract (4 U/ ml). Such genetically transformed *L. lactis* has been added to the feed of poultry, which increased phytate phosphorus uptake to levels comparable to the use of commercial phytase from *E. coli* (Pakbaten *et al*., 2019).

Another promising phytase-producing probiotic bacterium is *L. plantarum* that has been used as a host for heterologous expression of various proteins. This bacterium is also used as an inoculum in the preparation of grass silage and vegetable products, due to its ability to decrease pH, thus contributing to their preservation (Liu *et al*., 2022). Similarly, the phytase gene (*phyC*) from *B. subtilis* has been expressed in *L. plantarum* strain 755 (Peirotén and Landete, 2020), as expression levels are not sufficient for large-scale of phytase production. In general, lactobacilli are regarded not only as safe and possessing valuable probiotic properties, but also are used as hosts for heterologous expression of various proteins. Moreover, lactobacilli from the gastro-intestinal tract of animals have a remarkable ability for adhesion and colonization of the intestinal mucosa, tolerance to an acidic environment, and induce increased concentration of bile salts. Genetically transformed lactobacilli capable of degrading phytate and β-glucan have been used to improve food digestibility and reduce the risk of gastrointestinal disease in broiler chickens (Wang *et al*., 2014). According to the previous study conducted by Miao *et al*., (2013), combination of phytase activity and probiotic properties in transformed *L. lactis* opened new possibilities for developing functional foods with enhanced nutritional benefits, potentially contributing to the prevention and treatment of diseases. Furthermore, this study revealed that phytase produced in the genetically modified *L. lactis* exhibited activity within a wide pH range of 2.0-9.0 and temperatures from 20- 80 °C; with an optimum at 60 °C. The phytase characteristics and probiotic properties provided the transformed *L. lactis* with important nutritional applications used in the degradation of phytate during both food processing and digestion.

3. Application of bacterial phytase

Recently, the field of biotechnology has witnessed a surge of interest in harnessing the potential use of bacterial phytases in various applications. Phytase is naturally present in some plant tissues, but it is often added to animal feeds to improve phosphorus utilization and reduces the environmental impact of phosphorus excretion. In animal nutrition, supplementing feed with bacterial or fungal phytase enhances the digestibility of phosphorus, making it more readily available for absorption in the digestive tract. This is especially important in poultry, swine, and other monogastric animals that rely on plant-based diets. The phytase enzymes could be used in sustainable agriculture, animal nutrition, medicine, and environmental management (Handa *et al*., 2020).

