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Review Article

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Phytase - A Key to Unlock Phytate Complex

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ABSTRACT

Phytase is an enzyme that frees the inorganic phosphorous from the phytate complex. It addresses both the antinutritional and eutropication problem and hence finds an important role in the feed industry. Phytases had been classified into subgroups based on their catalytic mechanisms, pH optima (alkaline or acid phytases), or the order in which phosphates groups are liberated. The phytase can be supplemented or genetically transformed into plants or animals to improve the phosphorous utilisation in monogastric animals. Several phytases has been isolated from organisms like bacteria, fungi, yeast, plants. Among them, Aspergillus niger phytase has been commercialised first (Natuphos) and widely used since its active at pH 2.5 and 5. The quest for the search of new phytase with wider pH and thermostabilty still goes on.

Keywords*: phytase, classification, phytic acid, producing organisms, commercialisation.*

INTRODUCTION

Phytase

Phytases (EC 3.1.3.8), are a special class of phosphatases that catalyze the sequential hydrolysis of *myo*-inositol- (1,2,3,4,5,6)-hexakisphosphate or phytic acid (InsP6) to less phosphorylated *myo*-inositol derivatives and inorganic phosphate[24] (Figure 1).

Phytase reduces the antinutritional properties of phytic acid and eutrophication, caused by the excretion of undigested phytic acid by monogastrics because of the lack of adequate levels of phytase in their digestive tracts[15,9]. A number of phytase genes and proteins have been identified from plants and microbes including bacteria, yeast, and fungi. The first and probably the best characterized phytase is *Aspergillus niger PhyA* that is encoded by a 1.4 kb DNA fragment and has a molecular mass of 80 kDa, with 10 *N*glycosylation sites[22]. Average molecular masses of bacterial phytases are smaller than those of fungal phytases (40–55 vs. 80–120 kDa), mainly due to glycosylation differences[5]. The molecular masses of plant phytases isolated from corn, wheat, lupine, oat, and barley range from 47 to 76 kDa[20].

Classification

Phytases derived from plants and microbes can be classified into subgroups based on their catalytic mechanisms, pH optima (alkaline or acid phytases), or the order in which phosphates groups are liberated[39].

Catalytic mechanism[44,6]:

1. Histidine acid phytases (HAP)
2. β-propeller phytases (BPP)
3. Cysteine phytases or purple acid phytases (PAP)

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4. Protein tyrosine phosphatase (PTP)

pH optima[57]:

1. Acid phytases (includes those enzymes belonging to the HAP, PAP and PTP-like class of phosphatises)
2. Alkaline phytase (includes BPPs from Bacillus)

Carbon in the myo-inositol ring of phytate at which dephosphorylation is initiated[52]: According to the International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB), phytase feed enzymes fall into three categories depending on the site where the hydrolysis of the phytate molecule is initiated.

1. 3-phytases (myo-inositolhexakisphosphate -3-phosphohydrolase, E.C. 3.1.3.8) (preferentially liberates the P moiety at C3 position and includes microbial phytases except *S. Ruminantium*)
2. 6-phytases (myo-inositol hexakisphosphate 6-phosphohydrolase, E.C. 3.1.3.26) (preferentially liberates the P moiety at C6 position and includes plant phytases)
3. 5-phytases (myo-inositol hexakisphosphate 5-phosphohydrolase, E.C. 3.1.3.72) (preferentially liberates the P moiety at C5 position and includes phytases from *Selenomonas Ruminantium*)

The characteristic property of these phytase groups were given below in the Table 1.

