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Review

Phytases - Types, Sources, and Factors Affecting Their Activity

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Abstract

Phytases are a large group of enzymes that hydrolyze phytate and its complexes. This most abundant organic phosphate in the world is commonly found in plant-based foods. It can bind to essential minerals, making them less available for absorption. Enzymatic hydrolysis of phytates is the most beneficial method for reducing their content in foods and feeds. Phytase supplementation enables more efficient utilization of phytate phosphorus. The enzyme is produced by prokaryotic and eukaryotic microorganisms, plants, and animals. Several types of phytases, depending on certain structural and kinetic properties are described. Phytase activity is influenced by metal ions, surfactants, and various plant extracts.

Keywords: phytases, phytate, producers, inhibitors

Резюме

Фитазите са голяма група ензими, които хидролизират фитата и неговите комплекси. Този най-изобилен органичен фосфат в света обикновено се среща в растителните храни. Може да се свърже с основни микро- и макроелементи, което ги прави по-малко достъпни за усвояване. Ензимната хидролиза на фитатите е най-полезният метод за намаляване на съдържанието им в храните и фуражите. Добавянето на фитаза позволява по-ефективно използване на фитатния фосфор. Ензимът се продуцира от прокариотни и еукариотни микроорганизми, растения и животни. Описани са няколко типа фитази в зависимост от определени структурни и кинетични свойства. Активността на фитазата се влияе от метални йони, повърхностноактивни вещества и различни растителни екстракти.

Phytic Acids

The most abundant organic phosphate in the world is Inositol hexakisphosphate (InsP6), also known as phytic acid. In nature, phytic acid ($C_6H_{18}O_{24}P_6$, Fig. 1A) can exist as free acid, phytate, or phytin, depending on the physiological pH and metal salts (Amitha and Balaji, 2015). Phytates are natural compounds found in a wide variety of plant-based foods. They are commonly known as myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphates, and their levels can vary from 0.1 to 6.0% in different foods. While phytates have some beneficial properties, they can also bind to certain minerals in the digestive tract, making them less available for absorption (Gupta *et al*., 2015). Due to its chemical structure, phytate is an extremely stable molecule that contains high phosphate content, leading to a strong negative charge across a wide pH range. Phytate, which is composed of an inositol ring with six phosphate groups, has the ability to bind up to 12 protons and acts as a potent chelator that readily binds to mineral cations like copper, calcium, zinc, and iron (Castro-Alba *et al.,* 2019). Under normal physiological conditions, phytic acid binds also to amino acids and proteins and inhibits digestive enzymes (Fig. 1B). This makes phytic acid an antinutritive component in plant-based foods and feeds, and thus enzymatic hydrolysis of phytic acid is desirable (Pallauf and Rimbach, 1997).

Phytase

The hydrolysis of phytate to orthophosphate and lower substituted inositol phosphates is achieved enzymatically with phytase. This is the most beneficial method for reducing phytic acid content in plant foods as it can remove the maximum amount of phytic acid without reducing its mineral content. Phytases are myoinositol hexakisphosphate phosphohydrolases that catalyze the hy-

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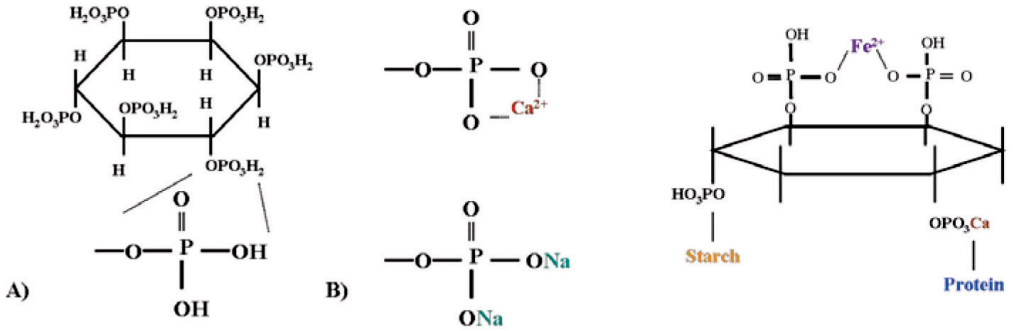

Fig. 1. A) Phytic acid (phytate, myo-inositol-1,2,3,4,5,6-hexakis dihydrogen phosphate); B) Modes of binding metal ions and organic compounds leading to various phytic acid complexes.

drolysis of phytic acid to inorganic phosphate and myoinositol phosphate derivative (Mullaney and Ullah, 2003). They catalyze the cleavage of phosphate from phytate in a stepwise hydrolysis reaction and release available forms of inorganic phosphorus (Fig. 2.) (Konietzny and Greiner, 2002). Phytase is the most widely used feed enzyme, and farmers increasingly rely on phytase supplementation to release phosphate from the feed phytate. As a result, the supplemented phytase activity covers more than 50% of the animals' requirement in phosphate (Willard *et al.*, 2022). Phytases can be used as additives in many food products which strengthens the interest of isolation of new and efficient phytase-producing microorganisms.

Thermostable microbial phytases are selected as well, which endure processing with the lowest production cost (Greiner and Konietzny, 2006). Phytase supplementation has enabled a more efficient utilization of phytate phosphorous (P) and the reduction of P pollution. It releases trace minerals too, such as iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) which are essential for maintaining health and immunity as well as animal growth, production, and reproduction (Santos *et al.*, 2015).

Sources of phytases

In the last decade, interest in the study and isolation of phytases from various sources has been continuously increasing due to their wide application in agriculture as well as their importance for environmental protection. Phytases are produced in nature by a wide range of microorganisms (bacteria, yeast, fungi), and in plant and animal tissue (Vohra and Satyanarayana, 2002). The studies concerning the presence of phytase activity in animal tissues are scarce. However, many years ago phytase activity was reported in the blood of lower vertebrates, birds, reptiles, fishes, batrachians, and sea turtles (Rapoport *et al.*, 1941; Lopez *et al.*, 2000). Summarized information about the main sources of phytase is presented in Table 1.

The use of microbial phytases has primarily been focused on enhancing mineral bioavailability and food processing in animal and human food products. While these enzymes show promise in the food industry, there are concerns regarding their safety when used as a dietary supplement for humans, particularly when derived from microorganisms. Several screening programs have been carried out aiming at the isolation of different groups

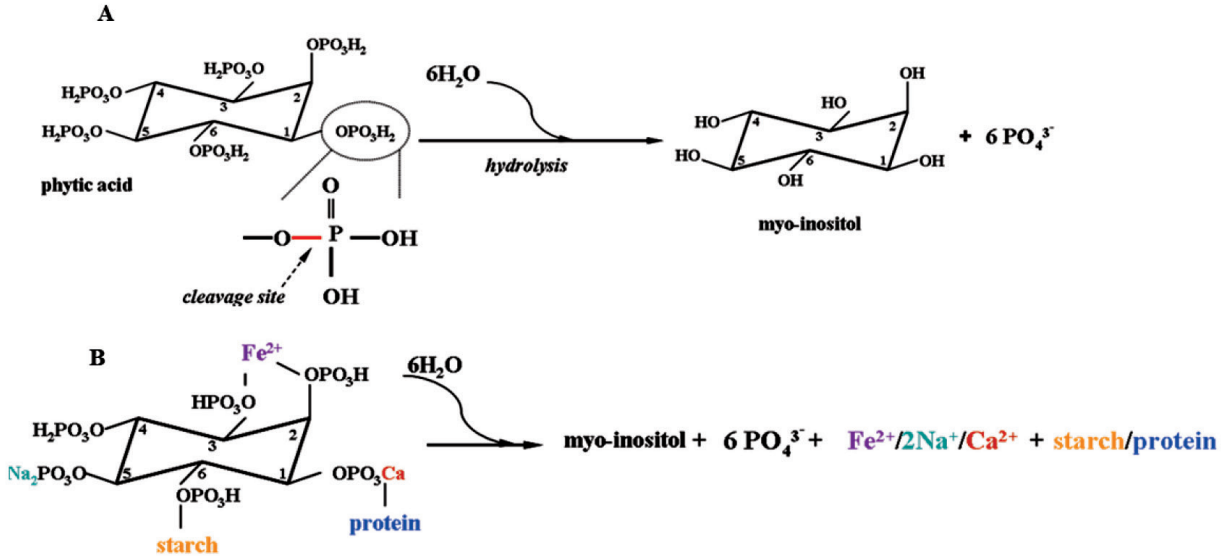

Fig. 2. Schematic diagram showing A) phytase mechanism of action; B) hydrolysis of phytate complexes

Table 1. Main sources of phytases

Source of phytase	Organism	References
Bacteria	*Bacillus sp.*	Manna *et al*., 2021
	Arthrobacter sp.	Rix *et al*., 2022
	Acinetobacter sp.	Sharma *et al*., 2020 (a)
	Lactic Acid Bacteria, Carnobacteriaceae, Enterococcaceae,	Mogal *et al*., 2017
	Lactobacillaceae,	Sumengen *et al*., 2012
	Leuconostocaceae, Streptococcaceae	
	Enterobacteriaceae	
Yeast	Candida sp.	Quan *et al*., 2002
	Pichia anomala	Vohra and Satyanarayana, 2002
	Pichia pastoris	Guo *et al*., 2007
	Cyberlindnera fabianii (basionym Hansenula fabianii)	Kaur *et al*., 2007
	Debaryomyces castellii CBS 2923	Watanabe *et al*., 2009
	Saccharomyces cerevisiae	Ragon *et al*., 2008
	Kodamaea ohmeri	Kłosowski *et al*., 2018
		Roopashri and Varadaraj, 2015
		Li *et al*., 2008
Fungi	*Penicillium sp.*	Ajith, *et al*., 2019
	Aspergillus sp.	Hassouni et al., 2006
	Myceliophthora sp.	Roopesh *et al*., 2006
	Mucor sp.	Sabu *et al*., 2002
	Rhizopus sp.	Kour *et al*., 2020
	Trichoderma sp.	Dailin *et al*., 2019
	Humicola nigrescens	Balla *et al*., 2014
Microalgae	Synechococcus bigranulatus	Klanbut *et al.,* 2004
	Synechococcus lividus	
	Chroococcidiopsis thermalis	
Plants	Wheat, rye, barley, pea, bean, soybean, maize, rice, lettuce,	Bouajila *et al.,* 2019
	spinach, grass, lily pollen, etc.	Steiner *et al*., 2007
	ferns	Faba- Rodriguez *et al*., 2022
		Liu *et al*., 2021
Animals	*Rattus* sp. (small intestine)	Rapoport *et al*., 1941
	Gallus domesticus (chickens, laying hens)	Abudabos, 2012 (a)
	fish	Abudabos, 2012 (b)
		Lopez *et al*., 2000

of bacteria, yeast and fungi having extracellular phytase activity. Scientific reports of new producers of this important enzyme are constantly being published.

