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REVIEW

Production of fungal phytases in solid state fermentation and potential biotechnological applications

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Abstract

Phytases are important enzymes used for eliminating the anti-nutritional properties of phytic acid in food and feed ingredients. Phytic acid is major form of organic phosphorus stored during seed setting. Monogastric animals cannot utilize this phytate-phosphorus due to lack of necessary enzymes. Therefore, phytic acid excretion is responsible for mineral deficiency and phosphorus pollution. Phytases have been reported from diverse microorganisms, however, fungal phytases are preferred due to their unique properties. *Aspergillus* species are the predominant producers of phytases and have been explored widely as compared to other fungi. Solid-state fermentation has been studied as an economical process for the production of phytases to utilize various agro-industrial residues. Mixed substrate fermentation has also been reported for the production of phytases. Physical and chemical parameters including pH, temperature, and concentrations of media components have significantly affected the production of phytases in solid state fermentation. Fungi produced high levels of phytases in solid state fermentation utilizing economical substrates. Optimization of culture conditions using different approaches has significantly improved the production of phytases. Fungal phytases are histidine acid phosphatases exhibiting broad substrate specificity, are relatively thermostable and protease-resistant. These phytases have been found effective in dephytinization of food and feed samples with concomitant liberation of minerals, sugars and soluble proteins. Additionally, they have improved the growth of plants by increasing the availability of phosphorus and other minerals. Furthermore, phytases from fungi have played an important roles in bread making, semi-synthesis of peroxidase, biofuel production, production of myo-inositol phosphates and management of environmental pollution. This review article describes the production of fungal phytases in solid state fermentation and their biotechnological applications.

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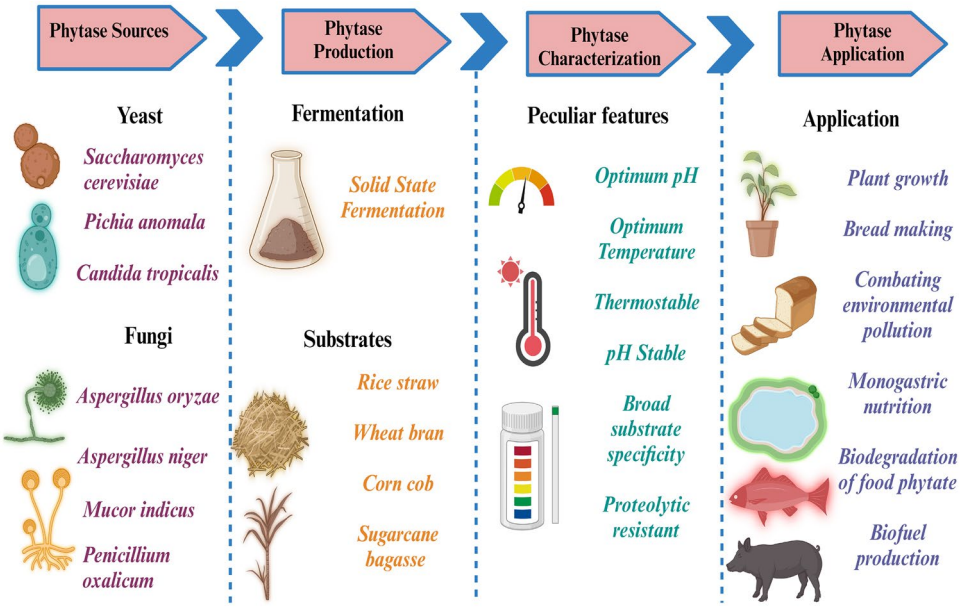
Graphical abstract



Keywords Fungal phytases · Solid-state fermentation · Nutrition · Thermostable · Plant growth promotion · Biofuel · Myo-inositol triphosphate

Introduction

Phosphorus (P) is a crucial element for animal and plant nutrition due to its critical role in their growth and development, but it does not have a natural replenishment cycle. All living organisms need an adequate amount of phosphorus because it is involved in the formation of cell membranes, nucleic acids, and enzyme regulation (Singh et al. 2011; Vashishth et al. 2023; Priya et al. 2023). As a result, animal diets must include sufficient inorganic phosphorus (Pi). Livestock and poultry are generally administered dicalcium phosphate as a part of their dietary intake to ensure the fulfillment of their requisite daily nutrient needs. This phosphorous and calcium supplement is incorporated into their feed to promote optimal growth and development, thereby enhancing the overall health and productivity of the animals (Jain et al. 2016; Singh et al. 2020).

Phosphorus, an essential mineral, predominantly exists in the form of phytic acid (PA), accounting for approximately 18–88% of the total phosphorus content in various plant-based sources. Phytate constitutes 1–5% of the weight in certain foodstuffs, including wheat bran, cereals, rice bran, legumes, and oilseeds (Singh and Satyanarayana 2011b, 2015; Moreira et al. 2014; Awad et al. 2014; Coban and Demirci 2014). Inability of monogastric animals to break down phytate P necessitates the addition of exogenous phosphorus to their diets, increasing the P load and resulting in large amounts of P excretion in feces in regions with high animal production. This leads to phosphorus pollution in the environment. Moreover, the defecation of unprocessed phytate along with inorganic phosphorus raises universal environmental concerns related to P eutrophication in areas with extensive cattle farming (Liu et al. 2022). Excessive phosphorus in the soil can be washed away during different weather cycles into various water bodies such as rivers and ponds, leading to rapid cyanobacterial blooms, growth of phytoplankton, algae, lack of oxygen, and the aquatic species death (Vats and Banerjee 2005; Singh et al. 2020).

Phytases have been found in mammals, plants, and microbes, with microbial phytases being predominantly researched for worldwide commercial applications (Singh et al. 2011; Singh and Satyanarayana 2011a; Jain et al. 2016; Kaur et al. 2017). Fungal phytases are potentially excellent candidates for eliminating anti-nutrients from plant-based diets due to their natural activity of breaking the phosphomonoester linkages present in the phytates (Singh et al. 2020; Kumari and Bansal 2021). Consequently, soil microorganisms have been studied for phytase production, although more sources are still required to be identified (Kalsi et al. 2016). Filamentous fungal species that can produce phytases during the fermentation process are *Aspergillus oryzae, A. fumigatus*, *Mucor piriformis*, *A. niger*, *A. carbonarius*, *Rhizopus oligosporus*, and *Cladosporium* species (Jatuwong

et al. 2020). Fungi produce high levels of phytases in solid state fermentation as compared to bacteria and other sources.

Phytases break down phytic acid into Pi and myo-inositol, effectively excluding their anti-nutritional effects (Sapna and Singh 2017a, 2017b; Singh et al. 2011, 2020; Singh and Satyanarayana 2011a). Phytic acid, mainly in the form of inositol triphosphates, serves as a vital component in the regulation of signaling and cellular functions in both plant and animal cells (Goyal et al. 2022). The presence of inositol phosphates (InsP 3) in signal transduction pathways can affect the control of the cell cycle, as well as the growth and differentiation of cancerous cells (Pujol et al. 2023). Phytase reduces the ability of phytic acid to form complexes with essential minerals, enzymes, and proteins (Fig. 1). Fungi, among microorganisms, are the primary producers of phytases, and their unique attributes, such as their wide range of substrates specificity, and the ability to function effectively across a broad spectrum of pH levels and temperatures, make them potentially valuable in multiple domains. This review article narrates the classification, fungal sources, and optimization of production process, peculiar features, and advanced biotechnological applications of fungal phytases. It also describes the importance of myo inositol triphosphate, an intermediate produced during hydrolysis of phytic acid.