Table 1. Characteristic property of phytases

Phytases	Group	Organism	Amino acid	Molecular mass	Optimum pH	Temperature range	Dephosphorylates at	Reference
Acid phytases	Group I - PhyA	*A. Niger, A. Fumigates, A. Terrus, Emericella nidulans, Myceliophthora thermophila, Talaromyces thermophilus*	465-469	Glycosylated: 62-128KDa, Unglycosylated: 48-50KDa	2.5, 5.5	55-60°C	3C in myo-inositol ring	62,58,59, 70,71
	Group II - PhyB	*A.niger (extrcellular), Saccharomyces Cerevisiae, Schizosaccharomyces Pombe*	453-479	Glycosylated: 65 KDa, Unglycosylated: 48-50KDa	2.5	55-60°C	3C in myo-inositol ring	8,34,71
	Group III - PhyC	*E. coli,* lysosomal acid phosphatises from rat and Human	354-439	Glycosylated: 42- 45 KDa, Unglycosylated: 48-50KDa	5.0- 6.0	40-60°C	6C in myo-inositol ring	7,70,36
Alkaline phytases	Group IV - PhyD	*Bacillus* and some plants, such as *T. lattifolia* pollen , *longiflorum* pollen and some legume seeds	383	Glycosylated: 42KDa,	7.0-8.0	55-70°C	C in myo-inositol ring	23,28,57

Mode of action

Phytase protein contains two conserved motifs, substrate-binding site and catalyzation domain. The substrate-binding motif is functional in binding to the substrate and is generally located at the N-terminal with a conserved sequence RHGxRxP. The catalyzation motif is found at the C-terminal and consists of distinct HD elements. At the tertiary level, a typical 'pocket' structure is shown based on the interaction of key residues in the motifs[45]. When the 'pocket' space is touched by the substrate, the conserved sequence RHGxRxP in the substrate-binding site interacts with the phosphate groups in the substrate to form a complex of enzyme-substrate. The HD elements in the catalyzation domain further function to release the phosphate group from the substrate[39].

Phytase enzyme catalyze the phytic acid hydrolysis in two steps: a nucleophilic attack from the histidine in the active site of the enzyme to the scissile phosphoester bond of phytic acid[66] and protonation of the leaving group by the aspartic acid residue of the HD motif[37]. But alkaline phytases (β−propeller phytases), lacks the RHGXRXP sequence motif and therefore It requires Ca^{2+} for both activity and thermostability[30]. β−propeller phytases have a narrow substrate range while requiring calcium for activity and only remove three phosphates from phytic acid to yield inositol trisphospahte as a final product[44].

Phytases from different source

Both prokaryotic and eukaryotic microbes have been used as a source of *phy* gene. A number of phytase producing organism has been identified from organisms like microbes, plants and mammals[13]. The biochemical properties of phytase from different sources was given below in table 2

Table 2. Biochemical properties of phytase from different sources

Sources	Organisms	Optimum Temperature	Optimum pH	*K*m (mmol/L)	*M* (kD)	Activity (U/ml)	Specific activity (U/mg)	References
Bacteria	*Bacillus* sp. DS11	37	6.5				20	30,31
	Bacillus amyloliquefaciens	37				2-3		32
	Bacillus licheniformes (168 phyA, phyL)						36.9, 23.6	57
	Enterobactor sp	37	5.5			505	8016	72
	Escherichia coli	37	7.5			200-700		17
	Klebsiella pneumonia	50	4.0		45			9
	Mycobacterium smegmatis	60	3,7		45		233.51	49
Yeast	*Pichia anomola*	30	5.6			68		67,69
	S.occidentalis	60	4.5					47
	Pichia anomala	60	4.0					15
	Candida krysei	40	4.6					15
Fungi	*Aspergillus ficcum* (phyA)	28				20-30	2090	53,54,61
	Aspergillus niger NCIM 564	30,37				108		68
	Aspergillus niger van Teighem	30	6-5			81.33	22,592	63,64
	Aspergillus ficcum (phyB)	27	5			10-20		58,59,60
	Aspergillus oryzae	37	6.4			370.7	20-3	55
	Aspergillus niger SK-57	30				68X 10^3	158	62
	Peniophora lycii (phyA)						400-1200	36
	Lentinus edodes	37	5		14			73
Plants	Canola seed	50	4.5~5	0.36; 0.25	70			29
	Zea mays	55	5	0.02; 0.03; 0.04; 0.117	71; 76			27, 35
	Soyabean seeds	55; 58	4.5~4.8; 4.5~5	0.05; 0.061	119; 72~130	389	2288	10 26
	Avena sativa	38	5.0	0.030	67			14
	Lupine seed LP11	50	5.0	0.08; 0.3; 0.13	57~64			20
	Buttercup squash	48	4.8		67			11

