Front. Agric. China 2010, 4(2): 195–209
DOI 10.1007/s11703-010-0103-1

REVIEW

Ruijuan LI, Jinfeng ZHAO, Chuanfan SUN, Wenjing LU, Chengjin GUO, Kai XIAO

Biochemical properties, molecular characterizations, functions, and application perspectives of phytases

Abstract As a kind of enzyme widely existing in eukaryotic species, especially in grains and oil seeds, phytases play an important role in the degradation of some phosphates containing organic molecules. So far, phytases derived from various species have been successfully used as animal feed additives. It has also been experimentally verified that phytases have a potential use in generating crop germplasm with high phosphorus use efficiency, based on their biochemical role in releasing Pi from the phytate and its derivatives. In this paper, the biochemical properties, molecular characterizations, functions and the potential application perspective of phytases are reviewed and commented on, aiming at the further exploration of the biochemical and molecular characterizations, and promotion of the application of phytases, a kind of important enzyme possessing potential use in animal feeding and creation of high P use crop cultivars, in the future.

Keywords phytase, biochemical property, molecular characterization, function, potential application perspective

1 Introduction

Acting as an enzyme (EC 3.1.3.26), phytase (myo-inositol hexakisphosphate phosphohydrolases) can catalyze the release of orthophosphate from myo-inositol hexakisphosphate (Ins P6) (Mullaney and Ullah, 2003), and plays an important role in breaking down the undigestible phytic acid (phytate) part that exists in grains and oil seeds, and thus release digestible phosphorus, calcium and other mineral nutrients. In the past several decades, phytase was widely used as an animal feed supplement, such as in poultry and swine husbandry, for monogastric animals that have no ability to generate endogenous phytase, for enhancing the nutritive value of plant material by releasing the inorganic phosphate from phytic acid (myo-inositol hexakisphosphate) and its derivatives. The phytases used in the animal feed have also alleviated the environmental phosphorus pollution resulting from the non-digested phosphorus in the animal feed.

On the other hand, acting as the macromolecular structure and indispensable in energy generation and metabolic regulation, phosphorus with adequate levels is critical to sustain the normal growth and development of all organisms (Carla and Elizabeth, 2001). However, phosphorus (P) deficiency in soil is a major constraint for agricultural production worldwide. It is noted that most soils around the world contain significant amounts of total soil P that occurs in inorganic and organic fractions and accumulates with phosphorus fertilization, and a major component of soil organic phosphorus occurs as phytate (O'Dell et al., 1991). Recently, transgenic plants with overexpression of phytases have shown an ability to utilize the phytate-bound phosphorus in rizhosphere, suggesting that phytases with high specific activities have a potential use in the creation of elite crop germplasm with high-P use efficiency. In this paper, several aspects such as the biochemical properties, molecular characterizations, biological functions and the potential use of phytases have been summarized, aiming at further understanding the biological mechanism and promoting the potential applications of phytases in animal and crop production.

Received September 14, 2009; accepted November 18, 2009

Ruijuan LI, Wenjing LU
College of Life Sciences, Agricultural University of Hebei, Baoding 071001, China

Chuanfan SUN
Technology Development Center of Rural Areas in China, The Ministry of Science and Technology of P. R. of China, Beijing 100045, China

Jinfeng ZHAO, Chengjin GUO, Kai XIAO (✉)
College of Agronomy, Agricultural University of Hebei, Baoding 071001, China
E-mail: xiaokai@hebau.edu.cn

2 Classifications of phytase

The first phytase described was extracted from rice by Suzuki et al. (1907). In the past two decades, lots of

phytases were isolated and characterized, including those of fungal phytases from *Aspergillus niger* (Piddington et al., 1993), bacterial phytases from *Escherichia coli* (Greiner et al., 1993), plant phytases from mung bean (Maiti et al., 1974), rice (Hayakawa et al., 1990), wheat (Nakano et al., 2000), a variety of other cereals (Greiner and Alminger, 2001) and legumes (Greiner et al., 2002a), and a mammalian phytase (Craxton et al., 1997).