Classification of phytases

Based on their optimal pH for activity, phytases have been classified as acidic or alkaline. Fungal phytases and certain bacterial phytases are acidic, whereas phytases from *Bacillus* spp. and plants are alkaline in nature (Jain and Singh 2017; Singh et al. 2020; Pragya et al. 2021). Phytases have been categorized into 3-, 5-, and 6-phytases based on their selective attack on phosphorus linked to inositol moiety (Kumar and Sinha 2018). Only 3-phytases are documented in microorganisms, whereas 6-phytases are found in plants. Based on their catalytic processes, Phytases can be categorized into four distinct groups, namely HAP (histidine acid phosphatases), CP (cysteine phosphatases), BPP (β-propeller phytase), and PAP (purple acid phosphatases). This classification depends on reaction mechanisms, amino acid sequences, biochemical properties, and 3D conformations. HAP phytases have been reported in microbes as well as plants. Natuphos, the earliest commercial phytase, is a HAP synthesized by *Aspergillus niger* var *ficuum* (Chen et al. 2015). The enzymes exhibit a shared catalytic site structure situated at the junction of the two domains, which includes the well-preserved N-terminal active site motif RHGXRXP and the C-terminal HD. In the two-step process, the first step involves the histidine from the RHGXRXP motif initiating a nucleophilic attack on the phosphorus, resulting in the formation of a covalent phosphohistidine intermediate. Concurrently, the aspartic acid from the HD motif serves as a proton donor to the oxygen atom within the cleavable phosphomonoester bond. The necessity for the protonation of the aspartate carboxylate group to enable proton donation to the departing group explains the preference for an acidic pH for the biocatalysis (Fan et al. 2016). During substrate hydrolysis, the N- and C-terminal portions of HAPs combine to create the catalytic core (Gessler et al. 2018).

Fungal and bacterial HAPs exhibit comparable architectures, although with certain distinct features. Among these enzymes, the main detection of BPP phytases is predominantly observed in *Bacillus* species and similar types of bacteria. These particular phytases are occasionally referred to as alkaline phytases owing to their highest catalytic efficiency within the pH range of 7.5 to 8.0. The three-dimensional configuration of BPP phytases comprises a hexameric assembly resembling a six-bladed propeller, providing a rationale for their nomenclature (Sanangelantoni et al. 2018). The phytases under consideration exhibit distinctive catalytic characteristics, demonstrating both resistance to proteases and specificity towards phytate substrates (Jain and Singh 2017). BPPs are characterized

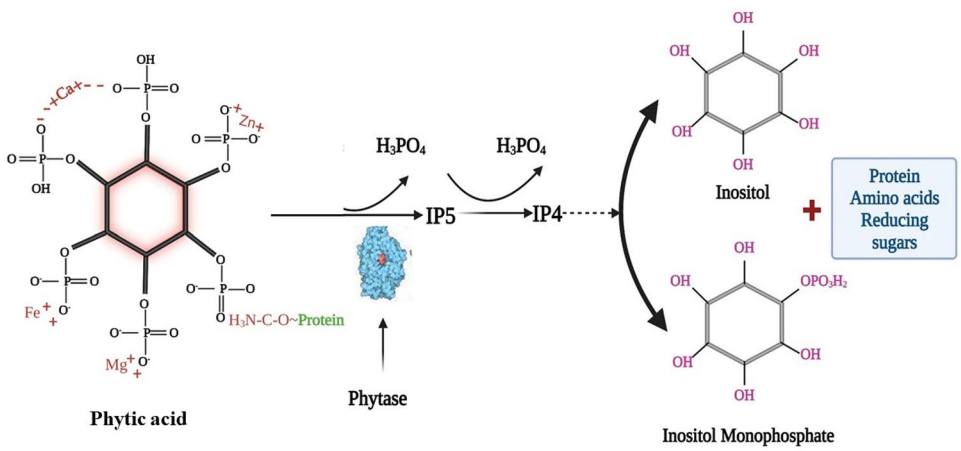


Fig. 1 Product liberation by the action of phytase on phytic acid

by their remarkable thermostability, dependence on Ca^{2+} ions, and an optimal enzymatic activity observed within the pH range of neutrality to alkalinity (Kaur et al. 2017; Jain and Singh 2017). In contrast, phytase from *B. licheniformis* required low Ca^{2+} for their catalytic activity (Borgi et al. 2014).

Purple acid phosphatases have been identified in mammals, plants, and fungi. These enzymes, referred to as purple or pink phytases, derive their name from the presence of Fe^{2+}, Fe^{3+}, Mn^{2+}, and Zn^{2+} in their catalytic centers. Found predominantly within plant structures, they exhibit resemblance to other plant PAPs. One specific PAP, known as GmPhy, is discovered in the developing cotyledons of sprouting soybean (*Glycine max* L. Merr.) and bears a structural resemblance to a kidney bean. This enzyme, described by Xiao et al. (2005), demonstrates comparable characteristics to other plant PAPs highlighted by Singh and Satyanarayana (2015). This specific phytase assembly has a limited range of catalytic activity. Notably, all PAPs in this group include five conserved blocks of metal-ligating residues, which are recognized as distinguishing features of GmPhy (Feder et al. 2020; Langeroudi et al. 2023). It is worth noting, however, that MtPHY1, a phytase produced from *Medicago truncatula*, lacks the entire set of all five blocks, separating it from other members of the group (Ma et al. 2012). In a recent study, a bacterium capable of producing PAP has been identified in earthworm castings (Ghorbani et al. 2018).

The CPs are phytases that belong to a new subfamily and share a catalytic mechanism with protein tyrosine phosphatases (Puhl et al. 2008; Gontia-Mishra and Tiwari 2013; Gruninger et al. 2014). They exhibit notable phytase activity in acidic environment and are selective for tyrosine phosphates. *Selenomonas ruminantium*, a ruminal bacterium, was the first organism to have this type of phytase identified (Mullaney and Ullah 2006). Figure 2 presents a comprehensive classification of phytases based on different criteria.

Sources and production of fungal phytases in solid state fermentation

Phytases have been identified in mammals, plants, and microbes, although microbial phytases have been largely studied for commercial uses globally (Jain and Singh 2017; Puppala et al. 2019; Pragya et al. 2023). Therefore, soil microorganisms have been extensively screened as sources of phytases; nonetheless, it is required to uncover novel sources (Kalsi et al. 2016). Phytases have been reported from microorganisms including fungi, yeasts and bacteria (Abd-ElAziem et al. 2015; Gaind and Singh 2015; Hellström et al. 2015; Singh et al. 2015; Jain et al. 2016). Fungal phytases