	Faba beans	50	5	0.148	65			19
	Hazel seed		5	0.162	72			2
	Mung beans	57	7.5	0.65	160			43
	Navy beans	50	5.3	0.018				41
	Peanut	55	5		22			12
	Rapeseed	50	5.2					42
	Scallion leaves	51	5.5	0.2				51
	Sunflower	55	5.2	0.29				1
	Tomato roots	45	4.3	0.038	164			38
	Typha latifolia pollen		8	0.017				23
	Barley	45; 55	5; 6	0.072; 0.19	67			16
	Rice	40	4.4; 4.6	0.17; 0.09	66; 61			25
	Rye	45	6	0.3	67			18
	Spelt	45	6	0.4	68			33
	Wholemeal wheat	55	5.15	0.3				50
	Wheat bran		5	0.49				46
	Wheat bran		5.6; 7.2	0.02; 0.2	47			40
	Wheat bran	45; 50	6; 5.5	0.0005; 0.0008	68; 66			48
	Crude extract wheat	45	6	0.83	65			3

Commercialisation of phytase

Phytase enzyme was first discovered by Suzuki et al. in 1907 during the course of rice bran hydrolysis studies, which found that the phosphatidylinositols exhibiting varying degree of phosphorylation were generated as intermediates or in some cases as end products. Society's awareness and increasingly demanding recent regulations worldwide on controlling the agricultural pollution, particularly phosphorus pollution with limits on the phosphorus content in manure, have intensified the phytase research. The focus has mainly been on its production and use as a means of reducing inorganic phosphorus supplementation. The growth of the market for phosphate to supplement animal feed fostered a critical step in the commercial development of phytase[65].

Milestones in phytase commercialisation

Year	Development
1962	First effort to make phytase as a commercial product at International Minerals and Chemicals, Skokie
1968	Sheih and Ware, isolated *Aspergillus ficuum*, which produced the highest yield of phytase with two pH optima, 5.5 and 2.5, and deposited it as NRRL 3135
1968	The microbial biochemistry group at IMC scaled the process and the animal nutrition group there successfully tested the enzyme for its use as feed additive
1968	the yields of phytase were not high enough to produce a product that would be competitive with the feeding of inorganic phosphorus and the project was terminated
1984	Technology developed at IMC was transferred to U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Southern Regional Research Center (SRRC)
1988	Production purification and characterisation of phytases from *A. ficcum* by Ullah
1991	Mullaney and his co workers Cloned *phyA* gene from *A. niger* NRRL 3135 in a λgt11 expression vector

1991	The researchers at Gist-Brocades cloned, sequenced, and overexpressed *phyA* from *A. niger* NRRL 3135, along with amyloglucosidase promoter, which resulted in 52-fold improvement of phytase yield. They also cloned the enzyme along with amyloglucosidase promoter and *A. niger* NRRL 3135 leader sequence into *A. niger* CBS 513.88, which resulted in 1400-fold improvement of phytase yield in one of the wild-type nonproducers. This bioengineered strain secreted 7.9 g/L of purified phytase with a specific activity of 2100 nkat/mg protein. The cloned gene was identical to that one cloned by the USDA group (GenBank acc. no. M94550).
1991	The nutrition group at Gist-Brocades tested the bioengineered enzyme extensively in swine and poultry.
1993	van Hartingsveldt and his co-workers, cloned and expressed the *phyA* gene from *A. niger* NRRL 3135, and the expression of multiple copies of *phyA* yielded up to 10-fold higher phytase activities than the native wildtype strain.
1993	*phyB* gene was cloned and expressed by Ehrlich and his co-workers
1993	researchers at Gist-Brocades group expressed *phyA* in tobacco seeds and canola plant and successfully tested the efficacy of transgenic plants as a source of phytase for monogastric animals.
1993	Researchers at Alko Ltd., Finland, in collaboration with Pan Labs, cloned the genes for phytase and pH 2.5 optimum acidphosphatase from *A. niger* var awamori
1996	After receiving approval from several countries and the Food and Drug Administration (FDA) as GRAS (generally recognized as safe) for use in food, phytase is being marketed as food additive in the United States from January 1996 as Natuphos.
1997	Meittinen-Oinonen and his coworkers overexpressed pH 2.5 acid-phosphatase in the *Trichoderma reesei* expression system, and the enzyme is now available on the market as Finase-F.
1997	Lassen and his coworkers reported phytase from a basidomycete, *Peniophora lycii*, and expressed in *Aspergillus oryzae* IFO 4177 (WO9828409 and US6060298). The initial studies suggested its capability of releasing phosphate from phytic acid at a high initial rate coupled with high specific activity. The enzyme is marketted as Bio-Feed phytase by DSM.
1997-2014	Several organisms have been screened for the production of phytase with higher catalytic property