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. Based on the catalytic mechanism, phytases can be referred to as histidine acid phytases (HAP), β-propeller phytases (BPP), cysteine phytases or purple acid phytases (PAP) (Mullaney and Ullah, 2003). Recently, a new class of phytase, i.e., the protein tyrosine phosphatase (PTP)-like phytases has been identified, based on the X-ray structure analysis of a novel phytate-degrading enzyme from *Selenomonas ruminantium* (Chu et al., 2004). On the other hand, based on their pH optima, phytases could be divided into acid and alkaline phytases. Acid phytases include those enzymes belonging to the HAP, PAP and PTP-like class of phosphatases, which has been identified more recently. Update, the BPPs from *Bacillus* are the only extensively characterized class of alkaline phytase (Kerovuo et al., 1998; Kim et al., 1998a; Tye et al., 2002).

According to the carbon in the myo-inositol ring of phytate at which dephosphorylation is initiated, phytases could be grouped into 3-phytases (myo-inositolhexakisphosphate -3-phosphohydrolase, E.C. 3.1.3.8), 6-phytases (myo-inositol hexakisphosphate 6-phosphohydrolase, E.C. 3.1.3.26) and 5-phytases (myo-inositol hexakisphosphate 5-phosphohydrolase, E.C. 3.1.3.72). Generally, phytases from microorganisms have been found to be 3-phytases (EC 3.1.3.8), and rarely 5-phytase, which are identified to be an extracellular phytate-degrading enzyme from *S. ruminantium* (Chu et al., 2004). Whereas grains and oil seeds of higher plants belong to 6-phytases (EC 3.1.3.26).

3 Structural characterizations of phytases

The native phytases from microbes and plants generally exist as complexes by binding distinct proteins and oligosaccharides which are mainly functional in sustaining the specific enzyme structure. Generally, the amount of oligosaccharides in phytase is approximately 27.3%. Based on modern biology and biotechnology, the primary structure of phytase first elucidated is derived from that of *Aspergillus ficuumhas*. Composition analysis of the amino acid types in phytase reveals that it is composed of 37% non-polar amino acids, 42% polar amino acids, 11.5% acidic amino acids, and 9.5% alkaline amino acids (Ullah and Dischinger, 1993). The secondary structure of this phytase was dissected by endo-protease degradation analysis, showing that this phytase includes 17.3% α-helixes, 29.1% β-folds, 32.6% corners, and 24.7% coils. Similar to acid phosphatases, the phytases subgrouped into acid phosphatase type possess two conserved domains and one similar spatial structure.

Phytase is acted as a monomer with two domains, which include an alpha domain and an alpha/beta domain. The active site in phytase involved in the catalytic reactions is located at an indentation between these domains. It is noted that the indentation is closed off at the back by an N-terminal lid. Basic amino acids at the active site play a crucial role in helping bind the negatively charged 3-phosphorus group on phytate and its derivatives.

X-ray crystallographic method provides a power tool for understanding the atomic movements involved in an enzymatic reaction in phytase. Based on high resolution X-ray analysis, the structural information of phytase such as the interaction mechanism between the enzyme molecule and the substrate, including the enzyme-substrate, enzyme-intermediate and enzyme-product(s) complexes, has preliminarily been elucidated. The crystal structure of *A. ficuum* phytase at 2.5 Å resolution scale indicates that there are three domains, including a large α domain, β domain and a small α domain, in the phytase. The large α domain and small α domain contain five α-helixes and four α-helixes, respectively, and the β-domain contains eight β-folds. Crystal structure analysis of *Escherichia coli* phytase at 2.2 Å resolution level also shows two domains as in two *A. ficuum* phytases. One contains 5 α-helixes and 2 β-folds and the other includes 6 α-helixes and 9 β-folds. Similar studies have also been conducted in a *Bacillus amyloliquefaciens* phytase and a *Debaryomyces castellii* phytase, suggesting the phytases derived from microbe species contain two conserved domains but with variable amounts of α-helixes and β-folds (Hartingsveldt et al., 1993; Ullah and Dischinger, 1993; Kostrewa et al., 1997; Jia et al., 1998; Lim et al., 2000; Ragon et al., 2009). At a resolution higher than 1.7 Å scale, Liu et al. (2004) observed four crystal structures of *Aspergillus fumigatus* phytases. It is found that the pH-dependent catalytic activity of *A. fumigatus* phytase could block the nucleophilic attack of the catalytic imidazole nitrogen, further preventing the substrate from binding with the enzyme. Additionally, two reaction product phosphates were observed at the active site, providing proof of a possible release pathway of the intermediate product after hydrolysis.

