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RECOMBINANT PHYTASE: ADVANCES IN PRODUCTION STRATEGIES AND INDUSTRIAL APPLICATIONS – A REVIEW

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ABSTRACT. *Phytase has become an essential enzyme in modern biotechnology as global demand for sustainable and efficient feed and food production continues to increase. By catalyzing the hydrolysis of phytic acid, phytase improves phosphorus bioavailability, enhances nutrient utilization, reduces dependence on inorganic phosphate supplementation, and limits phosphorus discharge into the environment. These benefits have contributed to rapid market expansion, with the global phytase market valued at approximately USD 0.6–0.7 billion in 2023 and projected to exceed USD 1 billion by 2030. Recent advances in genetic engineering, protein engineering, and molecular biology have accelerated the development of phytase variants with improved catalytic efficiency, thermostability, and tolerance to acidic conditions. A range of microbial expression systems, including bacterial, fungal, and yeast platforms, has been extensively explored to optimize enzyme production and functionality under industrial settings. Recombinant DNA technologies now allow precise tailoring of phytase expression in intracellular, extracellular, and cell surface display formats, each offering specific advantages for industrial application. Notably, cell surface display systems are attracting growing interest due to their potential to simplify downstream processing and lower production costs. This review provides a comprehensive overview of contemporary recombinant phytase production strategies, critically examining host selection, expression formats, and key factors influencing large-scale production. By addressing both technological advances and production challenges, this review aims to support the development of efficient, cost-effective, and environmentally sustainable phytase production platforms.*

INTRODUCTION

Phytases, also known as myo-inositol hexakisphosphate phosphohydrolases, are enzymes that catalyze the hydrolysis of phytic acid, releasing inositol and inorganic phosphate (Gocheva *et al*., 2024). These enzymes play a pivotal role in improving the bioavailability of necessary minerals, such as calcium, iron, and zinc, which are otherwise bound to phytic acid, an anti-nutrient commonly found in plant-based feeds (Salim *et al.*, 2023). Due to their ability to enrich the nutritional value of animal feed, phytases are widely used in the livestock and poultry industries (El-Hack *et al*., 2018). Phytases are classified as 3-, 4-, and 5-phytases according to the location of the first phosphate group they hydrolyse (Meegoda *et al.*, 2018). This classification is crucial because it affects how well enzymes function in different environmental settings, such as temperature and pH, which are important considerations for both industrial operations and animal digestion (Ravindran, 2013). The need for phytases that maintain their activity throughout a wide range of pH and temperature conditions has prompted a great deal of

study on the stability and effectiveness of enzymes, especially for use in feed processing and the manufacture of biofuel (Rebello *et al.*, 2017). Phytases are produced by a broad range of species, including bacteria, plants, and animals. Singh *et al*. (2024) stated that microbial phytases, especially those derived from bacterial and fungal sources, are favored for industrial applications due to their high production yield, simplicity of genetic manipulation, and suitability to commercial fermentation techniques. Recombinant phytases provide improved enzymatic features, such as increased resistance to proteolysis, improved catalytic efficiency, and higher thermostability. Both wild-type and recombinant phytases are used in industrial settings (Venkataraman *et al*., 2024). The performance and production efficiency of recombinant phytases have been further enhanced using genetic engineering approaches such as cell surface display systems, powerful promoters, and codon optimization (Zhou *et al*., 2022). In commercial phytase synthesis, the cell surface display technique works especially well because it enables direct enzyme presentation on microbial cell surfaces, streamlining purification procedures while increasing enzyme stability and activity (Greenstein *et al*., 2020).

Interest in the industrial uses of phytases and recombinant phytases is rising due to the field's increasing research and the more than 30 patents that have been filed on them. In-depth examination of physiological roles of phytase enzyme, structural and catalytic mechanisms, industrial uses, and developments in production technologies are all included in this review (Outchkourov & Petkov, 2019). Through an examination of the relative importance of various production techniques, this review aims to highlight significant advancements in phytase biotechnology research and future directions (Handa *et al.*, 2020).