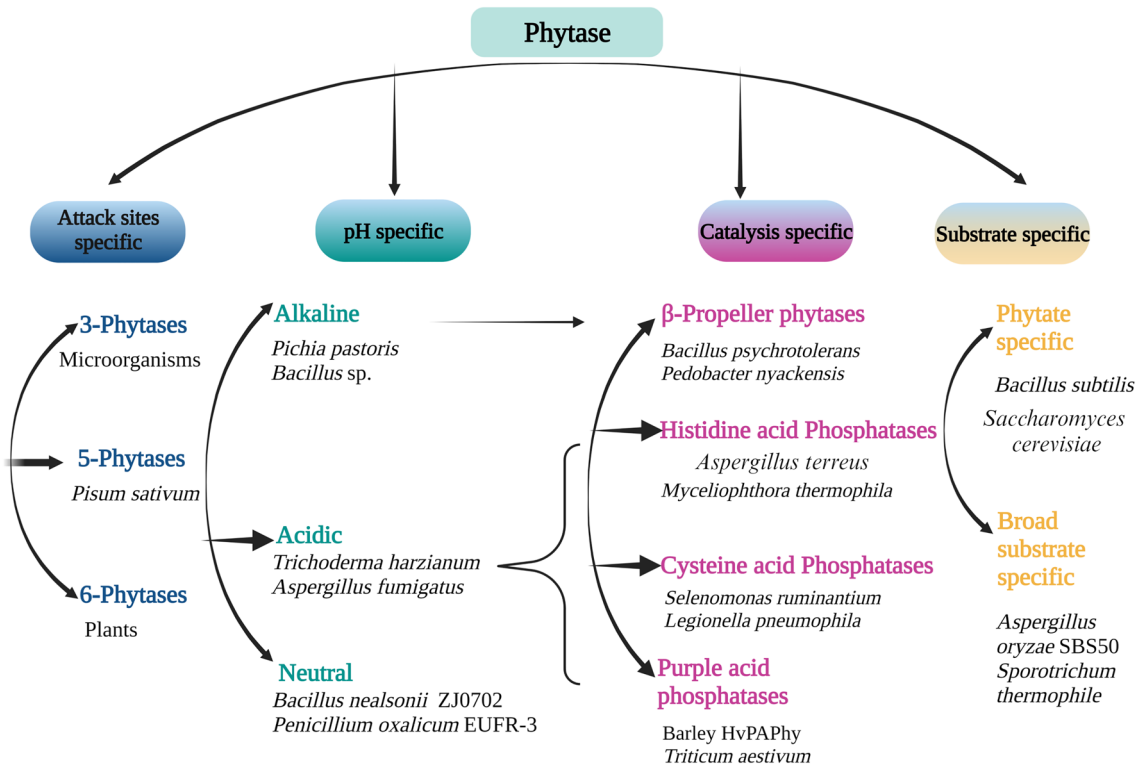


Fig. 2 Classification of phytases

are favored over bacterial phytases due to their desirable characteristics, including protease resistance, high activity/yield and extracellular nature (Song et al. 2019; Priya et al. 2023). Unlike unicellular fungi, most filamentous fungi are well-suited for both submerged and solid-state fermentations because of their filamentous growth, which covers a larger surface area and allows them to penetrate solid substrates (Rizwanuddin et al. 2023a).

Over a century ago, phytase activity in microbes was first discovered in *Aspergillus niger*. Since then, there have been numerous scientific reports on fungal phytases, with a particular focus on those derived from the species of *Penicillium*, *Rhizopus*, *Aspergillus*, *Thermomyces*, *Mucor*, and *Trichoderma*. Both mesophilic and thermophilic fungi are known to secrete phytases. Fungi, such as *Aspergillus oryzae* (Sapna and Singh 2014; Pragya et al. 2023), *Humicola nigrescens* (Bala et al. 2014), *Aspergillus flavus* (Haind and Singh 2015), *Sporotrichum thermophile* (Singh and Satyanarayana 2011a; Kumari et al. 2016), *Penicillium purpurogenum* (Awad et al. 2014)d *niger* (Kumari and Bansal 2021) have been recorded for their ability to degrade phytate. Fungi have certain advantages in phytate degradation, including their ability to extract P proficiently from soil, easy maintenance of culture, and high enzyme yields. Additionally, fungi exhale high concentrations of organic acids that functions as a chelator and play a significant role in Pi solubilization. Microbial phytases are safe biofertilizers and do not involve the use or release any harmful substances, making them beneficial for farmers practicing organic farming (Gessler et al. 2018; Shahryari et al. 2018; Jatuwong et al. 2020; Singh et al. 2020; Rizwanuddin et al. 2023).

Mesophilic microorganisms are recognized for producing more potent phytases when operating within the temperature range of 28 to 35 °C, which aligns with the optimal conditions for their growth and development (Sapna and Singh 2014; Kumari and Bansal 2021; Rizwanuddin et al. 2023). The temperature plays a critical role in regulating factors like water activity and humidity, affecting processes such as transport across cell membranes and cellular metabolism (Suresh and Radha 2015). Similarly, many filamentous fungi including *R. oligosporus* (Suresh and Radha 2015), *A.niger* and *N. sitophila* (Kanti et al. 2020), A. *aculeatus* (Saxena et al. 2020), A. *oryzae* (Sapna and Singh 2014), *A. flavus* (Onibokun et al. 2022), A. *niger* (Nascimento et al. 2022), and *A.oryzae* (Pragya et al. 2023) also secreted phytases maximally at 30 °C.

Most enzymatic activity and the transit of various components through cell membranes are influenced by the pH of the culture medium. High phytase secretion has been recorded in all filamentous fungi cultivated in SSF under acidic conditions (Singh and Satyanarayana 2008; Sapna and Singh 2014; Gupta et al. 2015; Tian and Yuan 2016; Kumari and Bansal 2021). Among all the pH tested, pH 5.0 supported high phytase production by *Aspergillus* sp. (Tian and Yuan 2016), *A. niger* (Gupta et al. 2015), *A. oryzae* (Sapna and Singh 2014; Pragya et al. 2023), and *A. niger* NT7 (Kumari and Bansal 2021). The extracellular pH influencing microbial production of phytase were pH 6.0 and 5.5 for high phytase production (Elkhateeb and Fadel 2022). In contrast, all the five fungi tested (*A. niger*, *A. fumigatus, A. flavus, Mucor rouxii*, and *P. purourogenum*), exhibited high phytase production at pH 7.0 (Sadaf et al. 2022). Production and properties of fungal phytases in SSF using various substrates are summarized in Table 1.

Solid state fermentation (SSF) presents the ability to use economical agro-industrial residues as substrate for the cultivation of fungi (Pragya et al. 2023). This bioprocess involves a minimal amount of free water within the interstitial space of the solid particles (Li et al. 2023). Nonetheless, the process provides sufficient moisture to sustain the growth and metabolism of microorganisms (Prado Barragán et al. 2016). The SSF approach has led to a reduction in operational costs associated with the regulation of temperature, agitation, pH, and aeration (Soccol et al. 2017; Srivastava et al. 2019; Piecha et al. 2023). Nowadays, a variety of fermentation techniques are often used in solid-state and submerged fermentation procedures to enhance the synthesis of enzymes generated by fungi (Mahendran et al. 2022; Dixit and Shukla 2023). The SSF system offers a number of advantages due to its low water, low aeration, easy fermentation medium, and low energy requirements (Kassim et al. 2022). Additionally, aeration is simple because an improved oxygen diffusion rate into wet solid substrate to assist microbial development prevents oxygen restriction from happening (Sapna and Singh 2014). Selecting the right substrate is a vital factor in ensuring the success of SSF process, as various factors such as substrate characteristics and the growth of microorganisms can have an impact on the SSF process (Kumar et al. 2021).

Fungi like *Aspergillus tubingensis*, *A. niger*, *A. flavus*, *A. ficuum*, and *Rhizopus oryzae*, which are filamentous in nature, are typically grown using SSF. However, SSF faces challenges such as inadequate nutrient utilization and limited biomass growth, resulting from a lack of free water content and the buildup of heat and moisture loss throughout the process. Essential substrates for fostering fungal growth and their metabolism include, wheat bran, rice bran, soybean meal, corn cobs, and citrus peels (Dahiya and Singh 2019; Rizwanuddin et al. 2023a). Triticale, containing grains like bali and barley, serves as a substrate for *A. niger* phytase production (Soccol et al. 2017; Rizwanuddin et al. 2023a), while corn bran and maize cob were employed for phytase production by *P. purpurogenum* (Awad et al. 2014). Natural substrate mixtures supply appropriate nutrients when compared to individual substrates and act as a support for microbial growth and development (Kumari et al. 2016). This is because the mixed substrate contains adequate nutrients