Bacteria

Some examples of phytase-producing bacteria are *Bacillus* sp*., Klebsiella* sp*., Escherichia coli, and Pseudomonas* sp. (Kumar and Sinha, 2018). Phytase activity was registered also in *Flavobacterium johnsoniae*, *Pseudomonas rhodesiae,* and *Pseudomonas* sp. (Kour *et al.,* 2020). Phytase obtained from *Bacillus subtilis* DR6 isolated from poultry farm soil is considered promising as a feed supplement in a monogastric animal diet. Phytase activity has been described for food-grade strains of the *Bifidobacterium* genus, which are endogenous inhabitants of the gastrointestinal tract. Phytase and phosphatase activities were screened in nine strains belonging to different species of the genus *Bifidobacterium*, representative of common intestinal isolates and probiotic species. It was found that *B. pseudocatenulatum*, *B. adolescentis*, *B. angulatum*, *B. globosum*, *B. longum*, possesed phytase activity (Haros *et al.,* 2005). Lactic acid bacteria (LAB) fermentation is a good approach to diminish the adverse effect of phytate-rich cereals such as pearl millet and maize, other cereals, and pseudocereals. These foods are a source of LAB displaying phytase activity, for instance, *L. plantarum* and *L. fermentum* isolated from the fermented teff meal injera and the pearl-millet fermented gruel ben-saalga

(Petrova *et al.,* 2022). Other authors have reported two strains of LAB (*L. amylovorus* and *L. plantarum*) isolated from sourdough that show a relatively high phytase activity. Phytate content was also found to decrease significantly after 20 h of lactic acid fermentation (Sharma *et al.*, 2020).

Fungi

A survey of fungi for the production of extracellular phytase has been reported and 58 strains of fungi (including *Aspergillus*, *Rhizopus,* and *Mucor* species) exhibited the ability to hydrolyze phytate (Howson and Davis, 1983). *Aspergillus ficuum* NRRL 3135 was reported to be a promising producer of the active phytase (Chelius and Wodzinski, 1994). Extracellular phytase has also been documented in *Aspergillus* species such as *A. amstelodami, A. candidus, A. flavus A. repens, A. carbonarius*, *A. fumigatus*, and *A. oryzae*) (Howson and Davis, 1983; Jatuwong *et al.,* 2020). A fungus called *Peniophora lycii*, isolated from the roots of *Lycium chinense* plants contains a phytase enzyme with strong activity. This enzyme could be useful in removing phosphorus from contaminated soil. According to Pontoppidan *et al*. (2007), *in vitro* simulations of pig gastrointestinal digestion showed that the phytase was stable and could degrade phytate and solubilize minerals. Recently, novel fungal phytases from various species including *Arthrobotrys soligospora* and *Penicillium polonicum* MF82 were isolated and characterized (Hou *et al.,* 2020, Kalkan *et al*., 2020). Some *Penicillium* species, isolated from various regions and sources (Brazilian caves, coffee plants, Himalayan regions) are reported as phytase producers (Alves *et al*., 2016; Kaur *et al*., 2017).

Although relatively low, phytase activity has also been detected in some edible mushrooms, such as *Agaricus bisporus, Grifola frondosa, Lentinula edodes, Pleurotus cornucopiae* (Collopy and Royse, 2004).

Yeast

There have been limited investigations of phytase in certain types of yeast, including *Sacharomices cerevisiae* and *Schwanniomyces castellii* (Lambrechts *et al.*, 1993; Kłosowski *et al*., 2018). However, researchers were able to successfully clone, purify, and characterize phytase (Hfphytase) from the yeast *H. fabianii* J640 (now known as *C. fabianii)* found in wastewater treatment facilities. This phytase was expressed in *P. pastoris.* (Watanabe *et al.,* 2009) Another phytase from the yeast *D. castellii* CBS 2923, a glycosylated protein that hydrolyzes the six phosphate bonds of phytate was characterized by Ragon *et al*, 2008. *Pichia anomala* and *Candida crusei* produce phytases that withstand high temperatures and acidity and therefore have the potential to be used in animal feed processing (Kumar and Sinha, 2018).

Microalgae

Only four species of blue-green algae, including *S. bigranulatus, S. lividus* DSK74, *S. lividus* SKP50, and *C. thermalis* were reported to possess intracellular phytase activity (Klanbut *et al.,* 2004). Surprisingly, to our knowledge, there are no more studies dedicated to phytase in microalgae despite the fact that algae are used as food for monogastric animals.

Plants

Phytase activities were found in many plants such as wheat, rye, barley, pea, bean, soybean, maize, rice, lettuce, spinach, grass, lily pollen, etc. High native phytase activities are present in cereals and cereal by-products. It was reported that phytase activity usually increases on germination, and germination has historically been used to induce this activity in cereals (Faba-Rodriguez *et al.*, 2022). It was established that legumens and oilseeds have lower enzyme activity compared to cereals (Konietzny and Greiner, 2002). The major problem in the production of plant phytases is that a cost-effective and efficient production of these enzymes is yet to be developed. The higher pH and thermal stability of microbial phytases compared to plant phytases have made microbial ones more investigated for industrial purposes (Bohn *et al*., 2008). The production of phytase from plants is not economically viable, as pre-treatment is required and the production procedure becomes time-consuming, troublesome, and expensive. So, the production of phytase from microbial origin is of greater potential.

Animals

Although phytase activity was recorded in intestinal tissues of some monogastric animals (fishes, hens, rats) it was generally accepted that the levels of this enzyme were insufficient to metabolize phytate (Lopez *et al.*, 2000; Abudabos, 2012). For example, the presence of enzyme was described in ER lumen of rat hepatic tissues, and the brush border membrane of the small intestine of some fishes, particularly representatives of *Oreochromis* and *Morone* (Kumar and Sinha, 2018). Phytase was recorded in the intestinal tissues of laying hens, where the enzyme activity varies with age (Abudabos, 2012).

Classification of phytases

Phytases can be classified according to different features such as stereospecificity, pH-optimum, structural and catalytic properties (Table 2).

Stereospecificity

This feature refers to the position of the first hydrolyzed phosphate. Currently, there are three classes of enzymes that dephosphorylate phytic acid at different positions on the inositol ring, producing different isomers of lower inositol phosphates: 3-phytase (EC 3.1.3.8), 5-phytase (EC 3.1.3.72), and 6-phytase (EC 3.1.3.26). The 3-phytases initiate dephosphorylation of phytic acid at the 3 positions of phytic acid and 5- and 6-phytases at position 5 and 6, respectively. However, all phytases demonstrate strong stereospecificity and preference for equatorial phosphate groups over axial groups, as noted by Lei and Porres in 2003.

Structural and catalytic properties

Even within each class of phytase, there can be structural differences, and not all enzymes within the same class use the same mechanism to hydrolyze phosphate from phytic acid. Thus, phytases have also been classified as histidine acid phytase (HAP), purple acid phytase (PAP**),** cysteine phytase (CP, protein tyrosine phosphatase), and β-propeller phytase (BPP) (Mullaney and Ullah, 2003; Singh and Satyanarayana, 2011; Dersjant-Li *et al.*, 2015).

The best-studied phytases belong to the class of HAPs, as most of the currently commercially available phytases belong to this group, such as the *E. coli* phytase. HAPs are produced by many microorganisms including *Sporotrichum thermophile*, *A. orizae*, *Penicillium oxalicum*, *Trichoderma harzianum*, *P. anomala*, *Klebsialla* sp., *Yersinia intermedia* (Singh *et al.*, 2020). They contain β-sheets surrounded by α-helices, more flexible structure, and are less resistant to high temperatures (Hermann, 2021). Their pH optima are mostly between pH 3.5-5.5. They share two conservative motifs containing histidine residues (RHGXRXP and HD) located at N- and C- termini, respectively. Enzyme action is characterized by a two-step mechanism for hydrolyzing phosphomonoesters (Mullaney and Ullah, 2003). HAPs have the ability to hydrolyze phytic acid and other types of phosphate esters, thus hav-

Table 2. Classification of phytases based on different features

Feature	Phytases	Mechanism/description/	References
Stereospecificity	3-phytases (EC 3.1.3.8)	dephosphorylation of phytic acid at the 3 position	Milko *et al.*, 2008 Zhu *et al.*, 2019
	5-phytases (E.C. 3.1.3.72).	dephosphorylation of phytic acid at the 5 position	
	6-phytases (EC 3.1.3.26)	dephosphorylation of phytic acid at the position 6	
Structural and catalytic properties	Histidine acid phosphatases (HAP),	Presence of conservative motifs with histidine in the active site; broad substrate specificity; mainly obtained by microorganisms	Singh *et al.*, 2020 Lei *et al.*, 2013 Singh and Satyanarayana, 2015 Ha *et al.,* 2000 Oh *et al.*, 2001 Mullaney and Ullah, 2003 Mullaney and Ullah, 2007 Makolomakwa, 2018
	Purple acid phosphatases (PAP),	Purple or pink color in solution due to the presence of a dinuclear iron centre; low activity; mainly reported in plants	
	β-propeller phytases (BPP),	Calcium dependent thermostable metalloproteins; six bladed tertiary structure; substrate of BPPs is calcium phytate**;** pH optima in neutral/ alkaline range.	
	Cysteine phytases (CP). EC 3.1.3.48 (PTP)	Contain catalytic cysteine; presence of larger and deeper pocket in the active site, facilitating the enzyme reaction; obtained by *S. ruminantium*.	
pH optimum	Acid phytases	pH optimum 2.0 – 6.0	Mullaney and Ullah, 2003 Singh and Satyanarayana, 2011
	Alkaline phytases	pH optimum 7.0 - 8.0	Konietzny and Greiner, 2002

ing a broad range of substrate specificity.