MATERIALS AND METHODS

This review was conducted through a comprehensive and systematic analysis of published literature focusing on recombinant phytase production strategies and their industrial applications. Relevant peer-reviewed research articles, reviews, book chapters, and doctoral theses were collected from major scientific databases, including Scopus, Web of Science, PubMed, Google Scholar, and ScienceDirect. The literature search covered publications primarily from 2000 to 2024, with emphasis on recent advances in recombinant DNA technology, enzyme engineering, and microbial expression systems.

Keywords used during the search included phytase, recombinant phytase, phytase production, cell surface display, yeast expression systems, fungal phytase, industrial enzymes, and phytase applications. Articles were selected based on their relevance to phytase classification, microbial sources, genetic engineering approaches, expression platforms (intracellular, extracellular, and surface display), biochemical properties, and industrial or environmental applications.

After initial screening of titles and abstracts, full-text articles were critically evaluated to extract information on enzyme characteristics, host systems, production strategies, and scalability. Only studies providing clear experimental data, comparative analyses, or significant technological insights were included. The selected literature was then categorized thematically to ensure structured discussion and critical comparison across different production platforms. This approach enabled an integrated assessment of current advancements, limitations, and prospects in recombinant phytase biotechnology.

RESULTS

Phytase

Sources of Phytase

The primary microbial sources of phytase include *Aspergillus niger, Bacillus subtilis, Escherichia coli*, and *Klebsiella pneumoniae* (Gocheva *et al*., 2023). These organisms are widely utilized in industrial processes due to their ability to ferment and produce large quantities of phytase efficiently (Jatuwong *et*

al., 2023). Advancements in genetic engineering have been applied to these microbes to enhance enzyme production, thermal and pH stability, and overall catalytic efficiency. As a result, these genetically modified strains are extensively used for large-scale phytase production in sectors such as agriculture, animal nutrition, and environmental management (Sharma & Satyanarayana, 2013).

The commercially significant phytase-producing microorganisms and plant sources are included in Table 1, along with their corresponding EC numbers. Due to their high enzyme output and stability under industrial processing settings, *Escherichia coli, Bacillus subtilis*, and *Aspergillus niger* are some of the most commonly utilized sources of phytase (Kumar & Sinha, 2018; Liu *et al*., 2022). Despite being less prevalent, plant-based phytases have drawn attention due to their possible use in biofortification and animal feed augmentation techniques (Liu *et al*., 2022).

Table 1. Commercially important phytase-producing organisms.

Microbial Sources	Plant Sources	EC Number	Refences
Escherichia coli	*Tomato roots*	EC 3.1.3.8	(Kumar & Sinha, 2018; Liu *et al*., 2022)
Bacillus subtilis	*Typha latifolia* pollen	EC 3.1.3.26	(Mittal *et al*., 2013; Liu *et al*., 2022)
Klebsiella terrigena	*Barley*	EC 3.1.3.26	(Kumar & Sinha, 2018; Liu *et al*., 2022)
Klebsiella pneumoniae	*Maize seedling*	EC 3.1.3.8	(Mittal *et al*., 2013; Liu *et al*., 2022)
Citrobacter braakii	*Wheat bran*	EC 3.1.3.8	(Mittal *et al*., 2013; Liu *et al*., 2022)
Lactobacillus sanfranciscensis	*Aspergillus niger*	EC 3.1.3.8	(Kumar & Sinha, 2018; Liu *et al*., 2022)
Aspergillus ficuum	*Aspergillus fumigatus*	EC 3.1.3.8	(Kumar & Sinha, 2018)
Pichia anomala	*Candida krusei*	EC 3.1.3.26	(Kumar & Sinha, 2018)
Saccharomyces cerevisiae	-	EC 3.1.3.8	(Kumar & Sinha, 2018)