Table 1 Production and properties of fungal phytases in solid-state fermentation

Fungal source	Substrate	Culture conditions			Phytase production (U/g)	Catalytic properties			References
		pH	Temp. (°C)	Incubation time (d)		pH	Temp. (°C)	K_m/V_{max}	
Penicillium oxalicum EUFR-3	Wheat bran	6	35	5	12.8 U/g	7	40		Kaur et al. (2017)
Aspergillus aculeatus APF1	Wheat bran	6	30	4		3	50	3.21 mM and 3.78 U/mg protein	Saxena et al. (2020)
Aspergillus niger NT7	wheat bran	5	35	5	208.30 ± 0.22 U/gds	Broad pH range	60		Kumari and Bansal (2021)
Pholiota adipose	Water hyacinth	6.5	30	7	17.02 ± 0.92 U/gds	5	42		Jatuwong et al. (2020)
Acremonium zeae	Corn meal	4	28	7	0.3 U day^{-1}	7	50		Pires et al. (2019)
Aspergillus flavus ITCC 6720	Mustard oil cake	6	37	6	112.25 U g^{-1}	7	45		Gaind and Singh(2015)
Aspergillus oryzae SBS50	Wheat bran and rice straw	5	30	5	1161.49 ± 27.23 U/g DMR	5	50	0.20mM and 416.5 nmol/sec	Pragya et al. (2021)
Rhizopus oligosporus MTCC 556	Rice bran	5.5	30	4	31.3 U/gds	5.5	50		Suresh and Radha (2015)

and inducers for phytase synthesis. Higher PA concentration in wheat bran has previously been described as the primary inducer of phytase synthesis by microorganisms in SSF (Gupta et al. 2014; Tanruean et al. 2021). Lignocellulosic components (cellulose and hemicellulose) of plant residues supply carbohydrates/sugars for rapid development and metabolism of fungi (Miao et al. 2019). Similarly, the increased PA content of wheat bran and mustard oil cake allowed *Rhizopus oligosporus* MTCC556 to produce the most phytase in SSF (Suresh and Radha 2015). *A. oryzae* used mixed agri- industrial substrate (Wheat bran + rice straw) for phytase production (Pragya et al. 2023). The use of mixed substrates in phytase production by fungi offers several advantages. Firstly, by combining various substrates such as agricultural residues, oilseed cakes, and organic waste materials, a rich nutrient composition can be achieved, promoting fungal growth and enzyme production (Kumari et al. 2016). Additionally, mixed substrates can provide a cost-effective alternative compared to single substrates, as they utilize readily available and potentially inexpensive feedstocks (Kanti et al. 2020). The utilization of mixed substrates also contributes to waste management and sustainability efforts, by converting organic waste materials into valuable products (de Oliveira Ornela and Souza Guimarães 2019). This method is favored by the fermentation industry due to its reduced time consumption, simplicity, cost-effectiveness, and the ease of enzyme extraction with water (Srivastava et al. 2019).

Optimization of phytase production in solid-state fermentation

The process of optimizing conditions in experiments conventionally involves using a method called one variable at a time (OVAT) optimization, where only one factor is altered at a time while the others remain constant to optimize the process/conditions (Sagar Verma et al. 2022). The OVAT optimization, also known as single factorial optimization, is a traditional experimental approach to optimize the entire system by altering one factor at a time. This approach helps to identify critical parameters, improve manufacturing yield, and is easy to understand and apply (Sagar Verma et al. 2022).

Statistical experiment design is an efficient approach for optimization, particularly in predicting interactions between variables and identifying significant components affecting phytase production. Using factorial design and response surface methodology, a combination of factors starting at a certain optimum factor response can be determined, leading to an increase in phytase production (Kumari and Bansal 2021, Pragya et al. 2023). Statistical optimization techniques

offer optimum media with minimal experiments in a short period, taking into account the interaction between selected components, which is crucial for enhancing the synthesis of phytases (Bhavsar et al. 2013; Sapna and Singh 2014; Kumari et al. 2016; Shahryari et al. 2018). The use of statistical methods in diverse bioprocessing procedures, particularly in the selection of main ingredients in the medium, has drawn considerable attention. In comparison to conventional methods, previous research has demonstrated that statistical methodology significantly enhanced phytase synthesis in SSF (Jatuwong et al. 2020; Kanti et al. 2020; Ahmed et al. 2021; Kumari and Bansal 2021) and is therefore, a preferred method for optimizing production of phytase (Ahmed et al. 2021).

Plackett-Burman design, a screening technique, efficiently identifies critical factors affecting phytase production. It allows for the screening of numerous factors in a relatively small number of experiments (Kumari and Bansal 2021). By assessing the main effects of factors, Plackett-Burman design (PBD) helps in selecting influential variables for subsequent optimization using other experimental designs (Ahmed et al. 2021). Factors such as pH, temperature, carbon and nitrogen sources, inducers, and trace elements have been identified as key variables influencing phytase production. Response surface methodology is a powerful statistical tool that combines mathematical models and experimental design to optimize process variables and their interactions (Wang et al. 2017). RSM helps to understand the complex relationships between factors and responses, enabling the identification of optimum conditions for phytase production. RSM also provides insights into the interactions between variables, enabling fine-tuning of the process for enhanced enzyme production (Shahryari et al. 2018). The combination of PBD and RSM offer a comprehensive approach for phytase production optimization (Wang et al. 2017) When compared to unoptimized conditions, phytase production has increased dramatically due to OVAT and statistical approaches (Table 2).

Peculiar features of fungal phytases

Phytases from distinct sources display different characteristics. Their qualities include glycoprotein nature, thermal stability, substrate specificity, enzyme levels, and protease-resistance (Singh and Satyanarayana 2015; Singh et al. 2020). Fungal phytases are mostly monomeric proteins with molecular weights in the range of 38 to 500 kDa and are secreted in larger quantities as compared to bacterial and other sources. For usage in the feed and food sectors, phytases that can tolerate protease action and acidic conditions are mostly preferable. Fungal and bacterial phytases respond differently to pepsin and trypsin (Singh et al. 2020). Fungal phytases are acidic phytases and have been studied in more depth than alkaline phytases due to their multifarious applications. Fungal phytases are highly useful in improving soil fertility and in aquaculture, where lower temperature conditions favour their activity and suitability under these conditions (Pragya et al. 2023; Priya et al. 2023).

Acidic phytases

Acidic phytases have been studied in more depth than alkaline phytases due to their roles in enhancing nutritional quality of food and feed ingredients. Fungal phytases are acidic phytases as compared to neutral and alkaline phytases from bacteria. The optimum pH of fungal phytases plays a critical role in determining their efficacy in catalyzing the breakdown of phytic acid. The search of an ideal phytase involves evaluating its efficacy within the stomachs of both humans and animals during the digestive process, where the pH levels typically range from 1.5 to 3.5. Phytases that remain active under acidic conditions can significantly enhance their applicability in the food industry. According to Saxena et al. (2020), the partially purified phytase obtained from *Aspergillus aculeatus* APF1 exhibited its highest level of activity at an acidic pH of 3.0. Furthermore, the purified phytase from *Aspergillus niger* BIONCL8 demonstrated a wide range of pH stability, reaching its peak activity at pH 2.1 (Bhandari et al. 2023). While a general pH range of 3.0 to 5.0 is commonly observed among many fungal phytases, variability exists among different fungal species (Filippovich et al. 2023). Phytase of *P. oxalicum* PJ3 (Lee et al. 2014), A. *niger* CFR335 (Shivanna and Venkateswaran 2014), *A.oryzae* SBS50 (Pragya et al. 2023) and *A. niger* (Neira-Vielma et al. 2018) were optimally active between pH 4.0–5.3. The maximum activity of phytase was obtained from *T. purpureogenus* NSA20 (Ahmed et al. 2021)d *polonicum* MF82 (Kalkan et al. 2020) at pH 5.5. Phytase of *Aspergillus flavus* showed optimal activity at pH 6.0 (Onibokun et al. 2022). The crude phytase from *A. niger* NT7 displayed activity under acidic conditions (Kumari and Bansal 2021). Recent advances in biotechnology and enzyme engineering have allowed for the manipulation of pH activity profile of fungal phytases (Zhou et al. 2022).