PAPs are metalloenzymes that exist as homodimeric glycoproteins with a dinuclear catalytic center composed of Fe^{2+} and Fe^{3+}, Zn^{2+}, Mg^{2+}, or Mn^{2+} (Oh *et al*., 2001; Dionisio *et al*., 2007). This underlines the fact that for some enzymes metal free phytate is required, whereas others can deal with chelated ions in the phytate structure. It was proposed that the weak activity of this enzyme is an advantage during seed germination because the slow release of phosphorus is of particular importance to germinating plants (Outchkourov and Petkov, 2019). PAPs were originally identified in germinating soybean seedlings and are found mainly in plant species (Dionisio *et al*., 2011).

CPs are active under acidic conditions and contain a catalytic cysteine residue in the active site. These enzymes consist of a small and a large domain with a specific configuration of α-helices and β-sheets. The larger domain harbors the catalytically important HCXXGXXR(T/S) sequence motif (Outchkourov and Petkov, 2019). The enzyme was first found and characterized in the ruminal bacterium, *Selenomonas ruminantium* (Mullaney and Ullah, 2007).

BPPs are known for their rigidity due to their six-bladed β-propeller architecture. They are more thermostable compared to other phytases and are highly specific for phytate. BPPs require Ca^{2+} ions for their activity and thermostability. Such enzymes are documented mainly in *Bacillius* represesntatives (*Bacillus* spp., *Bacillus psychrotolerans*), but also *Sphingomonas wittichii*, *Enterobacter* sp., *Geobacillus thermocatenulatus* (Wulandari *et al*., 2015; Kalsi *et al.*, 2016; Jain *et al*., 2018; Jorquera *et al.*, 2018; Sanangelantoni *et al*., 2018).

Range of pH-optima

Phytases can be categorized into acid, neutral, and alkaline phytases based on their pH optimum (Milko *et al*., 2008). Fungi are the main source of acidic phytases (Singh and Satyanarayana, 2011). They have their optimum in the pH range of 2.0 - 6.0. Among the bacteria, *E. coli* and *Klebsiella* phytases show the highest activity in the acidic pH region while *Bacillus* phytase performed best in a neutral pH (Kour *et al.*, 2020). Neutral phytases are less numerous and are isolated from few microorganisms (thermotolerant *A. flavus*, *B. subtilis* subsp. subtilis JJBS250, and *Bacillus nealsonii* ZJ0702) (Yu and Chen, 2013; Gaind and Singh, 2015; Jain *et al*., 2018). Phytase from *Alcaligenes* shows maximal activity in the pH range of 7.0 – 8.0 (Nassiri and Ariannejad, 2015). Other alkaline phytases are found in plants (*Typha latifolia* and *Lilium longixorum*), and in some bacteria (*Bacillus amyloliquefaciens*, *B. laevolacticus*) (Gulati *et al*., 2007; Singh *et al*., 2020).

Localization of phytases in the microbial cell

Phytase enzyme localization in microbial cells has been studied in various organisms. Microbial phytase activity in eukaryotes and prokaryotes could be extracellular or intracellular. It was generally accepted that most of the bacterial phytases are cell-bounded (with exceptions) whereas fungal enzymes are extracellular (Konietzni and Greiner, 2004). The majority of LABs are known to produce phytases intracellularly. However, in the last few years, reports have been trending on extracellular phytases produced by LABs (Sharma *et al*., 2020). For example, intracellular phytase in bacteria was found in some LABs as *Lactobacillus brevis.* Some bacteria possess intra- and extracellular phytase. Both activities were detected in *L. plantarum* (Sumengen *et al*., 2012).

Extracellular phytase activity

It was found in LAB strains isolated from boza (traditionally fermented beverage) such as *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus pentosus*, *Leuconostoc lactis*, and *Pediococcus pentosaceus*. It was found that *Pediococcus pentosaceus* EK1 strain showed the highest activity. This leads the authors to believe that traditionally produced bozas could be used as a potential starter culture reservoir for sourdough fermentation with significantly higher extracellular phytase activities, thus challenging opportunities to lower antinutritional factors, in particular, phytic acid or phytate in the foods for the consumers (Doğan and Tekiner, 2020). Fasimoye and coworkers (2014) purified extracellular phytase from *B. licheniformis* PFBL-03. Also, different strains *B. subtilis* were found to produce extracellular phytase (Javaid *et al*., 2022; Trivedi *et al*., 2022). Extracellular phytase activity was shown also in *Bacillus megaterium* (Kumar *et al.,* 2013), *Paenibacillus* sp. (Khianngam *et al.,* 2017), *Enterobacter cloacae* and other *Enterobacter* representatives (Yoon *et al*., 1996; Onawola *et al.,* 2019), and *E. coli* (Nagar *et al.*, 2021).

Phytases of *E. coli* were found to be periplasmatic enzymes, and *S. ruminantium* and *Mitsuokella multiacidus* have their phytases bounded by the outer membrane (Greiner *et al*., 1993; D'Silva *et al*., 2000).

In general, phytase enzymes are produced by fungi as part of their extracellular enzyme systems.

The phytase enzymes are secreted by the fungus into the surrounding environment, where they can interact with phytate molecules in the soil or in other organic material. It was reported production of an extracellular phytase from a thermophilic fungi *H. nigrescens* in solid-state fermentation (Bala *et al*., 2014) and *Rhizomucor pusillus*, isolated from composting soil (Chadha *et al*., 2004). Recently it was reported production of extracellular phytase by three thermophilic fungi: *Thermomyces lanuginosus, Talaromyces luteus,* and *Rhizomucor pusillus* (Kumar *et al*., 2021). Phytase enzymes have been observed to be localized within intracellular organelles such as the vacuole or the endoplasmic reticulum in certain species of fungi. This type of intracellular localization can assist the fungus in the preservation and recycling of phosphate, which is a crucial nutrient required for fungal growth and metabolism (Corrêa and de Araújo, 2020).

According to some studies yeasts are good candidates for phytase production and some of them have been already characterized (Ragon *et al*., 2009; Kłosowski *et al*., 2018; Pires *et al*., 2019). These enzymes show different localizations: in some species, phytases are extracellular enzymes, and in others are cell-bound or released in the periplasmic space. The extracellular phytase and cell-bound activity in *Cyberlindnera jadinii* was detected together with cell-bound activity in *Kluyveromyces marxianus*, and *Torulaspora delbrueckeii* (Capusoni *et al*., 2021). Extracellular phytase in *S. cerevisiae* was reported by some authors (Roopashri and Varadaraj, 2015; Kłosowski *et al*., 2018). However, some cases have been found, such as *Candida krusei* (Quan *et al*., 2002), *P. anomala,* and *S. castelli* (Kaur *et al*., 2007), that have a phytase associated with the cell. In another yeast*, Schwanniomyces occidentalis* phytase activity was determined in the cell wall fraction (cell wall-bound phytase), the protoplasm (intracellular phytase), and the culture medium (extracellular phytase). According to the results, it was concluded that the remaining 90% of enzymatic activity is associated with the cell walls and cell membrane-associated fraction (Molina *et al*., 2021).

Factors affecting phytase activity

Studying the influence of various substances on the enzymatic activity of phytases is of particular interest in recent years. Many authors have investigated the effect of various metal ions, inhibitors, detergents, organic solvents, and proteolytic enzymes on phytase activity.

The affinity site of phytases is the region of the enzyme that specifically recognizes and binds to phytic acid or its derivatives. The amino acid residues involved in the binding of phytic acid can vary between different classes and subtypes of phytases, but in general, they are located in a conserved region of the enzyme that is close to the active site. The binding of phytic acid to the affinity site is an essential step for the catalytic activity of phytases, as it positions the substrate in the optimal orientation for the hydrolysis reaction to occur. Some phytases have been shown to have allosteric sites that can be targeted by specific effectors. For example, some studies have found that the activity of BPP phytases can be modulated by allosteric effectors, such as sulfate or phosphate ions. The affinity of phytases for their substrates can also be affected by factors such as pH, temperature, and the presence of metal ions or other molecules that can compete for the binding site.

Effect of various ions

Phytase activity can be influenced by different ions, which can affect the stability, activity, and specificity of the enzyme. Some of the most common ions that affect phytase activity are Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Fe^{2+}/Fe^{3+}. In the literature, there is also information about the influence of phytase activity of other ions such as Ag^{2+}, Hg^{2+}, Co^{2}, $Al^{3+,}$ and Ba^{2+} (Aly *et al*., 2015).