Classification of Phytase

Phytases are classified based on their structural and catalytic properties, which influence their stability, substrate specificity, and suitability for industrial use. The major classes include: Histidine Acid Phosphatases (HAPs), predominantly found in bacteria, fungi, and plants. HAPs contain a conserved histidine residue crucial for their catalytic activity. They function effectively in acidic conditions, making them widely used in commercial applications, especially in food and feed industries (Bouajila *et al.,* 2020). β-Propeller Phytases (BPPs), mainly derived from bacterial species, are characterized by a β-propeller fold that provides broad pH tolerance and high thermal stability. Their ability to function in neutral to alkaline environments makes them especially valuable in animal feed formulations (Singh *et al.,* 2018). Purple Acid Phosphatases (PAPs) are mostly found in plants and some fungi. They exhibit unique substrate selectivity and pH stability and play an important role in plant phosphorus metabolism. However, they are less commonly used in commercial settings (Bhadouria & Giri, 2022). Although the primary function of Protein Tyrosine Phosphatases (PTPs) is not phytate degradation, some PTPs from microorganisms have shown potential for biotechnological applications. They possess a distinct catalytic mechanism and are under investigation for specialized uses (Singh *et al.,* 2018; Cangussu *et al.,* 2018).

Phytase Production Platforms

Phytase production is carried out using two primary approaches: wild-type production and recombinant production systems. Each method has distinct advantages and limitations, depending on the intended application (Bhavsar & Khire, 2014)

Production of Wild-Type Phytase

Phytase-producing bacteria are necessary for wild-type production. Due to its ease of use and affordability, this technique is frequently employed in large-scale fermentation. *Aspergillus niger* and other fungal strains are among the most widely employed species for the manufacture of phytase (Nagar *et al.,* 2021). Since it replicates the natural growing conditions of these fungi, solid-state fermentation (SSF) is the recommended technique for producing wild-type phytase (Santos, 2011). This method is

made both economically and environmentally feasible by using agricultural byproducts as substrates, such as soybean meal, rice bran, and wheat bran. However, the yield, thermostability, and specific activity of wild-type manufacturing are limited, frequently requiring further processing or enzyme purification (Katileviciute *et al.*, 2019).

Recombinant Phytase Production

The production of phytase has been transformed by recombinant DNA technology, which makes it possible to introduce phytase genes into host microorganisms, including *Saccharomyces cerevisiae, Pichia pastoris*, and *Escherichia coli*. This method offers greater catalytic efficiency, increased thermostability, increased enzyme yields, and resistance to proteolytic degradation (Kaur *et al.,* 2010). Some major benefits of producing recombinant phytase have been found. Optimised enzyme properties, improving pH and thermal stability made possible by genetic alterations, increase the applicability of the enzyme in a wider range of industrial settings (Rigoldi *et al.,* 2018). Compared to wild-type strains, recombinant strains can be designed for higher enzyme expression, which results in higher yields (Saxena, 2015). Recombinant systems offer improved control over the development and synthesis of enzymes, guaranteeing constant activity and quality (Huang *et al*., 2012). Modern advancements, such as codon optimization, the use of strong promoters, and cell surface display systems, have further improved recombinant phytase production (Han *et al*., 2018). The cell surface display strategy is particularly advantageous, as it allows phytase enzymes to be anchored on microbial cell membranes, facilitating direct enzyme application without the need for extensive purification (Pragya *et al*., 2023). Table 2 presents a comparative overview of recombinant phytases produced in recent years, highlighting their source organisms, host strains, expression vectors, optimal biochemical parameters, and industrial applications. The enzymes were expressed in various microbial systems such as *E. coli*, *Pichia pastoris*, *Kluyveromyces lactis*, and *P. griseoroseum*, using well-established vectors such as pET-28a (+), pPICZαA, and pYES2. These recombinant phytases exhibit diverse optimal temperatures (ranging from 50 to 60 °C) and pH levels, with specific activities varying significantly depending on the expression system and assay conditions. Applications primarily include animal feed supplementation, particularly in poultry and aquaculture, due to improved thermal stability and protease resistance. While the data provide valuable insight into enzyme performance, it should be noted that differences in assay substrates and definitions of unit activity may affect direct comparison across studies (Ribeiro *et al.,* 2015; Ranjan & Satyanarayana, 2016).