As a widely used feed additive, the *Aspergillus niger* PhyA phytase has been thoroughly studied to understand its structural basis liable to be heat inactivation during feed pelleting. Based on crystal structure comparisons with that of its close homolog, the thermostable *Aspergillus fumigatus* phytase (Afp), it is suggested that thermostability of PhyA is associated with several key residues (E35,

S42, R168, and R248), which were involved in the formation of a hydrogen bond network in the E35-to-S42 region and the ionic interactions between R168 and D161, and between R248 and D244. Loss-of-function mutations of above key residues (E35A, R168A, and R248A) singularly or in combination resulted in a decrease in thermostability, with the highest loss (25%, $P < 0.05$) in the triple mutant (*E35A/R168A/R248A*). Whereas, a set of corresponding substitutions such as quadruple mutant (*A58E/P65S Q191R/T271R*), retained 20% greater ($P < 0.05$) activity after being heated at 80°C for 10 min and had a 7°C higher melting temperature than that of wild-type PhyA by intensification of the hydrogen bond and ionic interactions. These results demonstrate that the hydrogen bond network together with the ionic interaction plays an important role in supporting the high thermostability of Afp and the feasibility of adopting these structural units to improve the thermostability of PhyA phytase and its homologues (Zhang et al., 2007).

Contrary to those derived from other microbial phytases, the phytase from *Selenomonas ruminantium* (SrPhy) shares no sequence homology with them. Crystal structure analysis reveals that SrPhy belongs to the dual-specificity phosphatase type, with its active site located near a conserved cysteine-containing (Cys241) P loop. It is also found that two other crystals form in which an inhibitor, myo-inositol hexasulfate is cocrystallized with SrPhy. In the "standby" and the "inhibited" crystal forms, the inhibitor is bound, respectively. In a pocket slightly away from the conserved P loop Cys241 and at the substrate binding site where the phosphate group is hydrolyzed, the pocket is held close to the –SH group of Cys241 in the enzyme. Further, mutagenesis studies verify that the P loop-containing phytase attracts and hydrolyzes the substrate (phytate) sequentially with a complicated mechanism (Chu et al., 2004).

4 Biochemical characterizations of phytases

Various phytases have been isolated from plants and microbes, and can be grouped based on their pH optima (alkaline or acid phytases), catalytic mechanisms (histidine acid phosphatases, β-propeller phytase, cysteine phosphatases or purple acid phosphatases), or stereospecificity of phytate hydrolysis (3- or 6-phytases). The physicochemical characteristics and catalytic properties of phytases from various sources indicate that it is an ester-hydrolyzing enzyme with an estimated molecular weight of 35–700 kDa depending upon the source of origin and is usually active within a pH range of 4.5–6.0 at 45–60°C. Generally, the phytases from bacteria have an optimum pH in neutral to alkaline range while in fungi the optimum pH range is 2.5–6.0. Phytases are fairly specific for phytic acid under the assay condition and it is possible to distinguish the phytase from the acid phosphatase incapable of degrading phytate. The molecular weight (MW) and the biochemical properties, such as optimum pH, temperature, specific activity, activator and inhibitor in the catalytic reactions of phytase previously reported in microbes, plants and recombinants are summarized in Table 1.

5 Biochemical catalytic mechanism of phyases

So far, the catalytic mechanism of the phytase superfamily enzymes has been extensively studied. It is noted that two indispensable motifs, including substrate-binding site and catalyzation domain, are conserved in the phytases. The former motif functional to bind the substrate is generally located at the N-terminal with a conserved sequence RHGxRxP. The latter 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 (Mullaney et al., 2000). 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 (Loewus and Murthy, 2000; Mullaney et al., 2000).

The data demonstrate that all of the phytases dephosphorylate the substrate (myo-inositol hexakisphosphate) in a stereospecific way by sequential removal of phosphate groups. The final products after thorough enzymatic reaction are inositol and Pi. However, the intermediates that occur orderly after the substrate hydrolysis vary in phytases. The hydrolysis of phytic acid by bacillus phytase PhyC could be done by the following pathways: phytic acid→DL-Ins(1,2,4,5,6)P/DL-Ins(2,3,4,5,6)P→Ins(2,4,5,6)P→Ins(2,4,6)P, or phytic acid→DL-Ins(1,2,3,4,5)P/DL-Ins(1,2,3,5,6)P→Ins(1,2,3,5)P→Ins(1,3,5)P (Kerovuo et al., 2000). Whereas, PhyAsr initiates hydrolysis of Ins P6 exclusively at the D-3 position and produces Ins(2)P predominantly (>80%) via D-Ins(1,2,4,5,6)P5→Ins(2,4,5,6)P4→D-Ins(2,4,5)P3→D-Ins(2,4)P2, (3,1,6,5,4) (Aaron, 2006).