In general, calcium ions can help to stabilize the structure of phytase enzymes, increasing their thermal stability and resistance to proteolysis. Calcium can also improve the activity of phytase enzymes, by increasing the binding affinity of the enzyme for its substrate. The unique Ca^{2+}-dependent catalytic properties of the phytase from *B. amyloliquefaciens* DS11 are reported by Oh *et al*. (2001). The binding of calcium ions in the active site is essential for enzyme activity. Phytase can degrade only the calcium-phytate complex. Interestingly, excess amounts of phytate and calcium ions which are not bound together appeared to be competitive inhibitors (Oh *et al*., 2001). While bacterial phytases are known to be strongly affected by metal ions, the activity of fungal phytases is not as heavily dependent on them (Wyss *et al*., 1999). Although it is well-established that the association of Ca^{2+} ions with β-propeller phytases can enhance their activity in *Bacillus* species, this type of dependency is not commonly observed in fungal phytases (Kerovuo *et al.,* 2000). Manganese is a divalent cation that can also affect the stability and activity of enzymes, and it has been shown to have both positive and negative effects on phytase

activity, as it can be seen from Table 3. Overall, the effect of Mn^{2+} ions on phytase activity can be complex and dependent on several factors, including the specific bacterial strain, the concentration of the ion, and the pH and temperature conditions of the reaction. However, Mn^{2+} ions can affect the activity of phytase enzymes, and understanding these effects can be important for optimizing phytase production and activity in different applications. Zinc ions are known to be important cofactors for some phytase enzymes, and can be required for their optimal activity (Strater *et al*., 1995; Olczak *et al.,* 2003). Zinc can also increase the stability of some phytase enzymes, by protecting them from proteolytic degradation. Phytases from various types and sources are affected by these ions in different ways and degrees. Our known examples from the available literature are summarized in Table 3.

Chelating agents such as EDTA and phthalate have been shown to stimulate the phytase activity of HAPs. This is because these agents can bind to metal ions, which would otherwise inhibit the activity of the enzyme by forming complexes with phytic acid. By removing these metal ions, chelat-

Table 3. Effect of metal ions on some microbial phytases

Source	Activation	Inhibition	Reference
Enterobacter sp. 4	nd	Zn^{2+}, Ba^{2+}, Cu^{2+}, Al^{3+}	Yoon *et al.,* 1996
B. subtilis subsp. subtilis JJBS250	Ca^{2+}, K^{+}, Co^{2+}	Mg^{2+}, Al^{2+}, Fe^{2+}	Jain *et al.,* 2018
Klebsiella pneumoniae 9-3B	Ca^{2+}	Zn^{2+}, Mg^{2+},Fe^{2+}, Co^{2+}, Mn^{2+}	Escobin-Mopera *et al.,* 2012
Weissella halotolerans	Co^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+}	Ag^{2+}, Zn^{2+}, Cr^{2+},Fe^{2+}	Demir *et al.*, 2017
Lactobacillus plantarum CRL1964	Co^{2+}	Ni^{2+}, Cd^{2+}, Cu^{2+}, Fe^{2+}	Sandez Penidez *et al.,* 2020
Lactobacillus pentosus CFR3	Co^{2+}	Hg^{2+}, Fe^{2+}	Amritha *et al.,* 2017
Lactobacillus brevi	Cu^{2+}, Mg^{2+}, Mn^{2+}, Hg^{2}, Zn^{2+}	Fe^{2+}, Co^{2+}, Ca^{2+}	Sümengen *et al.,* 2012
Aspergillus aculeatus	nd	$Al^{3+} > Mg^{2+} > Fe^{2+} > Ca^{2+} > Na^{2+}$	Saxena *et al.,* 2020
Aspergillus niger 7A1	Cu^{2+}, Mg^{2+}, Mn^{2+}, Hg^{2+}, Cd^{2+}, Ba^{2+}, Zn^{2+}	Ca^{2+}	Neira-Vielma *et al.,* 2018
A. niger CFR 335	$Cu^{2+} > Ca^{2+} > Mg^{2+} > Zn^{2+}$	$Mn^{2+} > Fe^{3+} > Al^{3+}$	Gunashree and Venkateswaran, 2015
A. ficuum	nd	Cu^{2+}, Hg^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}	Ullah and Cummins, 1988
Acremonim zeae	nd	Ca^{2+}, Ag^{2+}, Fe^{2+}, Cu^{2+}	Pires *et al.,* 2019
Kluyveromyces marxianus	Ca^{2+}, Ag^{2+}, Mg^{2+}	Zn^{2+}, Fe^{2+}	Pires *et al.,* 2019
A. fumigatus	Ca^{2+}, Cu^{2+}, Fe^{2+}	Zn^{2+}, Hg^{2+}, Al^{3+}	Sanni *et al.,* 2019
Streptomyces luteogriseus R10	Ca^{2+}, Mg^{2+}, Mn^{2+}	Ag^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}	Yoon *et al.,* 1996
Thermomyces lanuginosus IMI 096218	Fe^{2+}, Fe^{3+} Ca^{2+}, Mg^{2+},K^{+}	Ag^{2+}, Co^{2+}, Zn^{2+}	Bujna *et al.,* 2016
Malbranchea sulfurea	Zn^{2+}, Fe^{2+}, Fe^{3+}	Na^{+}, Li^{+}, Mg^{2+}, Hg^{2+}, Mn^{2+}, Cd^{2+}	El-Gindy *et al.,* 2009
Candida melibiosica 2491	Ba^{2+}, Mn^{2+}, K^{+}	Ag^{2+}, Hg^{2+}, Fe^{3+}, Hg^{2+}, Zn^{2+}>-Cu^{2+}> Co^{2+}	Georgiev *et al.,* 2018
S. cerevisiae MTCC 5421	nd	Hg^{2+}, Zn^{2+}, Ba^{2+}, Cd^{2+}	Roopashri and Varadaraj, 2015
P. anomala	nd	Cu^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+}	Vohra and Satyanarayana, 2002
Kodamaea ohmeri	Mn^{2+}, Ca^{2+}, K^{+}, Li^{+}, Na^{+}, Ba^{2+}, Mg^{2+}, Co^{2+}	Cu^{2+}, Hg^{2+}, Fe^{2+}, Fe^{3+}, Ag^{+}, Zn^{2+}	Li *et al.,* 2008
Peniophora lycii	nd	Fe^{2+}, Zn^{2+}	Santos *et al,* 2015

ing agents increase the availability of free phytic acid for the HAPs to hydrolyze (Wyss *et al*., 1999).

Phytase activity and surfactants

The addition of surfactants can significantly affect phytase activity. Surfactants can improve enzyme stability and enhance enzyme-substrate interactions, ultimately leading to increased phytase activity. However, the effects of surfactants on phytase activity are highly dependent on the type of surfactant used and the specific characteristics of the enzyme. A study shows that activity of protease-resistant and thermostable phytase from *B. subtilis subsp. subtilis* JJBS250 was increased about 2-fold by surfactants like Tween 20, Triton X-100, and Tween 80 as compared to control while SDS has an inhibitory effect. Similarly, in the case of *Sporotrichum thermophile and Thermomyces lanuginosus*, the addition of SDS decreases the phytase activity whereas it was enhanced in the presence of Tweens. The presence of SDS also inhibited the phytase activity in *Nocardia sp*. MB 36 and *Shigella sp.* CD2 (Jain *et al*., 2018). The detergents Triton X-100 and Tween-80 (non-ionic surfactants) at 0.05% inclusion level showed no effect on phytase activity from *A. foetidus* MTCC 1168. However, the reducing agent, 2-mercaptoethanol, and denaturing agents such as SDS and Tween-20 at similar inclusion levels showed inhibitory effects. The authors suggest that the enhanced stability of this enzyme could be attributed to glycosylation, similar to those observed in recombinant phytase (Ajith *et al*., 2019).

Effect of plant extracts on enzyme activity

Various compounds isolated from plants could influence the enzyme activity of phytase. Bekalu *et al*. (2017) reported that protein extracts of wheat, barley, rice, and maize have adverse effects on the enzyme activity of *A. ficuum* phytase. The maximum inhibition was achieved with grain protein extracts from infected wheat by *F. graminearum*. The observed suppressive effect is due to the protease activity of the aspartic proteinase type.

Polyphenols are a class of organic compounds that are commonly found in plant-based foods, including grains, fruits, and vegetables. They are known to have antioxidant properties and have been associated with various health benefits, such as reducing the risk of chronic diseases (Zhang *et al*., 2022). The inhibition of plant phytases by the polyphenols is described as a physiological phenomenon during germination. The polyphenol phloroglucinol (1,3,5-benzenetriol) non-competitively inhibited *Cucurbita maxima* phytase *in vitro* (Bekalu *et al*., 2017). By inhibiting phytase activity, polyphenols may reduce the effectiveness of phytase as a feed additive and impact animal growth and health.

Proteolityc stability

An important condition of a phytase to have a potential for commercial use is its proteolytic resistance. The addition of this enzyme promotes greater uptake of phosphorus and also contributes to a decrease in the levels of phosphorus excreted by animals. It is known that many proteolytic enzymes are secreted in the intestinal tract of animals, which could have a negative impact on the activity of phytases. Susceptibility to proteases usually limits the application of phytase. A lot of efforts are directed towards the selection of phytases resistant to various proteases. Previous studies have shown the presence of large numbers of phytases that retain their activity when treated with different proteolytic enzymes (Menezes-Blackburn *et al*., 2022). Protease resistance and high activity of enzymes facilitate their biotechnological and medical application. Protease-resistant phytases have been isolated from bacteria and fungi. Also, in recent years, efforts have been made to create protease-resistant phytases using bioengineering (Lei and Porres, 2003). Later, Jain *et al*. (2018) studied the purified phytase from *B. subtilis* subsp. *subtilis* JJBS250. They reported that the enzyme was resistant to different proteases (0.05%) such as trypsin and pepsin at 37^{o}C. The susceptibility of feed enzymes to proteolysis is crucial because it determines the site and rate of enzyme inactivation. The narrow pH range of current commercial phytase is a concern because it operates within a range similar to that of the stomach or crops, which may limit its effectiveness in the small intestine. The rate of inactivation caused by pepsin at acidic pH levels will likely be a limiting factor for the effectiveness of these enzymes (Kour *et al*., 2020). The effectiveness of four phytases derived from different sources, including *Bacillus, E. coli, Klebsiella*, and *A. niger*, was evaluated *in vitro* and *in vivo*. *Bacillus* phytase was found to be more heat-resistant, while *E. coli* and *Klebsiell*a phytases were more stable against proteolytic inactivation. Specifically, the *E. coli* and *Klebsiella* phytases retained 89.7% of their activity after one hour of exposure to pepsin at 40°C, as reported by Elkhalil *et al*. (2007).