Thermostable phytases

Temperature is a significant parameter that influence the activity of fungal phytases (Priya et al. 2023; Pragya et al. 2023). The optimum temperature of fungal phytases can vary depending on the fungal species. However, the optimal temperature for phytase activity typically ranges from 40 to 60 °C, depending on the sources of the enzymes (Goyal et al.

Table 2 Effect of different optimization strategies and substrates on phytase production by fungi in solid state fermentation

Microorganism	Substrate (s)	Optimization strategy*	Fold increase in phytase production	References
Aspergillus oryzae SBS50	Wheat bran	PBD & RSM	3.35	Sapna and Singh (2015)
Aspergillus oryzae SBS50	Wheat bran + Rice straw	PBD & RSM	2.29	Pragya et al. (2023)
Aspergillus niger	Groundnut oil cake	PBD & RSM	36.67	Buddhiwant et al. (2016)
Aspergillus niger	Wheat bran	OVAT & RSM	6.8	Kumari and Bansal (2021)
Sporotrichum thermophile	Sugarcane bagasse + wheat bran	PBD & RSM	11.6	Kumari et al. (2016)
Aspergillus ficuum	Wheat straw	OFAT & RSM	22.24	Shahryari et al. (2018)
Pholiota adipose	Water hyacinth	PBD & RSM	3.15	Jatuwong et al. (2020)
Thermomyces lanuginosus	Rice bran	OFAT & RSM	10.83	Berikten and Kivanc (2014)
Aspergillus niger	Wheat bran	RSM	2.9	Gupta et al. (2014)
Aspergillus ficuum	Waste vinegar residue	PBD & RSM	7.34	Wang et al. (2017)
Aspergillus oryzae	Soybean meal	RSM		Chen et al. (2013)
Aspergillus niger NCIM563	Wheat bran	PBD & RSM	3.08	Bhavsar et al. (2011)
Rhizopus oligosporus MTCC 556	Wheat bran + Mustard oil cake	OVAT	1.8	Suresh and Radha (2015)
Aspergillus niger Str3	Coconut oil cake + Rice bran	OVAT	8.9	Kanti et al. (2020)
Neurospora sitophila	Coconut oil cake + Rice bran	OVAT	11.8	Kanti et al. (2020)
Penicillium purpurogenum GE1	Corn cob + Corn bran	OVAT RSM	2.9	Awad et al. (2014)
Aspergillus ficuum PTCC5288	Wheat bran	PBD & RSM	20	Jafari-Tapeh et al. (2012)
Sporotrichum thermophile BJTLR50	Sesame oil cake	PBD & RSM	2.6	Singh and Satyanarayana (2008)
Talaromyces purpureogenus NSA20	Potato peel waste	RSM	1.57	Ahmed et al. (2021)
Aspergillus niger CFR 335 and *Aspergillus ficuum* SGA 01	Wheat bran, rice bran, and groundnut cake	OVAT	3–5	Shivanna and Venkateswaran (2014)
Thermoascus aurantiacus	Rice bran	OVAT		Tanruean et al. (2021)
A. awamori NRC- F18	Wheat bran	OVAT		Elkhateeb and Fadel (2022)

**PBD* plackett-burman design, *RSM* response surface methodology, *OVAT* one variable at a time

2022). The purified phytases from *A. fumigatus* (Sanni et al. 2019) and *A. niger* S2 (Sandhya et al. 2019) had temperature optima at 40 °C (Sanni et al. 2019). The ideal temperature for phytase derived from *Yersinia intermedia* (Lahiji et al. 2021) and *A. oryzae* (Pragya et al. 2023) was determined as 50 °C. Phytase from *P. polonicum* MF82 had optimal activity at 60 °C (Kalkan et al. 2020).

The pH and thermo-stability of phytases are critical factors that significantly influence their catalytic efficiency, functionality, and applicability in various industries (Pragya et al. 2021). In the feed and food processing industry, the feed is treated at a high temperature and this is the phase where phytases are typically added to the feed before pelletization. Therefore, the thermal stability of phytase is critical and required for this treatment (Coutinho et al. 2020). Phytase from *A. niger* displayed high thermostability, maintaining 70% of its activity at 80 °C (Neira-Vielma et al. 2018). Phytases derived from *A. fumigatus* and *A. niger* experienced denaturation at 50 and 70 °C, respectively (Wyss et al. 1998), while *Thermomyces lanuginosus* TL-7 phytase demonstrated high tolerance at 70 °C by maintaining 70% of its activity (Gulati et al. 2007). Phytase from

Aspergillus aculeatus APF1 showed high activity at 55 °C (Saxena et al. 2020) and from *A. niger* NT7 at 60 °C (Kumari and Bansal 2021). Phytase derived from *A. niger* remained stable even at 80 °C and retained 30% of its activity (Bhandari et al. 2023). Partially purified phytase from *A. oryzae* was thermostable upto 60 °C (Pragya et al. 2023).

Protease-insensitive phytases

Proteases are naturally present in the digestive systems of all living organisms. Therefore, the ability to withstand protease activity is highly esteemed among feed enzymes and is essential for their effectiveness as additives in animal feed (Gordeeva et al. 2023). Proteolysis, the enzymatic degradation of proteins, is a common challenge that enzymes face, especially during industrial applications and gastrointestinal transit. Fungal phytases are no exception, as they are exposed to proteolytic enzymes in animal digestive systems, food processing conditions, and various biotechnological processes (Jatuwong et al. 2020). The susceptibility of phytases to proteolytic degradation can reduce their efficacy and limit their applications. Fungal phytases exhibit significant diversity unlike bacterial ones in their ability to withstand the actions of pepsin and trypsin (Yu et al. 2015). *A. niger* phytase showed noticeable protease resistance (Ushasree et al. 2014). The protease susceptibility test revealed that the enzyme maintained 80% of its activity following exposure to pepsin. The enzyme retained 60% of its activity after treatment with higher levels of pepsin (Ushasree et al. 2014). Recombinant phytase of *S. thermophile* also showed resistance to proteases, as it retained 95% of its initial activity against pepsin and trypsin (Ranjan et al. 2015). The phytase from *P. polonicum* MF82 maintained its full activity after exposure to trypsin (Kalkan et al. 2020). Phytases from *A. oryzae* SBS50 (Sapna and Singh 2017a), *A. aculeatus* APF1 (Saxena et al. 2020)d *tubingensis* TEM 37 (Çalışkan-Özdemir et al. 2021) also observed high resistance to proteases. When subjected to trypsin, phytases from *A. niger*, *R. mucilaginosa*, and *A. oryzae* retained 10%, 75%, and 84% of their respective activities (Yu et al. 2015). Phytase from *P. polonicum* MF82 maintained 100% activity after exposure to trypsin (Kalkan et al. 2020).

Broad substrate specificity

Substrate specificity refers to the ability of an enzyme to recognize and bind specific substrates, initiating the enzymatic reaction. Fungal phytases possessing a wide range of substrate compatibility can efficiently break down phytate into myo-inositol monophosphate without significant buildup of intermediate compounds. Conversely, bacterial phytases with a limited substrate range lead to the accumulation of myoinositol tris- and bisphosphate as intermediates during the degradation of phytate (Singh et al. 2018; Kaur et al. 2021). Phytases from fungi showed broad-substrate specificity in contrast to bacterial phytases that are phytate-specific (Singh et al. 2011; Singh and Satyanarayana 2015; Jain et al. 2016; Jatuwong et al. 2020). The purified phytase from *A.niger* dephosphorylated various substrates such as, 1- naphthyl phosphate, phenyl phosphate and 2-naphthyl phosphate in addition to sodium phytate (Neira-Vielma et al. 2018). The purified recombinant phytase from *Myceliophthora thermophila* (syn. *Sporotrichum thermophile*) effectively hydrolyzed various organic phosphates besides phytic acid (Ranjan and Satyanarayana 2016). Moreover, the process of molecular docking of phytase with different substrates revealed distinct binding patterns (Singh et al. 2018). The docking simulations demonstrated a high binding affinity with phytic acid and ATP, while phosphoenol pyruvate and AMP exhibited the lowest binding affinity (Singh et al. 2018). The phytase from *Sporotrichum thermophile* possesses a larger catalytic pocket, contributing to its wide substrate specificity. This larger pocket of fungal phytases enables them to hydrolyze a diverse range of organic phosphates as compared to bacterial phytases (Kumari et al. 2016; Singh et al. 2018).