Table 2. Recombinant phytases produced in recent years: expression systems, biochemical properties, and industrial applications (data compiled from published studies).

Source Organism	Host Strain	Expression Vector	Optimum Temp (°C)	Optimum pH	Specific Activity	Km (mM)	Key Applications	EC Number	Reference
Dendroctonus frontalis	*E. coli*	pET-28a(+)	52.5	3.9	4135 U mg^{-1}	0.262	Animal feed additive	EC 3.1.3.8	(Tan *et al*., 2016)
A. niger NII08121	*Kluyveromyces lactis* GG799	pKLAC2	55	2.5 & 5.5	198 U mg^{-1}	N/A	Protease-resistant phytase for industrial use	EC 3.1.3.8	(Ushasree *et al*., 2014)
Aspergillus niger	*Pichia pastoris* GS115	pPIC9K	60	5.5	N/A	.148	Feed supplement with thermal stability	EC 3.1.3.8	(Hesampour *et al.,* 2015)
A. niger NII08121	*E. coli*	pET-21b	50	6.5	18 U mg^{-1}	N/A	Improved purification and protein yield	EC 3.1.3.8	(Vasude, Salim & Pandey, 2011)
Penicillium chrysogenum CCT 1273	*P. griseoroseum*	pYES2	50	5.1	2.86 ± 0.4 U μg^{-1}	N/A	Animal nutrition	EC 3.1.3.8	(Ribeiro, Queiroz & Araújo, 2015)
Sporotrichum thermophile	*Pichia pastoris* X-33	pPICZαA	60	5.0	480 ± 23 U mL^{-1}	0.147	Poultry and aquaculture feed additive	EC 3.1.3.8	(Ranjan & Satyanarayana, 2016)

*N/A indicates that the corresponding parameter was not reported in the original study.

Cell Surface Display System

An inventive method for creating recombinant phytase is the cell surface display system, which immobilises the enzyme on the surface of the host cell. By guiding the protein to the cell wall via a genetic cassette included in an expression vector, this method improves stability and streamlines downstream processing. Strong promoters such as GAL1 and GAL10 (Hossain *et al*., 2020), which stimulate high protein expression, and anchor proteins such as Sed1, Ccw12, Cwp1, and Cwp2 in yeast, which maintain the stability and integrity of the cell wall (Geetha *et al.,* 2019), are crucial parts of this system. Furthermore, effective protein immobilization is made possible by glycosylphosphatidylinositol (GPI) anchors, such as GCW61 in *Pichia pastoris*, which raises phytase activity to 6413.5 U g^{-1} (Müller, 2011). This technique is useful for both industrial and environmental applications since it not only increases stability and processing convenience but also provides environmental advantages, including improved ethanol production in Saccharomyces cerevisiae and effective phosphorus reduction (Kumari & Bansal, 2022). Different anchor proteins and genetic constructs have been used to successfully apply the cell surface display system across a range of expression hosts, as shown in Table 3.

Table 3. Surface display systems for recombinant phytase expression in various hosts.

Expression Host	Vector	Promoter	Anchor Protein	Phytase Activity	Reference
Candida amalonaticus CGMCC 1696	pPICZaA	AOX1	Gcw61p	6413.5 U g^{-1}	(Hossain *et al.,* 2020)
E. coli JM109	pMGK-AG	PGK1	α-agglutinin (C-terminal)	6.4 U g^{-1} (wet biomass)	(Li *et al.,* 2014)
Aspergillus niger	pPICZaA	AOX1	α-agglutinin (3'-half)	300 U g^{-1} (dry weight)	(Chen *et al.,* 2016)
Bacillus subtilis	Native OxdD motif	OxdD	OxdD	5.7×10^3 U g^{-1} (spore dry weight)	(Harnpicharnchai *et al.,* 2010)
	Codon-optimised *phyA* gene	CotG	CotG	91.62 U per 10^8 spores	(Potot *et al.,* 2010)