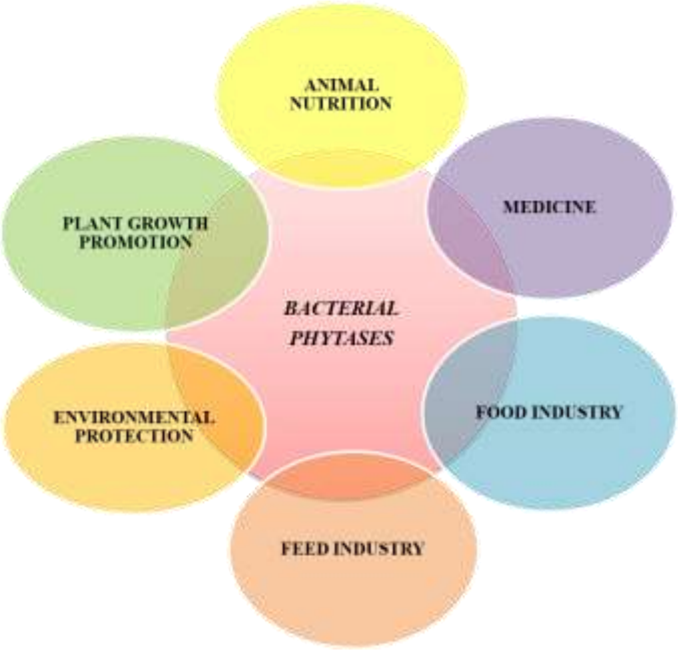

Fig. 2: Possible applications of bacterial phytases

By facilitating the hydrolysis of phytic acid, phytase helps in overcoming the anti-nutritional effects of phytic acid, which can otherwise bind essential minerals such as calcium, magnesium, and zinc, making them unavailable for absorption. The use of phytase in animal feeds contributes to improved nutrient utilization, reduced environmental pollution from phosphorus excretion, and overall better animal health and performance (Valente Junior *et al*., 2024). Initially, incorporating microbial phytases into animal feeds enhances phosphorus availability, thereby improving nutrient utilization and promoting animal growth. Additionally, this practice helps mitigate environmental pollution caused by phosphorus originating from animal wastes. Secondly, microbial phytases play a crucial role in enhancing mineral bioavailability and nutrient absorption in plant-based food items. All these characteristics counteract the adverse effects of phytic acid on human health. Moreover, these enzymes have the potential to enhance the taste and functional qualities of food, and they release bioactive compounds that contribute to beneficial health effects (Joudaki *et al*., 2023).

3.1. Enhancing nutrients availability in food and feed industries

In common feed ingredients, determination of total phosphorus, phytate phosphorus, and endogenous phytase activity is crucial for assessing the nutritional quality and formulating balanced diets for animals. Total phosphorus content represents the overall phosphorus concentration in the feed, including both organic and inorganic forms. However, a significant portion of phosphorus in plant based feed ingredients is present in the form of phytate phosphorus, which is indigestible for the monogastric animals, due to the lack of endogenous phytase enzymes (Abbasi *et al*., 2019).

3.2. Animal nutrition and aquaculture

Phytate, found in plant-based feed, poses as an anti-nutritional factor, contributing to mineral deficiencies in non-ruminant animals. The deleterious effects of phytate can be alleviated through the utilization of phytase, promoting the digestibility of trace minerals and amino acids, while simultaneously reducing phosphorus excretion into the environment. This, in turn, helps in minimizing several issues such as eutrophication in surface water and occurrence of algal blooms. Monogastrics like fish, poultry, and swine have little or no phytase activity in their intestine. Previous studies suggested positive effects of microbial phytase on the digestibility of trace minerals, phosphorus, phytate phosphorus, and amino acids, which are responsible for growth, performance, development, and overall health of non-ruminant animals (Rizwanuddin *et al*., 2023). It has been found that only 30 % of plant phosphorus is available to birds. The addition of exogenous microbial phytase; mostly of bacterial and fungal origins (*E. coli*, *Peniophora lycii*, *A. niger*, and *A. ficum*), facilitates the release of inorganic phosphorus and its better absorption by the poultry. At the same time, in this way, discharge of phosphorus into the environment is drastically reduced (Abbasi *et al*., 2019).

One of the effects of phytase activity is not only the release of inorganic phosphorus, but also increasing the availability of several mineral elements (*i.e*., Ca, Na, K, Mg, Zn) and amino acids (*i.e*., Valine, Cysteine, Threonine, Histidine, Phenylalanine, Lysine, Arginine, and Leucine), in addition to the production of inositol. There are data that all these “extra-products” obtained with the assistance of exogenous phytase positively affect composition of the natural microflora, immune protection, anti-oxidant status, and overall intestinal health in poultry and pigs (Valente Junior *et al*., 2024).

Studies conducted in recent years on the effect of dietary supplements in aquaculture have confirmed the benefits of phytases (Priya *et al*., 2023). This enzyme is produced by many microorganisms and plants, but does not exist in fish. It is highly recommended as a feed additive to improve the absorption of various nutrients. The use of microbial phytases in aquaculture has proven to have a positive effect on phosphorus uptake, and improves the growth of various fish species and increases their resistance to diseases. A study conducted by Wang *et al*., (2009) highlighted the positive impacts of microbial phytase on nutrient digestibility and bone mineralization in rainbow trout. Supplementation of microbial phytase in fish diets led to improved phosphorus retention and enhanced growth in Nile tilapia. Similarly, in a study reported by Lee *et al*., (2020), microbial phytases played a crucial role in improving fish health. Supplementation of fish feed with microbial phytases can improve the utilization of phosphorus from phytate, avoids the use of inorganic P in feed, minimizes P discharge in water bodies, decreases aquatic pollution, and preserves the aquatic environment. By optimizing phosphorus utilization through the use of microbial phytase, the aquacultural operations can reduce the environmental footprint and promote sustainability (Priya *et al*., 2023).