Products of phytate degradation

Phytases work by cleaving the phosphate groups from the inositol ring of phytate, leading to the formation of lower inositol phosphates, including inositol pentakisphosphate (InsP5), inositol tetrakisphosphate (InsP4), and ultimately inositol trisphosphate (InsP3) or even inositol bisphosphate (InsP2) (Gupta et al. 2014). Phytate degradation products using fungal phytases are mentioned in Table 3. The breakdown of phytate into these lower-phosphate forms not only improves the nutritional value of food and feed but also has positive environmental implications. The combination of *A. fumigatus* phytase and *A. niger* acid phosphatase successfully released all six phosphate groups results in production of myo-inositol 1-monophosphate (Wyss et al. 1999). Studies have also demonstrated that fungal phytases can effectively hydrolyze phytate and liberate phosphorus in the form of inorganic phosphate, reducing sugars and soluble proteins (Sapna Singh 2017). *Aspergillus oryzae* SBS50 phytase supplementation enhanced the release of inorganic phosphate, reducing sugars and soluble proteins from the flours (Pragya et al. 2023). The products of phytate degradation play a vital role in enhancing phosphorus availability for monogastric animals, such as poultry and swine, in their diets (Priya et al. 2023).

Table 3 Phytic acid hydrolysis products using fungal phytases

Fungal source	Substrate	Hydrolysis product	References
A.niger	Soyabean meal-based diets	InsP5	Zeller et al. (2015)
A. fumigatus	Sodium phytate	myo-inositol 2-monophosphate	Wyss et al. (1999)
A. niger	Sodium phytate	myo-inositol 2-monophosphate	Wyss et al. (1999)
3-phytase (Commercial phytase)	Malt	myo-inositol	Dulinski et al. (2020)
6-phytase (Commercial phytase)	Malt	myo-inositol	Dulinski et al. (2020)

Biotechnological applications of fungal phytases

Phytases are widely used in many sectors, including the food industry. Their function in the production of animal feed, human food, the production of bread, and the processing of various cereal grains has been investigated (Nadeem et al. 2023). Numerous studies have shown that the uses for microbial phytases in food and feed are the most promising. In addition, recent advancements in biotechnological research have led to the creation of microbial phytases that may aid in the promotion of plant growth and the reduction of environmental phosphorus pollution (Singh and Satyanarayana 2011a, 2015; Bhavsar et al. 2013). Fungal phytases have been tested for multifarious applications (Fig. 3). Phytases are predominantly sourced from genetically modified strains due to the limited protein production by wild-type strains in comparison to the commercial demand. The leading product in the market is Natuphos, which contains a phytase derived from *A. niger* var. *ficuum*. In 2016, BASF introduced an improved version of Natuphos known as Natuphos E (Correa et al. 2020). This upgraded version exhibits enhanced resistance to pepsin, tolerance to adverse pH conditions, sustained activity under high processing temperatures, and an extended shelf life. The possible microbial strains that have the ability to produce phytase, along with their potential functions, are outlined in Table 4.

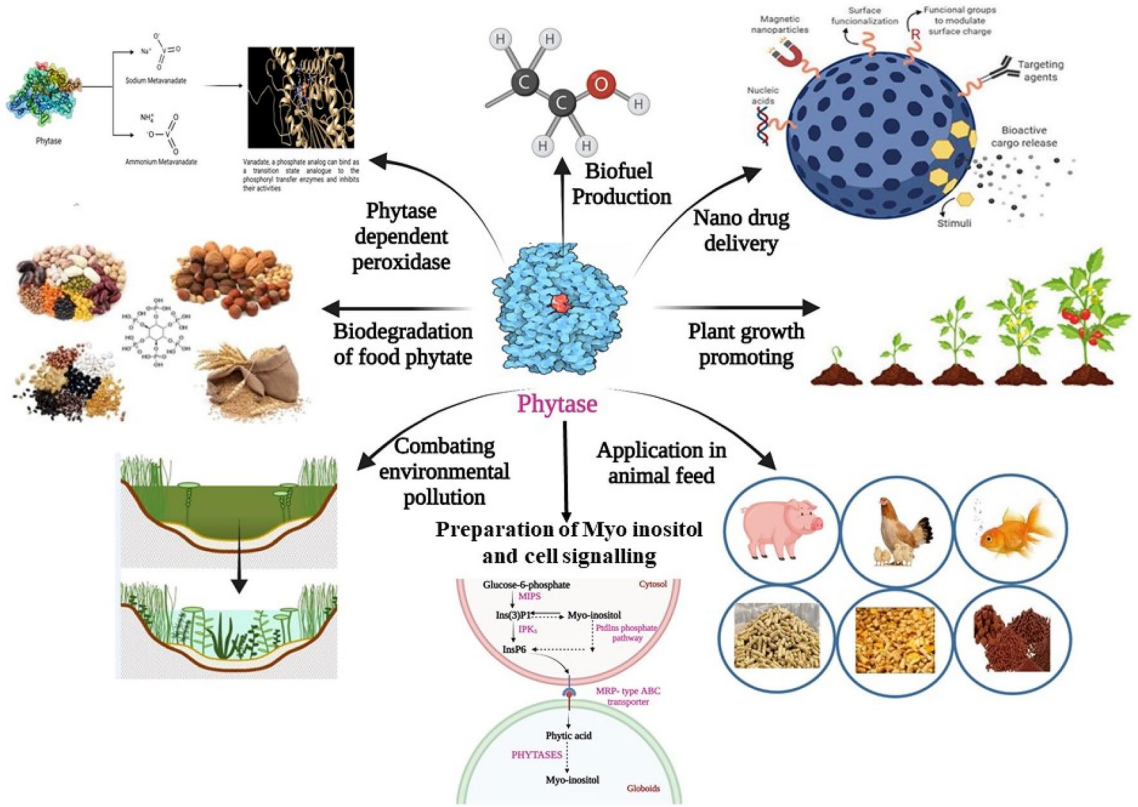


Fig. 3 Biotechnological applications of phytases

Table 4 Phytase producing fungi and their potential applications

Fungi	Applications	References
Aspergillus oryzae SBS50	Dephytinization of Wheat bran	Sapna and Singh (2014)
Sporotrichum thermophile	Dephytinization of poultry feed	Kumari et al. (2016)
Aspergillus awamori	Growth and seed germination	Kour et al. (2019)
Aspergillus flavus	Application in marine and poultry feed.	Gaind and Singh (2015)
Aspergillus niger	Solubilization of the rock phosphate and makin it available to plants	Din et al. (2019)
Humicola nigrescens	Dephytinization of flours	Bala et al. (2014)
Mucor indicus	Dephytinization of wheat and rice bran	Venkataraman and Vaidyanathan (2023)
Acremonium zeae	Dephytinization of Piglet diets	Pires et al. (2019)
Rhizopus arrhizus KB-2	Plant growth promotion	Evstatieva et al. (2020)
Aspergillus niger NT7	Dephytinization of cattle feed	Kumari and Bansal (2021)
Aspergillus niger	As poultry feed additive	Mahmood et al. (2023)

Improving monogastric nutrition

Most of the monogastric animals (poultry, piggery, fish) and humans use the plant-based foods to meet their nutritional requirements. However, many of nutritious components are not available for absorption during digestion owing to their interactions with the phytates (Kebreab et al. 2012; Priya et al. 2023). Therefore, phytase treatment is necessary in order to increase the bioavailability of minerals and nutrients with simultaneous reduction of anti-nutritional factor. Therefore, phytases used in food and feed industries must retain their activities in digestive tracts of monogastric animals (Bhandari et al. 2023). This is a requisite to ensure the successful biodegradation of phytate present in plant-based diets. Intestinal simulation studies have been conducted to study the efficacy of fungal phytases (Rodriguez et al. 2018; Lopes et al. 2021). Coutinho et al. (2020) observed similar action of immobilized and free phytase under simulated conditions. However, immobilized phytase exhibited high action at lower pH values than the free-phytase. In fishes, empty stomach pH varies from 5 to 7.0 that become highly acidic during digestion (Rodriguez et al. 2018). Therefore, highly acidic and acid-stable phytases are highly suitable for application in aquaculture (Moriarty 1973; Lopes et al. 2021; Priya et al. 2023).