Fungal phytases differ greatly in their ability to withstand the effects of gastrointestinal proteases like pepsin and trypsin. For instance, when exposed

to trypsin, *R. mucilaginosa*, *A. niger*, and *A. oryzae* phytases maintained 75, 10, and 84% of their activity, respectively. On the other hand, *P. polonicum* MF82 phytase retained 100% of its activity in the presence of trypsin. This exceptional resistance to proteases makes *P. polonicum* MF82 phytase particularly valuable for industrial applications (Yu *et al.,* 2015). Protease-resistant and acidic phytase was found in *A. aculeatus* APF1. The enzyme was partially purified and analyzed and was suggested to have practical application in the food industry (Saxena *et al.,* 2020).

Additionally, the phytase from *B. subtilis* showed a comparable pepsin resistance to *A. niger* phytase, whereas its susceptibility to pancreatin digestion was shown to be similar to the bacterial histidine acid phytases (Simon and Igbasan, 2002). The high pancreatin resistance of *B. subtilis* phytase and its high susceptibility to pepsin digestion was also confirmed by Kerovuo *et al.* (2000). Furthermore, plant phytases are considered to be more susceptible to inactivation by gastrointestinal proteases. Wheat phytase was reported to be less resistant to pepsin and pancreatin than phytases of *A. niger* (Phillippy, 1999). It also has to be remembered that recombinant enzymes may differ in proteolytic resistance compared with their wild-type counterparts, as reported for *E. coli* and *A. niger* phytases produced in *P. pastoris* (Rodriguez *et al.*, 1999).

The study of Zhao *et al*. (2010) involved cloning the phytase gene from *Penicillium* sp. in *P. pastoris* and examining its characteristics. Their results showed the presence of two specific amino acid sequences (RHGXRXP and HD) and indicated that the protein was a member of the HAPs family. The recombinant phytase enzyme produced by *P. pastoris* GS115 had several typical properties similar to phytases found in other fungal strains. Interestingly, the phytase enzyme from *Penicillium* sp. showed high resistance to pepsin, which is a valuable trait for its use as a feed supplement.

Application of phytases

Phytase is an enzyme that plays a crucial role in improving the bioavailability of phosphorus and other nutrients in animal feed. Phosphorus is an essential mineral for animals, and phytate, the primary form of phosphorus in plant-based feed, is not well absorbed by animals, leading to potential nutrient deficiencies and environmental pollution. Phytase is added to animal feed to break down phytate, making phosphorus and other nutri-

Table 4. Applications of phytases

Field	Application	Reference
Animal feed supplement	Feed enzyme of monogastric animals, (poultry, swine, and fish) improves the digestibility of phosphorous, improving fish health	Dersjant-Li *et al.,* 2015 Dang *et al.,* 2022 Attia *et al.,* 2021 Humer *et al.,* 2015
Food and feed industry	Bread making, detoxification of human foods, dephytinization of wheat flour, production of plant protein isolates, soybean milk processing	Dailin *et al.,* 2019 Petrova *et al.,* 2022 Thakur *et al.,* 2022 Rizwanuddin *et al.,* 2023 Greiner and Konietzny, 2006 Lopes *et al.,* 2021
Agriculture	Plant growth improvement	Kour *et al.,* 2020 Gaind and Nain, 2015 Singh and Satyanarayana, 2011
Pharmaceutical applications	Antioxidant in food products, anti-cancer agent, against coronary heart disease, against dental caries	Sharma *et al.,* 2020 Jatuwon *et al.,* 2020
Environmental protection	Soil remediation, treatment of toxic pollutants	Zhou *et al.,* 2022
Fuel industries	Improving the performance of thermostable α-amylase in the liquefaction stage, contribute to reducing the viscosity of fermentable sugars and improve their availability, reduce the levels of phytic acid in end-product	Vasudevan *et al.,* 2019 Khullar *et al.,* 2011 Shetty *et al.,* 2008

ents more available for absorption. Until the last few years, the application of exogenous phytase enzymes was mainly confined to animal feed, but recently scientists and entrepreneurs started to realize their great potential as food supplementation for human health. Removal of phytate, or at least part of it, by adding exogenous phytase can result in significant improvement of the bioavailability of essential minerals. Therefore, phytase seems to be a potential tool to reduce the risk of mineral deficiency among populations depending on unrefined cereals or pulses as a basal diet as well as for nutritionally vulnerable groups such as pregnant women, strict vegetarians, and dwellers of underdeveloped countries. Furthermore, the use of phytases in the processing and manufacturing of foods such as bread, plant protein isolates, corn steepliquor, etc., can positively influence the purity and yield of the products.

In the last decade, there has been increased interest in the potential application of microbial phytases for the detoxification of foods consumed by humans. Various applications of phytases are summarized in Table 4.

Conclusion

Even though phytase was first reported over a century ago, its commercial significance remains high. With the discovery of new uses for phytase, the demand for this enzyme has increased significantly. Bacteria and fungi isolated from various natural niches are intensively studied as potential phytase producers. The desirable characteristics of enzymes such as high activity and resistance to different inhibitors of the enzymes and the method of production the prime importance. Therefore, the influence of various potential inhibitors, inorganic and organic substances, as well as the mechanisms of inhibition, are being intensively studied. Modern approaches and molecular methods are also applied. These include heterologous expression of phytase genes in new hosts to increase the yield of the enzyme and obtain enzymes that are resistant to peptidases, high temperature, and adverse pH of the medium. Taken together, these recent findings and open questions continue to challenge scientists.

References

Abudabos, A. M. (2012). Intestinal phytase activity in chickens (*Gallus domesticus*). *Afr. J. Microbiol. Res.* **6**: 4932-4938.

Abudabos, A. M. (2012). Phytate phosphorus utilization and intestinal phytase activity in laying hens. *Ital. J. Anim. Sci.* **11**: e8.

Ajith, S., J. Ghosh, D. Shet, S. ShreeVidhya, B. D. Punith, A.V. Elangovan (2019). Partial purification and characterization of phytase from *Aspergillus foetidus* MTCC 11682. *AMB Express* **9**: 3.

Alves, N. M., L. H. S. Guimaraes, R. H. Piccoli, P. G. Cardoso (2016). Production and partial characterization of an extracellular phytase produced by *Muscodor* sp. under submerged fermentation. *Adv. Microbiol*. **6**: 23-32.

Aly, M., S. Tork, S. M. Al-Garni, S. A. Kabli (2015). Production and characterization of phytase from *Streptomyces luteogriseus* R10 isolated from decaying wood samples. *Int. J. Agric. Biol*. **17**: 515-522.

Amitha, J., S. Balaji (2015). Drug-likeness of phytic acid and its analogues. *Open Microbiol. J*. **9**: 141-149.

Amritha, G. K., P. M. Halami, G. Venkateswaran (2017). Phytate dephosphorylation by *Lactobacillus pentosus* CFR3. *Int. J. Food. Sci. Technol.* **52**: 1552-1558.

Attia, Y. A., F. Bovera, M. A. Al-Harthi, A. E. R. E. T. El-Din, W. Said Selim (2021). Supplementation of microbial and fungal phytases to low protein and energy diets: Effects on productive performance, nutrient digestibility, and blood profiles of broilers. *Agriculture* **11**: 414.

Bala, A., J. J. Sapna, A. Kumari, B. Singh (2014). Production of an extracellular phytase from a thermophilic mould *Humicola nigrescens* in solid state fermentation and its application in dephytinization. *Biocatal. Agric. Biotechnol*. **3**: 259-264.

Bekalu, Z. E., C. K. Madsen, G. Dionisio, H. Brinch-Pedersen (2017). *Aspergillus ficuum* phytase activity is inhibited by cereal grain components. *PLoS One* **12**: e0176838.

Bohn, L., A. S. Meyer, S. K. Rasmussen (2008). Phytate: Impact on environment and human nutrition. A challenge for molecular breeding. *J. Zhejiang Univ. Sci. B*. **9**: 165-191.

Bouajila, A., H. Ammar, M. Chahine, M. Khouja, Z. Hamdi, J. Khechini, A.-F. Z. Salem, A. Ghorbel, S. López (2019). Changes in phytase activity, phosphorus and phytate contents during grain germination of barley (*Hordeum vulgare L.*) cultivars. *Agrofor. Syst*. **94**: 1151-1159.

Bujna, E., J. Rezessy-Szabo, D. Nguyen (2016). Production and some properties of extracellular phytase from *Thermomyces lanuginosus* IMI 096218 on rice flour as substrate. *Mycosphere* **7**: 1576-1587.

Capusoni, C., I. Serra, S. Donzella, C. Compagno (2021). Screening for yeast phytase leads to the identification of a new cell-bound and secreted activity in *Cyberlindnera jadinii* CJ2. *Front. Bioeng. Biotechnol*. **9**: 662598.

Castro-Alba, V., C. E. Lazarte., B. Bergenståhl, Y Granfeldt (2019). Phytate, iron, zinc, and calcium content of common Bolivian foods and their estimated mineral bioavailability. *Food Sci. Nutr*. **7**: 2854-2865.

Chadha, B. S., G. Harmeet, M. Mandeep, H. S. Saini, N. Singh (2004). Phytase production by the thermophilic fungus *Rhizomucor pusillus*. *World J. Microbiol. Biotechnol.* **20**: 105-109.

