ACADEMICIA
An International Multidisciplinary Research Journal
(Double Blind Refereed & Peer Reviewed Journal)

DOI: 10.5958/2249-7137.2021.02364.8

A REVIEW ON ENZYMOLOGY, USES AND BIOTECHNOLOGY OF PHYTASE

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ABSTRACT

In addition to auxiliary enzymes, fungal proteins, as well as organic acids, phytase generated by filamentous fungus on chosen feed components increases feed digestibility and access to phytin in plant cells. Phytases are phosphohydrolases that start the process of removing phosphate from phytate one step at a time. These enzymes have long been used in animal feed to enhance phosphorus nutrition or decrease phosphorus contamination from animal manure. The use of phytases to improve human nutrition of important trace elements found in plant-derived foods is being investigated. This study focuses on the growing biotechnology utilized to create novel effective phytases with enhanced characteristics, as well as the fundamental biology and use of phytases.

KEYWORDS: *Biotechnology, Environmental Pollution, Mineral Nutrition, Phytase, Phytic Acid.*

1. INTRODUCTION

Phytase is necessary for increasing the nutritional content of feed as well as promoting animal development and health. It hydrolyzes phytate substrates to generate phosphorous in a free form that animals may ingest, reducing the need for inorganic phosphorus supplements. Furthermore, adding phytase to feed reduces the amount of phosphorus excreted in manure, resulting in a

reduced environmental effect from livestock production. Phytase for feed is generated via microbial strain fermentation and is mostly utilized in swine and poultry diets. Phytase is worth $300–350 million and accounts for approximately 40% of the entire feed enzyme market. The market price for phytase varies significantly, and the substance is available in various concentrations. The market price for a product standardized at 5000 FTU/g may range from $1 to $5 per kilogram1[1].

The cheapest phytase products are those made in China for the domestic market. However, comparing one product to another cannot be done only on the basis of FTU activity since other factors will influence the enzyme's performance. Four essential criteria distinguish one phytase from another without delving into complicated technical issues. Cereal and legume seeds acquire a significant quantity of phytic acid during ripening (myo-inositol1,2,3,4,5,6-hexakis dihydrogen phosphate). As a consequence, the majority of these seeds and co-products contain 1–2% phytic acid, which accounts for more than 60% of their physico - chemical parameters. A significant part, if not all, of phytic acid in seeds is probably in the form of phytate salts. Although phytate is the primary source of energy and phosphorus for seed germination, simple-stomached animals have limited access to the bound phosphorus. To fulfill the nutritional demand for phosphorus, inorganic phosphorus, a non-renewable and costly mineral, is added in swine, poultry, and fish diets. Meanwhile, in places where extensive animal husbandry is practiced, unutilized phytate phosphorus from plant feeds is excreted, creating an environmental contaminant. Excess phosphorus in the soil washes into lakes and the sea, producing eutrophication and promoting the development of aquatic species that may generate neurotoxins that are harmful to people. Furthermore, positively charged divalent cations bind with negatively charged phytic acid. As a result, the bonded metals are poorly absorbed in the small intestine. This is due in part to widespread human nutritional deficits in calcium, iron, and zinc in developing nations where plant-based diets are the norm. Overall, problems in animal feeding, environmental protection, and human health have spurred the rapid development of phytase research and innovation[2].

1.1 Phytase Characteristics:

The quantity of inorganic phosphate generated per minute from a certain substrate at a specific pH and temperature is used to determine phytase activity. Phytase activity or function is influenced by the enzyme's intrinsic characteristics as well as the action circumstances, much like other enzymes. The following characteristics of phytase are important in practice:

The substrate specificity as well as affinity of several phytases have been extensively studied. Plant phytases and certain fungal enzymes, such as the one from A. fumigatus, seem to have a wider substrate specificity or are responsible for breaking down the lower inositol phosphates, while microbial phytases appear to have a strong affinity for phytic acid. Although most phytases may degrade phytic acid to inositol monophosphate ester Bacillus sp. phytases hydrolyze every second phosphate preferentially over the neighboring ones, degrading the phytic acid molecule to inositol triphosphate[3].

Phytase converts inositol, phosphate, as well as other divalent minerals from phytate. Phytate is a dihydrogen phosphate of myo-inositol-1,2,3,4,5,6-hexakis that includes 14–28 percent phosphorus as well as 12–20 percent calcium. Phytate also chelates iron and zinc trace elements (1 to 2%) between phosphate groups within a single phytate molecule or between two phytate

molecules. Phytase is the sole enzyme that can start phosphate hydrolysis in the inositol ring of phytate at carbon 1, 3, or 6. Calcium, iron, zinc, or other metals are released when phytase removes the phosphate group.