The addition of phytase with citric acid to granulated feed has the potential to benefit the environment in carp farming, as it can reduce the phosphorus excretion from fish, thereby mitigating its environmental impact (Maly et al. 2023). The research findings indicated that using phytase as a feed additive for *Tilapia* sp. offers numerous advantages with no adverse effects. Among various fungal species tested, *Aspergillus tubingensis* demonstrated the highest yield of phytase (Mahendran et al. 2022). Mahmood et al. (2023) reported that supplementation of *A. niger* phytase to poultry diets improves the growth rate of broiler chickens, leading to increased body weight gain. *Aspergillus niger* BIONCL8 strain demonstrated a substantial reduction in phytate content in six poultry feed ingredients, making it a potential supplement for improving poultry feed (Bhandari et al. 2023). Because phytase has a specific target application, it cannot be considered universally ideal for both in vivo and in vitro use in all situations. For instance, in poultry, neutral phytases perform better, while acidic phytases are more effective in piggery. Additionally, the temperature optima for swine or poultry diets differ from those in aquaculture, leading to the use of distinct microbial phytases for various applications (Rizwanuddin et al. 2023a).

Bread making

The enzyme phytase is employed more prevalently in the food industry as compared to the feed industry, a preference mainly attributed to its unique property of complexing with crucial minerals like iron, zinc, and calcium in the human body (Longin et al. 2023). This distinctive characteristic of phytase facilitates its extensive utilization in the food sector, emphasizing its role in enhancing the bioavailability of these essential minerals, thereby contributing significantly to human health. This binding reduces mineral deficiencies and increases the bioavailability of essential minerals, ultimately improving the health of individuals who lack sufficient minerals (Handa et al. 2020; Rizwanuddin et al. 2023a). Addition of fungal phytase to whole wheat breads resulted in improved bread making (Goyal et al. 2022). Phytase of *P. anomala* effectively dephytinized whole wheat unleavened flat Indian breads like naan and tandoori (Joshi and Satyanarayana 2015). Phytase of *S. thermophile* has effectively reduced phytic acid in breads with concomitant amelioration of nutrition (Singh et al. 2011). Recombinant phytase of *S. thermophile* resulted in dephytinization of roti,

naan, tandoori and bread with improved nutritional properties (Ranjan and Satyanarayana 2016).

Synthesis of peroxidases

Vanadium is an inhibitor of acid phosphatases due to similarity with phosphate. Vanadate-treated acid phosphatase demonstrated the activity of a peroxidase (Tanaka et al. 2005; Sharma et al. 2020). Histidine acid phosphatases (HAP-phytases) showed similarities with vanadium haloperoxidases by substitution of phosphate with vanadate (Renirie et al. 2003). These haloperoxidases have great potential as catalysts in oxidative reactions/processes. Fungal phytases are HAP-phytases, therefore, can easily be converted into peroxidases due to incorporation of vanadate in their active sites (Velde et al. 2000). Phytase was synthesized as a CLEA and vanadium-haloperoxidase activity due to incorporation of vanadate into active site of HAP-phytase was employed in thioanisolesulfoxidation in the presence of hydrogen peroxide. This enzyme showed selectivity, and recyclability with high conversion rate (Correia et al. 2008). Vanadate ion incorporated into the *P. anomala* and *S. thermophile* phytases converted into haloperoxidases (Joshi and Satyanarayana 2015; Singh et al. 2018). Molecular docking studies also supported the sharing of binding site by vanadate with phytic acid (Joshi and Satyanarayana 2015; Singh et al. 2018). A notable increase in peroxidase activity was detected in the case of *A.oryzae* phytase when it was exposed to ammonium metavanadate as compared to sodium metavanadate. Additionally, there was increase in haloperoxidase activity with concomitant decline in phytase activity (Pragya et al. 2023).

Plant growth promotion

In many regions of the globe, phosphorus is a crucial macronutrient for agricultural crops that restricts plant development and crop yield (Singh et al. 2020). In the soil, phosphorus and other chemicals combine to create insoluble complexes. Soil P exists in two forms viz. organic and inorganic. The organic form predominantly comprises phytates, which account for approximately 50–80% of the total soil phosphorus pool (Singh et al. 2020). The specific proportion depends on the particular soil type and this form of phosphorus is often derived from plant residues and compost materials. Conversely, the inorganic form, often denoted as Pi, primarily consists of apatite that is complexed with other elements such as calcium, iron, and aluminum phosphate. Additionally, phosphorus can also be adsorbed onto clay particles in the soil matrix, enhancing its retention in the soil environment. Recent empirical investigations suggest potential strategies for optimizing the acquisition of phosphorus from soil phytate by plants (Rizwanuddin et al. 2023b). One such approach entails the inoculation of soil with a specific microbial strain known to produce the enzyme phytase. An alternate strategy involves the direct addition of phytase to the soil. Both methods aim to enhance the bioavailability of phosphorus from phytates, thereby potentially improving nutrient uptake and plant productivity (Ige et al. 2011). *Phialocephala fortinii* DSE2 demonstrated the capacity to colonize the roots of *Vaccinium macrocarpon*, a different plant species. This colonization resulted in an increase in the plant's phosphorus content and overall biomass. Additionally, the fungus exhibited the ability to hydrolyze phytates and accumulate polyphosphates (Mikheev et al. 2022). Out of the fungi examined, *Chaetomium globosum* displayed the most effective extracellular phytase, facilitating the mobilization of soil organic phosphorus for plant nutrition (Dhariwal et al. 2023).

Biofuel production

Phytic acid a prevalent compound in grain-derived raw materials has an inherent propensity to form complexes with multivalent cations including zinc, iron, calcium, and magnesium as well as proteins and starch (Mikulski et al. 2015). This complexation hampers its availability to yeast during the alcohol fermentation sequence. The chelation with polysaccharides confers a degree of resistance to enzymatic degradation, consequently reducing the quantity of sugars eligible for fermentation. The interaction with starch could occur directly via hydrogen bonds or indirectly through affiliated proteins. A feasible mitigation strategy to this issue 35 involves the hydrolysis of phytic acid utilizing phytase. The liberation of inositol from phytic acid has the potential to augment the ethanol endurance of yeast, thereby facilitating enhanced ethanol generation (Khullar et al. 2011; Mrudula Vasudevan et al. 2019). Moreover, the utilization of phytase serves to enhance the accessibility of liberated phosphorus, minerals, and vitamins to fermenting yeast. This, in turn, increases the production of ethanol and prevents the interference of phytic acid with minerals like Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{2+}. These minerals have a destabilizing effect on amylases, particularly those sourced from *Bacillus* sp. and *A. niger*, which are commonly employed in ethanol manufacturing (He et al. 2017). In another study, a heat and acid-resistant phytase obtained from the thermophilic mold *Thermomyces lanuginosus* SSBP was utilized to enhance the production of bioethanol from *Colocasia esculenta*. By reducing the phytate content in the starch of *Colocasia esculenta* from 1.43 mg/g to 0.05 mg/g, this enzyme increased the number of fermentable sugars available and decreased the viscosity, resulting in a significant 1.59-fold increase in ethanol yield (Makolomakwa et al. 2017). A cell-bound phytase from *Williopsis saturnus* NCIM 3298 was utilized in the saccharification of corn. It was observed that the saccharification

of phytase-treated corn resulted in enhanced production of reducing sugars. Furthermore, the bioethanol production was also increased 18 % using phytase-treated corn hydrolysate (Pable et al. 2019). Further studies need to be carried out on the role of fungal phytases in biofuel production from lignocellulosic substrates.