Chelius, M. K., R. J. Wodzinski (1994). Strain improvement of *Aspergillus niger* for phytase production. *Appl. Microbiol. Biotechnol*. **41**: 79-83.

Collopy, P. D., D. J Royse (2004). Characterization of phytase activity from cultivated edible mushrooms and their production substrates. *J. Agr. Food Chem*. **52**: 7518-7524.

Corrêa, T. L. R., E. F. de Araújo (2020). Fungal phytases: from genes to applications. *Braz. J. Microbiol.* **51**: 1009–1012.

Da'Silva, C. G., H. D. Bae, L. J. Yanke, K. J. Cheng, L. B.

Selinger (2000). Localization of phytase in *Selenomonas ruminantium* and *Mitsuokella multiacidus* by transmission electron microscopy. *Can. J. Microbiol.* **46**: 391-395.

Dailin, D. J., S. Z. Hanapi, E. A. Elsayed, D. Sukmawati, N. I. W. Azelee, J. Eyahmalay, V. Siwapiragam, H. El Enshasy (2019). Fungal Phytases: Biotechnological Applications in Food and Feed Industries. In: Yadav, A., S. Singh, S. Mishra, A. Gupta (Eds.), Recent Advancement in White Biotechnology Through Fungi. Fungal Biology. Springer, Cham, pp. 65-99.

Dang, D. X., S. G. Chun, I. H. Kim (2022). Feeding broiler chicks with *Schizosaccharomyces pombe*-expressed phytase-containing diet improves growth performance, phosphorus digestibility, toe ash, and footpad lesions. *Anim. Biosci*. **35**: 1390-1399.

Demir, Y., M. Şenol Kotan, N. Dikbaş, Ş. Beydemir (2017). Phytase from *Weissella halotolerans*: purification, partial characterisation and theeffect of some metals. *Int. J. Food Prop*. **20**: 2127-2137.

Dersjant-Li, Y., A. Awati, H. Schulze, G. Partridge (2015). Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *J. Sci. Food. Agric*. **95**: 878-896.

Dionisio, G., P. B. Holm, H. Brinch-Pendersen (2007). Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) multiple inositol polyphosphate phosphatases (MINPPs) are phytases expressed during grain filling and germination. *Plant Biotechnol. J*. **5**: 325-338

Dionisio, G., C. K. Madsen, P. B. Holm, K. G. Welinder, M. Jorgensen, E. Stoger, E. Arcalis, H. Brinch-Pedersen (2011). Cloning and characterization of purple acid phosphatases from wheat, barley, maize, and rice. *Plant Physiol*. **156**: 1087-1100.

Doğan, M., İ. H. Tekiner (2020). Extracellular phytase activites of lactic acid bacteria in sour-dough mix prepared from traditionally produced Boza as starter culture. *Food Health* **6**: 117-127.

El-Gindy, A. A., Z. M. Ibrahim, U. F. Ali, O. M. El-Mahdy (2009). Extracellular phytase production by solid-state cultures of *Malbranchea sulfurea* and *Aspergillus niveus* on cost-effective medium. *J. Agric. Biol. Sci*. **5**: 42-62.

Elkhalil, E. A., K. Männer, R. Borriss, O. Simon (2007). In vitro and in vivo characteristics of bacterial phytases and their efficacy in broiler chickens. *Br. Poult. Sci.* **48**: 64-70.

Escobin-Mopera, L., M. Ohtani, S. Sekiguchi, T. Sone, A. Abe, M. Tanaka, V. Meevootisom, K. Asano (2012). Purification and characterization of phytase from *Klebsiella pneumoniae* 9-3B. *J. Biosci. Bioeng.* **113**: 562-567.

Faba-Rodriguez, R., Y. Gu, M. Salmon, G. Dionisio, H. Brinch-Pedersen, C.A. Brearley, A. M. Hemmings (2022). Structure of a cereal purple acid phytase provides new insights to phytate degradation in plants. *Plant Commun.* **3**: 100305.

Fasimoye, F., F. M. Olajuyigbe, M. D. Sanni (2014). Purification and characterization of a thermostable extracellular phytase from *Bacillus licheniformis* PFBL-03. *Prep. Biochem. Biotechnol*. **44**: 193-205.

Gaind, S., L. Nain (2015). Soil–phosphorus mobilization potential of phytate mineralizing fungi. *J. Plant Nutr.* **38**: 2159-2175.

Gaind, S., S. Singh (2015). Production, purification and characterization of neutral phytase from thermotolerant *Aspergillus flavus* ITCC 6720. *Int. Biodeter. Biodegr*. **99**: 15-22.

Georgiev, D., G. Dobrev, S. Shilev (2018). Purification and properties of a phytase from *Candida melibiosica* 2491. *Emir. J. Food Agric*. **30**: 927-934.

Greiner, R. T., U. Konietzny, Kl-D. Jany (1993). Purification and characterization of two phytases from *Escherichia coli*. *Arch. Biochem. Biophys.* **303**: 107-113.

Greiner, R., U. Konietzny (2006). Phytase for food application. *Food Technol. Biotech.* **44**: 125-140.

Gulati, H. K., B. S. Chadha, H. S. Saini (2007). Production and characterization of thermostable alkaline phytase from *Bacillus laevolacticus* isolated from rhizosphere soil. *J. Ind. Microbiol. Biotechnol*. **34**: 91-98.

Gunashree, B., G. Venkateswaran (2015). Extracellular phytase from *Aspergillus niger* CFR 335: purification and characterization. *J. Food Sci. Technol.* **52**: 4558-4564.

Guo, M. J., Y. P. Zhuang, J. Chu, S. L. Zhang, A. S. Xiong, R. H. Peng, Q. H. Yao (2007). Production and purification of a novel thermostable phytase by *Pichia pastoris* FPHY34. *Process. Biochem*. **42**: 1660-1665.

Gupta, R. K., S. S. Gangoliya, N. K. Singh (2015). Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. *J. Food Sci. Technol.* **52**: 676-684.

Ha, N. C., B. C. Oh, S. Shin, H. J. Kim, T. K. Oh, Y. O. Kim, B. H. Oh (2000). Crystal structures of a novel, thermostable phytase in partially and fully calcium-loaded states. *Nat. Struct. Biol*. **7**: 147-153.

Haros, M., M. Bielecka, Y. Sanz (2005). Phytase activity as a novel metabolic feature in *Bifidobacterium*. *FEMS Microbiol. Lett*. **247**: 231-239.

Hassouni, H., M. I. Alaoui, I. G. Perraud, C. Augur, S. Roussos (2006). Effect of culture media and fermentation parameters on phytase production by the thermophilic fungus *Myceliophthora thermophila* in solid state fermentation. *Micol. Aplicada Int*. **18**: 29-36.

Herrmann, K. R. (2021). Phytase engineering for efficient phosphate recovery from press cakes [dissertation]. Aachen: RWTH Aachen University.

Hou, X., Z. Shen, N. Li, X. Kong, K. Sheng, J. Wang, Y. Wang (2020). A novel fungal beta-propeller phytase from nematophagous *Arthrobotrys oligospora*: characterization and potential application in phosphorus and mineral release for feed processing. *Microb. Cell Fact.* **19**: 1-13.

Howson, S. J., R. P. Davis (1983). Production of phytate hydrolyzing enzyme by some fungi. *Enzyme Microb. Technol.* **5**: 377-382.

Humer, E., C. Schwarz, K. Schedle (2015). Phytate in pig and poultry nutrition. *J. Anim. Physiol. Anim. Nutr*. **99**: 605-625.

Jain, J., A. Kumar, D. Singh, B. Singh (2018). Purification and kinetics of a protease-resistant, neutral, and thermostable phytase from *Bacillus subtilis subsp. subtilis* JJBS250 ameliorating food nutrition. *Prep. Biochem. Biotechnol*. **48**: 718-724.

Jatuwong, K., N. Suwannarach, J. Kumla, W. Penkhrue, P. Kakumyan, S. Lumyong (2020). Bioprocess for production, characteristics, and biotechnological applications of fungal phytases. *Front. Microbiol*. **11**: 188.

Javaid, A., M. Yasinzai, K. S. Kiyani (2022). Enhanced phytase production from indigenous *Bacillus subtilis* KT004404 by response surface methodology to be used as poultry feed supplement. *Pak. J. Zool.* **55**: 893-904.

Jorquera, M. A., S. Gabler, N. G. Inostroza, J. J. Acuna, M. A. Campos, D. Menezes-Blackburn, R. Greiner (2018). Screening and characterization of phytases from bacteria isolated from Chilean hydrothermal environments. *Microb. Ecol*. **75**: 387-399.

Kalkan, S. O., E. Bozcal, E. E. Hames Tuna, A. Uzel (2020). Characterisation of a thermostable and proteolysis resistant phytase from *Penicillium polonicum* MF82 associated with the marine sponge *Phorbas* sp. *Biocatal. Biotransform.* **38**: 469-479.

Kalsi, H. K., R. Singh, H. S. Dhaliwal, V. Kumar (2016) Phytases from *Enterobacter* and *Serratia* species with desirable characteristics for food and feed applications. *Biotech* **6**: 64.

Kaur, P., G. Kunze, T. Satyanarayana (2007). Yeast phytases: present scenario and future perspectives. *Criti. Rev. Biotechnol.* **27**: 93-109.

Kaur, R., A. Saxena, P. Sangwan, A. N. Yadav, V. Kumar, H. S. Dhaliwal (2017). Production and characterization of a neutral phytase of *Penicillium oxalicum* EUFR-3 isolated from Himalayan region. *Nusantara Biosci*. **9**: 68-76.

Kerovuo, J., I. Lappalainen, T. Reinikainen (2000). The metal dependence of *Bacillus subtilis* phytase. *Biochemi. Biophys. Res. Commun.* **268**: 365-369.