The pathway of hydrolysis by phytate-degrading enzymes seems to be unique for each species, and these enzymes, Ins P6, and its derivatives may play a variety of roles in biological systems (Aaron, 2006). To date, there is little knowledge of the sequence in which phytases hydrolyze phosphate groups from phytate and the Ins P6 derivatives, which is listed in Table 2. However, it is known that most characterized phytases hydrolyze Ins P6 in a stepwise manner, yielding myo-inositol pentakis-, tetrakis-, tris-, bis-and mono-phosphate products (Konietzny and Greiner, 2002). The biochemical mechanisms in the phytase involved still need to be further explored.

Table 1 The biochemical properties of phytases previously reported which are derived from various species

phytase source	MW/kDa	optimum pH	optimum temperature/°C	specific activity at 37°C/(U·mg^{-1})	activators	inhibitors	Km/(μmol·L^{-1}) phytate	references
microbial phytases								
WT *A. niger*	39	2.62, 5.05	55, 58	ND	Ba Ca	Hg, Cu, Zn, Fe, Al, Pb, Ag	0.929	Sariyska et al., 2005
A. niger phyA-NRRL 3135*	85–100	2, 5	55–58	50–103	ND	F, Zn, Ca, Hg	40	Ullah and Gibson, 1987
A. niger phyA-SK-57*	60	2, 5.5	–	ND	ND	ND	18.7	Nagashima et al., 1999
A. niger phyB*	68	2.5	50	ND	ND	ND	103	Ullah and Cummings, 1987
*A. terreus**	60	5.5	70	142–196	ND	Cu, Fe	38.7	Mitchell et al., 1997
*A. fumingatis**	60	4, 6.5	60	23–28	ND	ND	W10	Pasamontes et al., 1997; Wyss et al., 1999
*M. thermophilia**	63	5.5–6.0	ND	42	ND	ND	37.6	Mitchell et al., 1997
E. coli(P2)	42	6	ND	ND	ND	ND	130	Dassa et al., 1992
*E. coli*2.5 APase	45	6.3	55–60	811–1800	ND	ND	5000	Greiner et al., 1993
B. subtilis	36	7.5	55–60	9–15	Ca	Ba, Sr, B, Cd	35	Powar and Jagannathan, 1982
A. oryzae	40	2.7, 5.5	50	11	ND	ND	ND	Shimizu, 1993
A. caespitosus	ND	5.5	80	ND	ND	ND	ND	Guimaraes et al., 2004
E. nidulans	ND	6.5	ND	29–33	ND	ND	ND	Wyss et al., 1999
T. lanuginosus	ND	6.0	65	110	ND	ND	ND	Berka et al., 1998
P. simplicissimum	ND	4.0	55	3	ND	ND	ND	Tseng et al., 2000
P. lycii	ND	5.5	58	1080	ND	ND	ND	Ullah and Sethumadhavan, 2003
Cladosporium	ND	3.5	40	909	ND	ND	ND	Quan et al., 2004
S. castellii	ND	4.4	77	418 (70 C)	ND	ND	ND	Segueilha et al., 1992
P. anomala	ND	4.0	60	ND	ND	ND	ND	Vohra et al., 2002
C. krusei	ND	4.6	40	1210	ND	ND	ND	Quan et al., 2002
K. terrigena	ND	5.0	58	205	ND	ND	ND	Greiner et al., 1997
K. pneumoniae	ND	5.0, 5.5	50, 60	224, 297	ND	ND	ND	Sajidan et al., 2004
K. aerogenes	ND	4.5, 5.2	68		ND	ND	ND	Tambe et al., 1994
P. agglomerans	ND	4.5	60	23	ND	ND	ND	Greiner, 2004b
C. braakii	ND	4.0	50	3457	ND	ND	ND	Kim et al., 2003
P. syringae	ND	5.5	40	769	ND	ND	ND	Cho et al., 2003
L. sanfranciscensis	ND	4.0	45	ND	ND	ND	ND	Angelis et al., 2003
Cry. laurentii ABO 510	ND	5.0	62	ND	ND	Hg^{2+}, Zn^{2+}, Cd^{2+}, Ca^{2+}	21	Staden et al., 2007
B. myloliquefaciens	ND	7.0–8.0	70	20	ND	ND	ND	Kim et al., 1998a
plants and the rat intestine								
Buttercup squash	67	4.8	48	ND	ND	ND	ND	Goel and Sharma, 1979