Environmental pollution management

Phosphorus is a crucial element for both plant and animal nutrition, as it plays a significant role in growth and development processes (Kumar et al. 2016; Mir et al. 2022). However, the indiscriminate and persistent use of P can lead to various environmental issues. By increasing the bioavailability of phosphorus in animal feed, the addition of fungal phytases helps in reducing aquatic P pollution and hence, representing an essential strategy to address environmental pollution (Zhou et al. 2022). Phosphorus is vital for metabolic and regulatory functions in living organisms, including animals and humans, and is required for proper growth and development. PA, a primary source of phosphorus in food, can bind with metal ions and form an undigested, insoluble complex called phytate. This complex lower phosphorus bioavailability due to the limited activity of phytases in monogastric animals and humans, highlighting the importance of phytases in these organisms. Fungal phytases exhibit significant potential for sustainable phosphorus management through efficient utilization of soil phytate (Vashishth et al. 2023).

Organophosphate pesticides are commonly used in agriculture for inhibiting the growth of insects and pests. These pesticides remain in soil for prolonged duration due to poor degradation and hence, result in biomagnification in food chain. These pesticides are highly toxic to animals and humans due to adverse effects on nervous system. Fungal phytases have been shown effective in degradation of these organic phosphorus pesticides (Shah et al. 2017). Phytase from *Aspergillus niger* NCIM 563 degraded 72% of chlorpyrifos at pH 7.0 and 35 °C. Phytase also degraded monocrotophos and methyl parathion up to 53 and 77%, respectively. Chlorpyrifos was degraded up to 91% at 50 °C (Shah et al. 2017).

Role of phytase in nano-drug delivery

Recently, nanoparticles-loaded protein drug carriers are considered as promising materials for cancer and other therapies. The current development of a nanoscale drug delivery system harnesses the enzymatic properties of phytase, coupled with a platinum coating, presenting a novel therapeutic approach for the treatment of various cancer cell lines, specifically THP-1, Hep-G2, and MCF-7 (Sodhi et al. 2022). Materials from biological sources hold a distinct advantage for developing novel materials with potential applications (Wang et al. 2008). Unique structural characteristics of proteins make them naturally compatible with biological systems. These attributes enable proteins to effectively encapsulate diverse substances, including drugs, food components, and nutrients, in aqueous solutions, positioning them as robust delivery carriers (Hermenson et al. 2007). Soni et al. (2015) devised a method to create self-assembled nanospheres of the phytase, which significantly enhanced their effectiveness by incorporating platinum nanoparticles and the anticancer drug curcumin. The process of self-assembly involving the phytase within the ionic liquid,1-butyl-3-methylimidazolium tetrafluoroborate, leads to the creation of functionally active phytase nanospheres. A remarkable increase in anticancer effect was observed with phytase nanosphere (25%), platinum-phytase nanosphere (37%), phytase curcumin (78%) and platinum-phytase-curcumin nanosphere (90%). This innovative methodology potentially introduces a new paradigm in targeted cancer therapy to bridge the gap between nanotechnology and oncology (Sodhi et al. 2022).

Synthesis of myo-inositol phosphates

Fungal phytases catalyze the hydrolysis of phytic acid and generate myo-inositol phosphates intermediates. Lower myo-inositol phosphate derivatives have an important role in cell signaling pathways and mobilization of calcium ions from intracellular spaces (Jain et al. 2016). Plant-based materials are rich in inositol polyphosphates, primarily in the form of phytic acid or its salt (Gonzalez-Uarquin et al. 2020). Super-dosing effects of phytases have shown improvements in weight gain and overall performance as compared to the standard phytase dosage (Cowieson et al. 2011). This high dose of phytase facilitates almost complete degradation of phytate and increases the levels of inositol and intermediates (Walk et al. 2014). It is important to note that lower inositol polyphosphate esters exhibit greater solubility with lesser anti-nutritional effect (Schlemmer et al. 2001). Fungal phytases play a role in the gradual release of phosphate groups from phytate, generating intermediate products such as penta- (IP5), tetra- (IP4), tri- (IP3), di- (IP2), and mono- (IP1) phosphate esters of inositol (Table 3). When exogenous phytase is add to the animal diet, it initiates the hydrolysis of phytate in the acidic conditions of the stomach or gizzard, thereby releasing these lower esters into the intestinal tract (Lee et al. 2018). The animal's own alkaline phosphatase then completes the process by hydrolyzing IP1, resulting in the release of free inositol (Pirgozliev et al. 2017).

Release of Ins (1,4,5)P3 triggers the release of Ca^{2+} from internal stores (Irvine et al. 1984). Inositol plays a fundamental role in signal transduction in various tissues, including the brain, kidneys, reproductive organs, and others,

responding to neurotransmitters, hormones, and growth factors. Multiple genes are involved in inositol metabolism and related pathways (Kiani et al. 2021). Partial degradation of dietary InsP is carried out by phosphatases, phytases, microbial phytases, and pancreatic phospholipases in the digestive tract (Walk et al. 2018). In humans, nearly all (99.8%) of the myo-inositol is absorbed by the gastrointestinal tract (Kiani et al. 2021). Cowieson et al. (2015) demonstrated that plasma inositol levels increased in broiler chickens when they were fed with phytase-supplemented diets. These findings imply that dietary phytases leads to increased dephosphorylation of phytate, resulting in enhanced release of inositol phosphates, which play important role in cell signaling pathways (Lee et al. 2018).

Conclusions

This article discusses the production of phytases by fungi in solid state fermentation and their industrial applications. Fungal phytases are secreted in large amounts using economical substrates in SSF. Fungal phytases have features of ideal phytases, which are suitable for applications in food and feed industries. Fungal phytases are acidic, thermostable and protease-resistant. These properties make them suitable for improving nutrition of monogastric animals including humans, pigs, poultry and fishes. Fungal phytases have garnered significant interest in food production and feed industries, aiming to enhance nutrition quality and reduce phosphorus pollution. Investigating various biological properties of fungal phytases is essential to enhance their activity and stability for both nutritional and industrial purposes. However, only a limited number of fungal strains have been studied for phytase production, necessitating the identification of novel fungal species with advanced phytase characteristics and stability levels. Among fungi, *Aspergillus niger* and *Aspergillus oryzae* are classified as 'Generally Recognized as Safe' (GRAS) status by the FDA. Plants-based food and feed materials have been employed in poultry, piggery and aquaculture for economical production at large scale. The price of this commodity has increased due to growing demand for fishmeal in aquaculture, the livestock and poultry industries, and piggery production. It has been proven both *in-vitro* and *in-vivo* that addition of phytase to diets has improved the bioavailability of nutrients including phosphorus, sugars, minerals and proteins for absorption by the body. Phytase supplementation has reduced the excretion of phytic acid significantly and hence, resulting in mitigation of environmental phosphorus pollution. Thermophilic fungi have been explored as a potential source for phytases that are highly stable as compared to mesophilic fungi. Therefore, there is need to explore more natural resources for the isolation of thermophilic fungi for phytase production. Furthermore, cloning and protein engineering of potential phytase-producing fungi can provide valuable advantages. The growing demand for phytases offers opportunities for discovering catalysts with improved properties suitable for industrial implementation.

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Data availability All the data are included in the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

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