Khianngam, S., Y. Pootaeng-On, A. Sonloy, J. Kajorn-Aroonkij, S. Tanasupawat (2017). Characterization and comparison of phytase production by *Bacillus* and *Paenibacillus* strains from Thai soils. *Malays. J. Microbiol.* **13**: 318-325.

Khullar, E., J. K. Shetty, K. D. Rausch, M. E. Tumbleson, V. Singh (2011). Use of phytases in ethanol production from E□Mill corn processing. *Cereal Chem.* **88**: 223-227.

Klanbut, K., Y. Peerapornpisarn, C. Khanongnuch, M. Ishii (2004). Phytase from some strains of thermophilic bluegreen algae. *Jpn. J. Phycol*. **2**: 57-60.

Kłosowski, G., D. Mikulski, O. Jankowiak (2018). Extracellular phytase production by the wine yeast *S. cerevisiae* (Finarome strain) during submerged fermentation. *Molecules* **23**: 848.

Konietzny, U., R. Greiner (2002). Molecular and catalytic properties of phytate-degrading enzymes (phytases). *Int. J. Food Sci. Technol*. **37**: 791-812.

Konietzny, U., R. Greiner (2004). Bacterial phytase: Potential application, *in vivo* function and regulation of its synthesis. *Braz. J. Microbiol*. **35**: 11-18.

Kour, D., T. Kaur, N. Yadav, A. A. Rastegari, B. Singh, V. Kumar, A. N. Yadav (2020). Chapter 10 - Phytases from microbes in phosphorus acquisition for plant growth promotion and soil health. In: Rastegari, A. A., A. N. Yadav, N. Yadav (Eds), New and Future Developments in Microbial Biotechnology and Bioengineering, Elsevier, pp. 157-176.

Kumar, D., S. Rajesh, P. Balashanmugam, L. J. Rebecca, P. Kalaichelvan (2013). Screening, optimization and application of extracellular phytase from *Bacillus megaterium* isolated from poultry waste. *J. Mod. Biotechnol*. **2**: 46-52.

Kumar, V., A. K. Sinha (2018). Chapter 3-General aspects of phytases. In: Nunes, C. S., V. Kumar (Eds.), Enzymes in human and animal nutrition. Academic Press, London, pp. 53-72.

Kumar, R. R., E. R. Reddy, S. Girisham, S. M. Reddy (2021). Production of phytase by three thermophilic fungi. *Ind. J. Applied. Microbiol*. **23**: 18-26.

Lambrechts, C., H. Boze, L. Segueilha, G. Moulin, P. Galzy (1993). Influence of culture conditions on the biosynthesis of *Schwanniomyces castellii* phytase. *Biotechnol. Lett*. **15**: 399-404.

Lei, X. G., J. D. Weaver, E. Mullaney, A. H. Ullah, M. J. Azain (2013). Phytase, a new life for an «old» enzyme. *Annu. Rev. Anim. Biosci*. **1**: 283-309.

Lei, X. G., J. M. Porres (2003). Phytase enzymology, applications, and biotechnology. *Biotechnol. Lett*. **25**: 1787-1794.

Li, X., Z. Chi, Z. Liu, J. Li, X. Wang, N. Y. Hirimuthugoda (2008). Purification and characterization of extracellular phytase from a marine yeast *Kodamaea ohmeri* BG3. *Mar. Biotechnol.* **10**: 190-197.

Liu, C., C. Yang, Q. Yang, J. Wang, Y. Liu (2021). Effect of divalent metals on phytase activity and enzymatic kinetics of As-hyperaccumulation ferns. *Acta Agric. Scand. Section B.-Soil Plant Sci.* **71**: 112-123.

Lopes, M. M., T. C. Coutinho, J. O. Malafatti, E. C. Paris, C. P. Sousa, C. S. Farinas (2021). Immobilization of phytase on zeolite modified with iron(ii) for use in the animal feed and food industry sectors. *Process Biochem*. **100**: 260-271.

Lopez, H.W., F. Vallery, M.A. Levrat-Verny, C. Coudray, C. Demigne, C. Remesy (2000). Dietary phytic acid and wheat bran enhance mucosal phytase activity in rat small intestine. *J. Nutr*. **130**: 2020-2025.

Makolomakwa, M. (2018). Production, characterization and applications of two distinct phytases from *Thermomyces lanuginosus* SSBR [dissertation]. Durban University of Technology.

Manna, S. K., N. Maitra, C. Bandopadhyay, L. Nandi, S. Samanta (2021). Phytate mineralizing bacteria and their phytase activity from different matrices of tropical floodplain wetlands. *Explor. Anim. Med.* **11**: 220-228.

Menezes-Blackburn, D., R. Greiner, U. Konietzny (2022). Phytases: biochemistry, enzymology and characteristics relevant to animal feed use. In: Bedford et al. (Eds.), Enzymes in farm animal nutrition, 3rd edn., CABI, GB, pp. 103-123.

Milko, J., M. Oscar, M. Fumito, M. Petra, L. M. De La Maria (2008). Current and future biotechnological applications of bacterial phytases and phytase-producing bacteria. *Microbes Environ.* **23**: 182-191.

Mogal, C., A. D. Singh (2017). Mehta, Isolation and biochemical characterization of phytase from different sources. *J. Appl. Biotechnol. Bioeng*. **2**: 152-155.

Molina, D. C., G. N. Poisson, F. Kronberg, M. A. Galvagno (2021). Valorization of an Andean crop (yacon) through the production of a yeast cell-bound phytase. *Biocatal. Agric. Biotechnol*. **36**: 102116.

Mullaney, E., A. Ullah (2003). The term phytase comprises several different classes of enzymes. *Biochem. Biophys. Res. Commun.* **312**: 179-184.

Mullaney, E., A. Ullah (2007). Phytases: Attributes, catalytic mechanisms and applications. In: Turner, B. L., A. E. Richardson, E. J. Mullaney (Eds.), Inositol phosphates linking agriculture and the environment. Cambridge, MA, pp. 97-110.

Nagar, A., A. Kamble, H. Singh (2021). Preliminary screening, isolation and identification of microbial phytase producers from soil. *Environ. Exp. Biol*. **19**: 11-22.

Nassiri, M., H. Ariannejad (2015). Comparative analysis of

peripheral alkaline phytase protein structures expressed in *E. coli*. *Rep. Biochem. Mol. Biol*. **4**: 10-18.

Neira-Vielma, A. A., C. N. Aguilar, A. Ilyina, J. C. Contreras-Esquivel, M. D. G. Carneiro-da-Cunha, G. Michelena-Álvarez, J. L. Martínez-Hernández (2018). Purification and biochemical characterization of an *Aspergillus niger* phytase produced by solid-state fermentation using triticale residues as substrate. *Biotechnol. Rep*. **17**: 49-54.

Oh, B.-C., B. S. Chang, K. H. Park, N. C. Ha, H. K. Kim, B. H. Oh, T. K. Oh (2001). Calcium-dependent catalytic activity of a novel phytase from *Bacillus amiloliquefaciens* DS11. *Biochemistry* **40**: 9669-9676.

Olczak, M., B. Morawiecka, W. Watorek (2003). Plant purple acid phosphatases - Genes, structures and biological function. *Acta Bioch. Pol*. **50**: 1245–1256.

Onawola, O. O., I. S. Akande, W. O. Okunowo, A. A. Osumtoki (2019). Isolation and identification of phytase-producing *Bacillus* and *Enterobacter* species from Nigerian soils. *Nig. J. Biotech*. **36**: 127-138.

Outchkourov, N., S. Petkov (2019). Phytases for Feed Applications. In: Vogel, A., O. May (Eds.), Industrial Enzyme Applications. John Wiley & Sons, Ltd, Chichester, UK, pp. 255-285.

Palallauf, J., G. Rimbach (1997). Nutritional significance of phytic acid and phytase. *Arch. Tierernahr*. **50**: 301-319.

Petrova, P., A. Arsov, F. Tsvetanova, T. Parvanova-Mancheva, E. Vasileva, L. Tsigoriyna, K. Petrov (2022). The complex role of lactic acid bacteria in food detoxification. *Nutrients* **14**: 2038.

Phillippy, B. Q. (1999). Susceptibility of wheat and *Aspergillus niger* phytases to inactivation by gastrointestinal enzymes. *J. Agric. Food Chem.* **47**: 1385-1388.

Pires, E. B. E., A. J. de Freitas, R. L. Salgado, V. M. Guimarães, F. A. Pereira, M. R. Eller (2019). Production of fungal phytases from agroindustrial byproducts for pig diets. *Sci. Rep.* **9**: 1-9.

Pontoppidan, K., D. Pettersson, AS. Sandberg (2007). *Peniophora lycii* phytase is stabile and degrades phytate and solubilises minerals in vitro during simulation of gastrointestinal digestion in the pig. *J. Sci. Food. Agric.* **87**: 2700-2708.

Quan, C. S., S. D. Fan, L. H. Zhang, Y. J. Wang, Y. Ohta (2002). Purification and properties of a phytase from *Candida krusei* WZ-001, *J. Biosci. Bioeng.* **94**: 419-425.

Ragon, M., A. Aumelas, P. Chemardin, S. Galvez, G. Moulin, H. Boze (2008). Complete hydrolysis of myo-inositol hexakiphosphate by a novel phytase from *Debaryomyces castellii* CBS 2923. *Appl. Microbiol. Biotechnol*. **78**: 47-53.

Ragon, M., F. Hoh, A. Aumelas, L. Chiche, G. Moulin, H. Boze (2009). Structure of *Debaryomyces castellii* CBS 2923 phytase. *Acta Crystallogr. Sect. F: Struct. Biol. Cryst. Commun.* **65**: 321-326.

Rapoport, S., E. Leva, G. M. Guest (1941). Phytase in plasma and erythrocytes of various species of vertebrates. *J. Biol. Chem*. **139**: 621-632

Rix, G. D., C. Sprigg, H. Whitfield, A. M. Hemmings, J. D. Todd, C. A. Brearley (2022). Characterisation of a soil MINPP phytase with remarkable long-term stability and activity from *Acinetobacter sp. PLoS ONE* **17**: e0272015.