The first phytase product, which entered the feed market was manufactured by Gist Brocades (now DSM) and sold by BASF under the trade name Natuphos[21]. Later on many companies started commercial production of phytases and their list is given in table 3.

Table 3. Commercial production information of microbial phytases[4]

Company	Country	Phytase source	Production strain	Trademark
AB enzymes	Germany	*Aspergillus awamori*	*Trichoderma reesei*	Finase
Alko Biotechnology	Finland	*A. oryzae*	*A. oryzae*	SP, TP, SF
Alltech	USA	*A. niger*	*A. niger*	Allzyme phytase
BASF	Germany	*A. niger*	*A. niger*	Natuphos
Biozyme	USA	*A. oryzae*	*A. oryzae*	AMAFERM
DSM	USA	*P. lycii*	*A. oryzae*	Bio-Feed Phytase
Fermic	Mexico	*A. oryzae*	*A. oryzae*	Phyzyme
Finnfeeds International	Finland	*A. awamori*	*T. reesei*	Avizyme
Genencor International	USA	*P. simplicissimum*	*Penicillium funiculosum*	ROVABIO
Roal	Finland	*Aspergillus awamori*	*T. reesei*	Finase
Novozymes	Denmark	*A. oryzae*	*A. oryzae*	Ronozyme® Roxazyme®

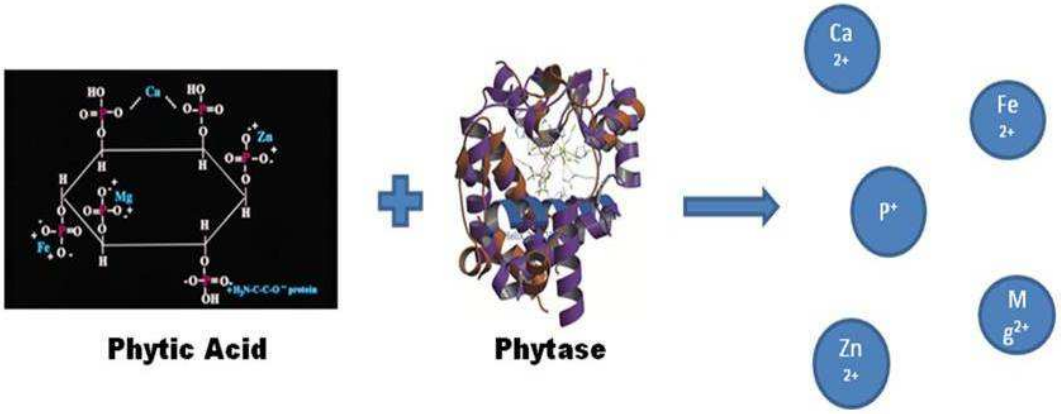

Fig. 1 Schematic illustration of phytase function.

Phytase, initiates the removal of phosphate groups from phytate at the carbon ring positions 1 or 3 (3-phytase) or 6 (6-phytase), releasing free inorganic phosphate, Ca^{2+}, Zn^{2+}, Mg^{2+} and Fe^{2+}

CONCLUSION

Phytase supplementation in feed and food will not only lower the inorganic P supplement in diet but also improves the micronutrient utilisation. Search for new phytase producing organism from different sources and engineering the phytase enzyme with higher heat tolerance, higher catalytic activity will widen its use in food and feed processing. Also optimising the culture condition and downstream processing of phytase expression will increase the phytase production economically.

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