3.3. Human nutrition

Phytases have potential applications in the food industry; particularly in the production of functional foods. Addition of phytases to foods of plant origin has been shown to reduce the amount of the anti-nutrient phytate, which results in an increase in the availability of absorbable minerals and other essential nutrients (Alkay *et al*., 2024). A lot of investigations suggest that utilization of lactic acid bacteria with phytase activity or their phytases hold significant potential for enhancing the nutritional content of different varieties of bread (Dahiya *et al*., 2020).

According to the previous study conducted by Nuobariene *et al*., (2015), lactic acid bacteria isolated from sourdough were found to possess both extracellular and intracellular phytase activity. Among the bacterial isolates, several strains such as *Pediococcus pentosaceus*, *Lactobacillus panis*, *Lactobacillus reuteri*, and *Lactobacillus fermentum* have shown the most promising results. When *L. panis* and/or *L. fermentum* had been used to ferment whole grain dough, a reduction in Na-phytate concentration of up to 74 % was observed. These findings were in line with the previous degradation of Na-phytate achieved using *L. sanfranciscensis* CB1 as a starter culture for sourdough fermentation. Furthermore, phytase activity was maintained upon repeated reuse of all the three bacterial strains as starters.

The ability of probiotic microorganisms to produce phytase has not yet been thoroughly investigated. Another scientific approach is the transfer of phytase genes in probiotic strains with the aim of obtaining recombinant microorganisms that possess both desired characteristics. During cereals and legumes fermentation, *Weissella confusa* mk.zh95 and *Pediococcus pentosaceus* are considered as sources of phytase, which has improved the bioavailability of minerals. Both lactic acid bacteria have been isolated from the sourdough of wheat flour–mung bean and identified by Mohammadi-Kouchesfahani *et al*., (2019). In a study conducted by Ghamry *et al*., (2023), a comparative analysis had been made among the metabolic characteristics of three new strains of *L. apis*, *L. plantarum*, and *Saccharomyces cerevisiae*, and microorganisms traditionally used in cereal fermentation. *L. apis* degraded phytic acid in fermented wheat bran in a significantly higher level compared to *S. cerevisiae* and *L. plantarum*. This bacterial strain also improved the volatile profile and enhanced the antioxidant activity of fermented wheat bran. Moreover, it significantly increased the level of conditional amino acids and branched chain amino acids, and remarkably increased the contents of organic acids and water-soluble vitamins in wheat bran, exhibiting encouraging fermentation characteristics.

3.4. Plant growth promotion

One of the key applications of bacterial phytases lies in promoting sustainable agriculture. The enzymatic activity of phytases aids in breakdown of organic phosphorus compounds present in soil, making phosphorus more accessible to plants. This promotes plant growth, enhances crop yields, and reduces the dependence on chemical phosphorus fertilizers. The use of bacterial phytases in sustainable agricultural practices aligns with the broader goals of minimizing the environmental footprint associated with conventional farming (Singh *et al*., 2020). Idriss *et al*., (2002) provided strong evidence that *B. amyloliquefaciens* FZB45 was able to degrade extracellular phytate and was important for plant growth stimulation under phosphate limitation. Phytase-producing microorganisms isolated from Himalayan soils and identified as *Advenella* spp. (three strains) and *Cellulosimicrobium* sp. (a single strain), have proven to possess a number of activities that stimulate plant growth; mainly production of ammonia, siderophores, and indole acetic acid. Additionally, they also have the ability to suppress the phytopathogenic *Rhizoctonia solani* and possess plant growth promoting activities. *Advenella* strains have increased the inorganic phosphorus content and stimulated the growth of *Brassica juncea*. It has been established that these bacteria are suitable to be used in the production of biofertilizers, as they possess the necessary characteristics (Singh *et al*., 2014). Other authors have reported the existence of 73 bacterial isolates from grass rhizosphere (*Qinghai-Tibetan Plateau*) with extracellular phytase producing activity. The findings of this study indicated that the use of bacterial inoculants can facilitate restoration of the phosphorus deficient pastures and soils, thereby enhancing grass growth (Li *et al*., 2023). In an investigation devoted to the possibilities of improving the values of soybean meal by increasing the level of degradation of anti-nutritional factors such as phytic acid, glycinin, and β-conglycinin, a *Pseudomonas* PY-4B strain with high protease and phytase activity has been recorded and proved to be safe (Lin *et al*., 2023). These findings have been based on ability of this strain to facilitate the release of phosphorus from organic compounds in the soil; as bacterial phytases enhance nutrient availability for plants, thus fostering increased crop yields. Optimization of phytate utilization in food, plant growth, and animal nutrition not only enhances nutritional outcomes for the individuals but also contributes to a sustainable approach by minimizing the release of excess phosphorus into the environment. These improve the agricultural productivity and underscore the significance of microbial contributions in promoting long-term environmental sustainability within the farming practices.