Various expression hosts and surface display systems have been employed to enhance phytase activity and stability for industrial applications. Hosts such as *Candida amalonaticus, E. coli, Aspergillus niger*, and *Bacillus subtilis* utilize vectors with specific promoters (e.g., AOX1, PGK1, CotG) and anchor proteins such as α-agglutinin, Gcw61p, or OxdD to facilitate efficient surface display (Hossain *et al.,* 2020; Li *et al.,* 2014; Chen *et al.,* 2016; Harnpicharnchai *et al.,* 2010; Potot *et al.,* 2010). Among them, *C. amalonaticus* and *B. subtilis* systems show notably high phytase activities, making them promising platforms for cost-effective phytase production in feed and environmental sectors.

Economic Implications and Industrial Applications

In industrial applications, recombinant phytases provide substantial financial advantages, particularly in the areas of environmental control and animal feed. For instance, supplementation of poultry feed with recombinant *Aspergillus niger* phytase has been shown to reduce inorganic phosphate supplementation by up to 30%, while simultaneously lowering phosphorus excretion into the environment (Tan *et al.,* 2016; El-Hack *et al*, 2018; Venkataraman *et al*., 2024; Bhavsar & Khire, 2014). Enzymes with increased stability and activity can be engineered to reduce phytate in feed and enhance nutrient absorption more effectively. For instance, supplementation of broiler feed with recombinant *Aspergillus niger* phytase increased phosphorus and calcium digestibility by 15–25%, improving growth performance and reducing phosphate excretion (Handa *et al.,* 2020). Additionally, by lowering the requirement for phosphate supplementation and minimizing environmental phosphorus pollution, recombinant phytases help to make animal rearing more sustainable (Kumar *et al.,* 2015; Gocheva *et al*., 2024). In order to enhance the nutritional value of foods or as possible treatment agents for phosphate-related illnesses, recombinant phytases are also being investigated for usage in the food and pharmaceutical sectors (Shunmugam, 2014; El-Hack *et al*, 2018).

DISCUSSION

There are distinct advantages and disadvantages to producing phytase from various microbiological sources and expression hosts, which are important for industrial applications. To move beyond descriptive reporting, a comparative evaluation of the major recombinant phytase expression platforms is necessary to identify systems with the highest industrial relevance. Comparative evaluation of recombinant phytase expression systems indicates that yeast-based platforms, particularly *Pichia pastoris* and *Saccharomyces cerevisiae*, provide high expression efficiency, appropriate post-translational modifications, and scalability suitable for industrial production (Bhavsar & Khire, 2014).

Bacterial hosts such as *Bacillus subtilis* further enhance industrial feasibility through efficient secretion and cell surface display, significantly reducing downstream processing costs. In contrast, filamentous fungi remain commercially dominant in feed industries due to their robustness in large-scale fermentation, despite comparatively limited genetic flexibility (Ranjan & Satyanarayana, 2016; Kaur *et al*., 2022). It is commonly known that *Aspergillus* species, especially *A. niger*, are very adaptable and easily genetically modified. For example, high yield levels were obtained by *A. niger NII 08121* produced in *Kluyveromyces lactis* GG799 (Tan *et al.,* 2016). At 826.33 U mL^{-1}, another strain, *A. niger 563*, produced a notably higher amount of phytase than its wild-type equivalent (Salaet *et al*., 2021). Expression systems based on yeast have also shown potential. For instance, employing yeast cell surface display technology, *Pichia pastoris* KM71, which expresses *A. niger* phytase, showed high specific activity (300 Ug^{-1} cell dry weight) (Müller, 2011). In a similar vein, *A. japonicus C03* showed beneficial glycosylation patterns and significant phytase activity (Geetha *et al*., 2019).