Rizwanuddin, S., V. Kumar, P. Singh, B. Naik, M. Chauhan, P. E. J. Saris, V. Kumar (2023). Insight into phytase-producing microorganisms for phytate solubilization and soil sustainability. *Front. Microbiol.* **14**: 912.

Rodriguez, E., J. M. Porres, Y. Han, X. G. Lei (1999). Different sensitivity of recombinant *Aspergillus niger* phytase (r-PhyA) and *Escherichia coli* pH 2.5 acid phosphatase (r-AppA) to trypsin and pepsin in vitro. *Arch. Biochem. Biophys.* **365**: 262-267.

Romano, N., V. Kumar (2018). Phytase in animal feed. In: Nunes, C. S., V. Kumar (Eds.), Enzymes in Human and Animal Nutrition. Academic Press, London, pp. 73-88.

Roopashri, A. N., M. C. Varadaraj (2015). Functionality of phytase of *Saccharomyces cerevisiae* MTCC 5421 to lower inherent phytate in selected cereal flours and wheat/pearl millet-based fermented foods with selected probiotic attribute. *Food Biotechnol*. **29**: 131-155.

Roopesh, K., S. Ramachandran, K. M. Nampoothiri, G. Szakacs, A. Pandey (2006). Comparison of phytase production on wheat bran and oilcakes in solid-state fermentation by *Mucor racemosus*. *Bioresour. Technol*. **97**: 506-511.

Sabu, A., S. Sarita, A. Pandey, B. Bogar, G. Szakacs, C. R. Soccol (2002). Solid-state fermentation for production of phytase by *Rhizopus oligosporus*. *Appl. Biochem. Biotechnol*. **102**: 251-260.

Sanangelantoni, A. M., M. Malatrasi, E. Trivelloni, G. Visioli, C. Agrimonti (2018). A novel β-propeller phytase from the dioxin-degrading bacterium *Sphingomonas wittichii* RW-1. *Appl. Microbiol. Biotechnol*. **102**: 8351-8358.

Sandez Penidez, S. H., M. A. Velasco Manini, C. L. Gerez, G. C. Rollán (2020). Partial characterization and purification of phytase from *Lactobacillus plantarum* CRL1964 isolated from pseudocereals. *J. Basic. Microbiol*. **60**: 787-798.

Sanni, D. M., O. T. Lawal, V. N. Enujiugha (2019). Purification and characterization of phytase from *Aspergillus fumigatus* isolated from African Giant Snail (*Achatina fulica*). *Biocatal. Agric. Biotechnol*. **17**: 225-232.

Santos, T., C. Connolly, R. Murphy (2015). Trace element inhibition of phytase activity. *Biol. Trace Elem. Res.* **163**: 255-265.

Saxena, A., M. Verma, B. Singh, P. Sangwan, A. N. Yadav, H. S. Dhaliwal, V. Kumar (2020). Characteristics of an acidic phytase from *Aspergillus aculeatus* APF1 for dephytinization of biofortified wheat genotypes. *Appl. Biochem. Biotechnol*. **191**: 679-694.

Sharma, N., S. Angural, M. Rana, N. Puri, K. K. Kondepudi, N. Gupta (2020). Phytase producing lactic acid bacteria: Cell factories for enhancing micronutrient bioavailability of phytate rich foods. *Trends Food Sci. Technol*. **96**: 1-12.

Sharma A., O. Ahluwalia, A. Tripathi, G. Singh, A. Kumar (2020). Phytases and their pharmaceutical applications: Mini-review. *Biocat. Agric. Biotech.* **23**: 101439.

Shetty, J. K., B. Paulson, M. Pepsin, G. Chotani, B. Dean, M. Hruby (2008). Phytase in fuel ethanol production offers economical and environmental benefits. *Int. Sugar J.* **110**: 160-174.

Simon, O., F. Igbasan (2002). *In vitro* properties of phytases from various microbial origins. *Int. J. Food Sci. Technol*. **37**: 813-822.

Singh, B., T. Satyanarayana (2011). Microbial phytases in phosphorus acquisition and plant growth promotion. *Physiol. Mol. Biol. Plants.* **17**: 93-103.

Singh, B., T. Satyanarayana (2011). Phytases from themophilic molds: their production, characteristics and multifari-

ous applications. *Process Biochem*. **46**: 1391-1398.

Singh, B., T. Satyanarayana (2015). Fungal phytases: Characteristics and amelioration of nutritional quality and growth of non-ruminants. *J. Anim. Physiol. Anim. Nutr*. **99**: 646-660.

Singh, B., I. Boukhris, Pragya, V. Kumar, A. N. Yadav, A. Farhat-Khemakhem *et al*. (2020). Contribution of microbial phytases to the improvement of plant growth and nutrition: A review. *Pedosphere* **30**: 295-313.

Steiner, T., R. Mosenthin, B. Zimmermann, R. Greiner, S. Roth (2007). Distribution of phytase activity, total phosphorus and phytate phosphorus in legume seeds, cereals and cereal by-products as influenced by harvest year and cultivar. *Anim. Feed Sci. Technol*. **133**: 320-334.

Strater, N., T. Klabunde, P. Tucker, H. Witzel, B. Krebs (1995). Crystal structure of a purple acid phosphatase containing a dinuclear Fe(III)-Zn(II) active site. *Science* **268**: 1489-1492.

Sumengen, M., S. Dincer, A. Kaya (2012). Phytase production from *Lactobacillus brevis*. *Turk. J. Biol*. **36**: 533-541.

Thakur, N., S. K. S. Patel, P. Kumar, A. Singh, N. Devi, K. Sandeep, D. Pandey, D. Chand (2022). Bioprocess for hyperactive thermotolerant *Aspergillus fumigatus* phytase and its application in dephytinization of wheat flour. *Catal. Lett.* **152**: 3220-3232.

Trivedi, S., I. Husain, A. Sharma (2022). Purification and characterization of phytase from *Bacillus subtilis* P6: Evaluation for probiotic potential for possible application in animal feed. *Food Front.* **3**: 194-205.

Ullah, A. H., B. J. Cummins (1988). *Aspergillus ficuum* extracellular phytase immobilization on glutaraldehyde□activated silicate. *Ann. N. Y. Acad. Sci.* **542**: 102-106.

Vasudevan, U. M., A. K. Jaiswal, S. Krishna, A. Pandey (2019). Thermostable phytase in feed and fuel industries. *Bioresour. Technol*. **278**: 400-407.

Vohra, A., T. Satyanarayana (2002). Purification and characterization of a thermostable and acid-stable phytase from *Pichia anomala. World J. Microbiol. Biotechnol.* **18**: 687-691.

Watanabe, T., H. Ikeda, K. Masaki, T. Fujii, H. Iefuji (2009). Cloning and characterization of a novel phytase from wastewater treatment yeast *Hansenula fabianii* J640 and expression in *Pichia pastoris*. *J. Biosci. Bioeng*. **108**: 225-230.

Willard, E. C., A. Sundquist, V. Glitsoe, Sorbara, J. O., P. Tamez-Hidalgo, C. Heine, C. V. Chatelle (2022). A new rapid and quantitative assay to determine the phytase activity of feed. *ACS Omega* **7**: 5292-5299.

Wulandari, R., E. N. Sari, A. Ratriyanto, H. Weldekiros, R. Greiner (2015). Phytase-producing bacteria from extreme regions in Indonesia. *Braz. Arch. Biol. Technol*. **58**: 711-717.

Wyss, M., R. Brugger, A. Kronenberger, R. Rémy, R, Fimbel, G. Oesterhelt, M. Lehmann, A. P. Van Loon (1999). Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases): catalytic properties. *Appl. Environ. Microbiol.* **65**: 367-373.

Yoon, S. J., Y. J. Choi, H. K. Min, K. K. Cho, J. W. Kim, S. C. Lee, Y. H. Jung (1996). Isolation and identification of phytase-producing bacterium, *Enterobacter* sp. 4, and enzymatic properties of phytase enzyme. *Enzyme Microb. Technol*. **18**: 449-454.

Yu, P., Y. Chen (2013). Purification and characterization of a novel neutral and heat-tolerant phytase from a newly isolated strain *Bacillus nealsonii* ZJ0702. *BMC Biotechnol.* **13**: 1-7.

Yu, P., X. T. Wang, J. W. Liu (2015). Purification and characterization of a novel cold-adapted phytase from *Rhodotorula mucilaginosa* strain JMUY14 isolated from Antarctic. *J. Basic Microbiol.* **55**: 1029-1039.

Zhang, Z., X. Li, S. Sang, D. J. McClements, L. Chen, J. Long, A. Jiao, Z. Jin, C. Qiu (2022). Polyphenols as plant-based nutraceuticals: health effects, encapsulation, nano-delivery, and application. *Foods* **11**: 2189.

Zhao, Q., H. Liu, Y. Zhang (2010). Engineering of protease-resistant phytase from *Penicillium* sp.: High thermal stability, low optimal temperature and pH. *J. Biosci. Bioeng*. **110**: 638-645.

Zhou, Y., A. N. Anoopkumar, A. Tarafdar, A. Madhavan, M. Binoop, N. M. Lakshmi, K. B. Arun, R. Sindhu, P. Binod, R. Sirohi, A. Pandey, Z. Zhang, M. K. Awasthi (2022). Microbial engineering for the production and application of phytases to the treatment of the toxic pollutants: A review. *Environ. Pollut.* **308**: 119703.

Zhu, D., Q. Wu, L. Hua (2019). 3.01 - Industrial Enzymes. In: Moo-Young, M. (Ed.), Comprehensive Biotechnology. (Third Edition), Academic Press, Elsevier, pp. 1-13.