1.2 Phytase nomenclature:

Phytases are inositol phosphate esters and inorganic phosphate phosphohydrolases that catalyze the stepwise phosphate splitting of phytic acid (IP6) or phytate to lower inositol phosphate esters (IP5-IP1) (Figure 1). Plants and microorganisms, including bacteria, yeast, and fungus, have been shown to contain phytase genes and proteins. Aspergillus nigerPhyA, which is encoded by a 1.4 kb DNA fragment and has a molecular mass of 80 kDa and 10 Nglycosylation sites, will be the first and possibly best described phytase.

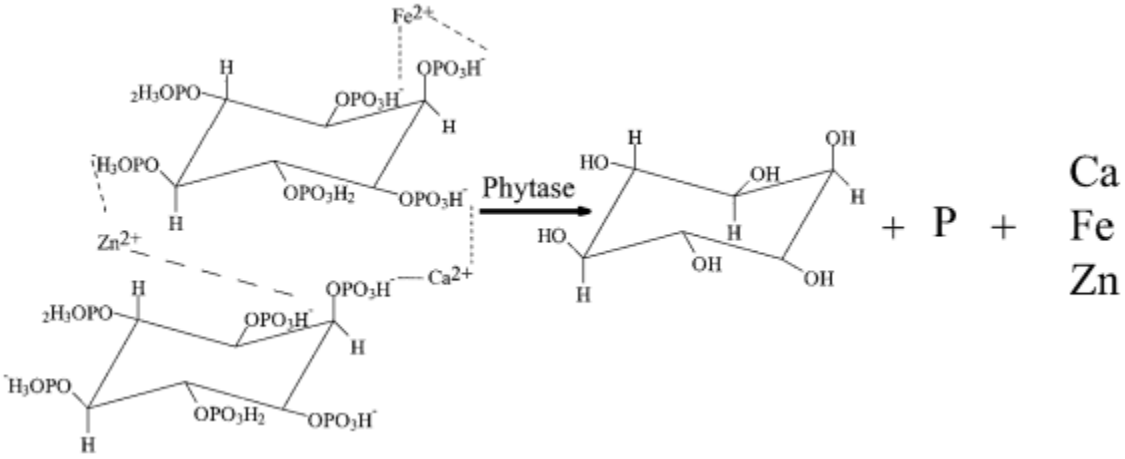

Figure 1:Phytase hydrolyzes phytate to produce inositol, phosphate, or other divalent chemicals[4]**.**

1.3 Optimal pH and temperature:

The pH optimal range for most isolated phytases is 4.5–6. Phytases from Bacillus sp., on the other hand, exhibit pH opticalities that are neutral or alkaline. The pH profile of A. Niger phytase (phyA) shows two pH maxima at 2.5 and 5.5, with a drop in activity between these two sites (Han & Lei 1999). To maintain a high specific activity at the stomach pH, mutagenesis was used to eliminate the decrease in activity in the pH range 3–5. Most plant and microbial phytases prefer temperatures between 45 and 60 degrees Celsius. These relatively high optimum temperatures prevent phytases from fully activating at swine or chicken stomach temperatures (37–40 C), resulting in even worse phytase efficacy in fish.

1.4 Thermostability:

Because commercial feeds are often pelleted, a process that involves high temperatures (60–80°C) and steam, all feed enzymes must be heat stable to prevent significant activity loss. The capacity of any particular phytase, like other proteins, to withstand heat denaturation, such as in hyperthermophile animals, and/or to refold properly into the native-like, fully active conformation following heat denaturation determines its thermostability. Environmental factors such as buffer specificity may have an impact on the latter assumption.

1.5 Resistance to proteolysis:

A high resistance to hydrolytic degradation by digestive proteinases in the digestive system is required for an efficient phytase. Pepsin and trypsin sensitivity differs between fungal and bacterial phytases, and the latter seems to be more resistant to proteolytic destruction than the former. Site-directed mutagenesis may be used to block or modify the protease-sensitive sites of phytases, which are usually found in exposed loops on the surface of the molecules[5].

1.6 Phytase's current and future uses:

Phytase has traditionally been utilized primarily, if not exclusively, as a feed additive in diets for pigs, poultry, and to a lesser degree, fish. Phytase may replace about 1 g inorganic phosphorus supplementation and decrease total phosphorus excretion by 30–50%, according to many laboratory studies and field trials

1.7 Phytase biotechnology:

If the demand for phytase arose from increased environmental awareness of phosphorus contamination caused by animal manure, biotechnology has accelerated its development to the present level. Phytase was first discovered to hydrolyze phytate phosphorus in chick diets 30 years ago. Due to the poor activity output and expected high cost of the traditional phytase fermentation method, commercialization has been impossible for many years. Large quantities of the enzyme may be generated for animal feed at cheap prices thanks to the advent of heterologous microbial expression methods.