Bacillus subtilis has proven beneficial in bacterial systems because of its efficient downstream processing and ease of purification. Comparative studies show that the surface display systems of *B. subtilis* and *S. cerevisiae* both exhibit noticeably higher amounts of phytase synthesis. Furthermore, increased phosphorus digestibility has been seen in hosts such as *Lactococcus lactis* that express *E. coli* phytase. The animal feed business has also benefited from fungi such as *Penicillium chrysogenum CCT 1273* and *P. griseoroseum* (Ribeiro *et al.,* 2015). One significant development that has made purification simpler, improved thermal stability possible, and made it economically viable for commercial usage is the immobilization of phytase on the cell surface. Although these advantages have drawn attention to surface display technologies, other methods, such as intracellular and extracellular expression systems, also increase the efficiency of phytase synthesis. Despite these advantages, these systems suffer from lower overall yield, restricted enzyme flexibility, and limited substrate accessibility due to anchoring constraints (Ribeiro *et al.,* 2015).

Despite the advantages of recombinant phytase expression systems, several limitations remain. Bacterial hosts, such as *E. coli* and *Bacillus subtilis*, may face challenges in proper folding and post-translational modifications, which can reduce enzyme stability and activity (Huang *et al.,* 2012; Ranjan & Satyanarayana, 2016). Yeast-based systems, including *Pichia pastoris* and *Saccharomyces cerevisiae*, generally provide higher yields and suitable secretion but can introduce undesired glycosylation patterns and impose metabolic stress on the host, limiting overall expression efficiency (Hossain *et al*., 2020; Geetha *et al*., 2019). Cell surface display approaches simplify downstream processing and allow direct enzyme application; however, enzyme accessibility may be restricted, and substrate interaction can be suboptimal due to anchoring constraints (Potot *et al*., 2010; Müller, 2011). Acknowledging these drawbacks is essential for selecting and optimizing host systems for industrial-scale phytase production.

CONCLUSION

The industrial significance of current developments in phytase production platforms is compiled in this study. Optimizing a number of factors, such as host strain selection, substrate cost and availability, and recombinant synthesis ease, is essential for industrial-scale production. Furthermore, attaining high-yield production depends on phytase expression (Xie, 2020). Intracellular and extracellular expression technologies have shown significant success in addition to surface display techniques. Strong expression

capabilities are provided by yeast-based platforms such as *P. pastoris* and *S. cerevisiae*, but simpler downstream processing is offered by bacterial systems such as *B. subtilis* and *E. coli*. Furthermore, the commercial production of phytase, especially for use in animal feed, still depends on fungal sources (Xie, 2020). Commercial phytase production for animal feed still relies primarily on fungal sources such as *Aspergillus niger* and *Penicillium* species due to their high extracellular enzyme yield and industrial suitability (Abd El-Hack *et al*., 2018; Bhavsar & Khire, 2014). Ongoing advancements in expression hosts and biotechnological methods are crucial due to the growing need for high-yield, economical, and thermally stable phytase (Kaur *et al.,* 2022). Future studies should concentrate on incorporating cutting-edge genetic engineering techniques, refining fermentation tactics, and investigating new host systems in order to enhance phytase production (Siddique *et al.,* 2022). In order to meet changing market demands, industrial phytase production can become more sustainable and efficient by tackling these issues. Although current studies highlight the advantages of various recombinant phytase production systems, more comparative data and industrial case studies are needed to draw stronger, evidence-based conclusions. Future research should focus on generating comprehensive experimental and application-based examples to reinforce these findings.

Abbreviations: In this review, the following abbreviations are used: SSF, solid-state fermentation; HAP, histidine acid phosphatase; BPP, β-propeller phytase; PAP, purple acid phosphatase; PTP, protein tyrosine phosphatase; RA, research assistant; EC, enzyme commission number; GPI, glycosylphosphatidylinositol; Km, Michaelis-Menten constant; U, unit of enzyme activity; AOX1, alcohol oxidase 1 promoter; PGK1, phosphoglycerate kinase 1 promoter; CotG, Bacillus subtilis coat protein G; pET, pET expression vector series; pPIC, Pichia pastoris expression vector; pKLAC, Kluyveromyces lactis expression vector; and pYES, yeast expression vector.

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