3.5. As biofertilizers

Synthetic fertilizers are integral to modern agriculture, fostering increased crop yields. However, their wide application raised environmental concerns, including soil degradation, water pollution, and disruption of the microbial communities. The scientific community is actively seeking sustainable alternatives that promote soil health and mitigate environmental impacts. Biofertilizers have proven to enhance plant growth and development by augmenting the accessibility of both macro and micronutrients within the plant system. Utilization of bacterial phytase as a biofertilizer offers several advantages over traditional synthetic alternatives, as they are natural for the soil, cheaper, and ecofriendly. The main role of microbial phytases as biofertilizers is solubilization of phytate and release of phosphate into a plant-absorbable form. Due to their phytate-mineralizing ability, several microbial genera, including *Azotobacter*, *Azosporillum*, *Phosphobacteria*, and *Rhizobium* are suitable for use as biofertilizers (Taj and Mohan, 2022). Moreover, microorganisms such as *Burkholderia* spp. (Luang-In *et al*., 2021), *Advenella* spp. and *Cellulosi microbium* sp. PB-09 (Singh et al., 2014), *Enterobacter* spp., and *Pantoea* spp. are capable of removing phosphate residues from phytate, thereby improving plant growth and development. In this way, microbial phytases, taking a significant part in the phosphorus cycle and being a successful alternative to artificial fertilizers are attracting more attention as biofertilizers (Rizwanuddin *et al*., 2023). One of the significant environmental benefits of bacterial phytases lies in their ability to mitigate phosphorus pollution. Traditional animal feeds often contain high levels of inorganic phosphorus, leading to excessive excretion of phosphorus-rich wastes. Bacterial phytases enable the utilization of phytic acid-bound phosphorus, reducing the need for supplemental inorganic phosphorus in feed formulations. This, in turn, minimizes the environmental impact associated with phosphorus runoff into water bodies, addressing concerns related to eutrophication and water quality.

3.6. In pharmaceutics

Phytase, traditionally recognized for its role in nutrition, has emerged as a versatile enzyme with significant pharmaceutical potentials. Its applications in bone health, antioxidant protection, digestive well-being, anti-inflammatory interventions, and metabolic modulation highlight its therapeutic capability. The existing studies have examined the potential applications and impacts of phytases on the treatment of socially significant diseases, including cancers, coronary heart disease, osteoporosis, and human papilloma virus (HPV) (Sharma *et al*., 2020b).

Conclusion

Bacterial phytases represent a valuable assist in the biotechnological toolbox, offering solutions to several challenges in animal nutrition, environmental sustainability, and agricultural practices. As researches in this field continue to advance, optimization of bacterial phytases production through genetic engineering holds promise for further improving their efficiency and applicability. Harnessing the potentials of bacterial phytases underscores their roles in fostering a more sustainable and environmentally conscious approach to food production and resource. Despite the promising applications of bacterial phytases, challenges remain in optimizing their production, stability, and efficacy. New researches can be directed toward the exploration of phytases of extremophilic origins, where they retain their activities under various unfavorable conditions. Nowadays, scientists are actively exploring genetic engineering and fermentation techniques to enhance the production of these enzymes. Additionally, efforts are underway to identify novel bacterial phytases with improved properties for specific valuable applications.

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Conflict of interest

No conflicts of interest are to be declared.

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