1.7.1 Expression of microbes:

systems Submerged or solid-state fermentation of filamentous fungus overexpressing phytase (i.e., Aspergillus species) provides high quantities of phytase at cheap cost. Recently, there has been a lot of study on the usage of methylotrophic yeast. Stretptomyceslividans and Lactobacillus plantarum have both been shown to produce phytase. Combining phytase with the beneficial probiotic lactic acid bacteria is possible with the latter expression method. In soybean or alfalfa seeds, a fungal phytase has been effectively expressed.

The most significant sources of phytase are bacteria and fungus. phytase sources from microbes. For commercial phytase synthesis, A. niger, Aspergillus ficuum, Aspergillus fumigatus, and S. cerevisiae are frequently utilized yeast strains. the hydrolysis of dietary phytate by exogenous microbial phytase. They discovered that chicks fed maize–soybean meal diets containing Aspergillus preparations had better phosphorus utilization. Phytase produced from S. cerevisiae is particularly important for bread making. Similar to cell-bound phytase from Pichia anomala and Candida krusei, cell-bound phytase from Pichia anomala and Candida krusei has potential uses in food processing since it is stable at high temperatures and acidity[6].

1.7.2 Plants and animals that are transgenic:

To enhance rice iron bioavailability to humans, transgenic rice has been created to over-express alleles encoding for phytase from Aspergillus fumigatus, ferritin from Phaseolus vulgaris, and a cysteine rich metallothionein-like protein. The plant was crossed with a rice line that produces -carotene, which was recently created. Meanwhile, overexpressing phytase in the salivary glands of mice and pigs has resulted in transgenic mice and pigs[7].

1.8 Protein engineering is a technique for modifying proteins:

Although phytases have different characteristics, no one wild-type enzyme is optimal or suitable for use in the field. An 'ideal' phytase, in theory, should be catalytically efficient, proteolysis-resistant, thermostable, and inexpensive. In fact, this good's phytase may never be discovered or produced. However, genetic modifications have been effective in improving single or several phytase characteristics[8].

1.9 Phytase-related issues:

Phytase has several advantages, but it also has certain drawbacks that need further study. Phytate, a powerful chelator of iron and zinc, may function as an antioxidant in plant meals, reducing free radical production caused by these metals. Indeed, pigs given phytase for four months were more susceptible to high-iron-induced lipid peroxidation in the colon than control pigs. Low-phytic acid grain, on the other hand, may have a negative impact on human health, particularly in those who have large iron reserves as a result of high dietary intakes of readily accessible iron from animal products or high dietary intakes of fruits that substantially increase non-heme iron absorption. As a result, extreme care should be used while spreading the low-phytic acid grain approach beyond animal production. The second question is whether supplementary phytases hydrolyze phytate-phosphorus from digesta faster than the animals can absorb it, releasing more free phosphorus into the environment than when the animals are not given phytase. By upgrading local exhaust systems and wearing all protective gear and masks with P2 filters, hypersensitivity symptoms may be prevented[9], [10].

2. DISCUSSION

Phytase is necessary for increasing the nutritional content of feed as well as promoting animal development and health. It hydrolyzes phytate substrates to release phosphorous in a free form that animals may ingest, reducing the need for inorganic phosphorus supplements. Furthermore, adding phytase to feed reduces the amount of phosphorus excreted in manure, resulting in a reduced environmental effect from livestock production. Phytase for feed is generated via microbial strain fermentation and is mostly utilized in swine and poultry diets.These enzymes have long been used in animal feed to enhance phosphorus nutrition and decrease phosphorus contamination from animal manure. The use of phytases to improve human nutrition of important trace elements found in plant-derived foods is being investigated. This study focuses on the growing biotechnology utilized to create novel effective phytases with enhanced characteristics, as well as the fundamental biology and use of phytases.

3. CONCLUSION

As phytase becomes more widely utilized throughout the globe, research and technology linked to the enzyme have rapidly developed into a new fascinating area. Supplemental phytases clearly enhance dietary phytate-phosphorus utilization in food-producing animals and decrease phosphorus pollution from animal waste in places where extensive animal production is practiced. The potential of phytase in enhancing human nutrition and health, as well as creating particular phytic acid or inositol-derived products, is gaining traction and will continue to grow as a new phytase path. Biotechnology has shown to be a very successful technique for creating and enhancing phytase enzymes and their delivery systems, and it will continue to be so.

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