(*Continued*)

phytase source	MW/kDa	optimum pH	optimum temperature/°C	specific activity at 37°C/(U·mg^{-1})	activators	inhibitors	Km/(μmol·L^{-1}) phytate	references
Canola seed	70	4.5–5.0	50	ND	ND	ND	360,250	Kim and Eskin, 1987
Legume seeds	ND	8.0	ND	ND	ND	ND	ND	Scott, 1991
L. esculentum	164 dimer	4.3	ND	ND	Mg	PO_4,Cu, Mo_7O_{24}, Hg	38	Li et al., 1997
P. mungo	153	7.5	ND	ND	ND	F, EDTA	400	Maiti and Biswas, 1974
P. vulgaris	ND	5.5	ND	ND	Ca, Mg	Pi, phytate	150	Gibbins and Norris, 1963
H. vulgare P1	68	6	ND	ND	none	Hg, Cu, Zn	72	Greiner and Alminger, 1999
A. sativa	67	5	ND	ND	none	Fe, Cu, Zn	30	Greiner and Alminger, 1999
T. aestivum	47	5	ND	ND	none	Hg, Cu, Zn	490	Nagai and Funahashi, 1962
T. spelta	68	5.5	ND	ND	none	Hg, Cu, Zn, phytate	400	Konietzny et al., 1994
Zea mays seedling	76 dimer	4.8	55	2.3	Ca	Zn, Fe	117	Laboure et al., 1993
Zea mays root (3 isoforms)	71 dimer	5.0	35–40	5.7	none	Pb, Zn, Al	24, 25, 43	Hbel and Beck, 1996
rice	66, 61	4.4, 4.6	40	ND	ND	ND	170,90	Hayakawa et al., 1989
crude extract wheat	65	6.0	45	ND	ND	ND	830	Bohn et al., 2007
wheat PHY1	68	6.0	45	127	ND	ND	0.5	Nakano et al., 1999
wheat PHY2	66	5.5	50	242	ND	ND	0.8	Nakano et al., 1999
Spelt D21	68	6.0	45	262	ND	ND	400	Konietzny et al., 1995
rye	67	6.0	45	517	ND	ND	300	Greiner et al., 1998
oat	67	5.0	38	307	ND	ND	30	Greiner and Alminger, 1999
barley P1	67	5.0	45	117	ND	ND	72	Greiner et al., 2000
barley P2	67	6.0	55	43	ND	ND	190	Greiner et al., 2000
soybean	70 dimer	4.5–5.0	55–58	2.4	ND	ND	50	Gibson et al., 1988
peanut	22	5.0	55	ND	ND	ND	ND	Gonnety et al., 2007
sunflower	ND	5.2	55	ND	ND	ND	290	Agostini and Ida, 2006
rapeseed	ND	5.2	50	ND	ND	ND	ND	Mahajan and Dua, 1997
Hazel seed	72	5.0	ND	ND	ND	ND	162	Andriotis and Ross, 2003
navy beans	ND	5.3	50	ND	ND	ND	18	Lolas and Markakis, 1977
mung bean	160	7.5	57	0.5	ND	ND	650	Mandal et al., 1972
scallion leaves	ND	5.5	51	500	ND	ND	200	Phillippy et al., 1998
faba bean	65	5.0	50	636	ND	ND	148	Greiner and Alminger, 2001
lupine L11	ND	5.0	50	539	ND	ND	ND	Greiner et al., 2002b
lupine L12	ND	5.0	50	607	ND	ND	ND	Greiner et al., 2002b
lupine L2	ND	5.0	50	498	ND	ND	ND	Greiner et al., 2002b

(*Continued*)

phytase source	MW/kDa	optimum pH	optimum temperature/°C	specific activity at 37°C/(U·mg^{-1})	activators	inhibitors	Km/(μmol·L^{-1}) phytate	references
T. latifolia pollen	ND	8.0	ND	ND	ND	ND	17	Hara et al., 1985
lily pollen	88	8.0	55	0.2	ND	ND	81	Jog et al., 2005
tomato root	164	4.3	45	205	ND	ND	38	Li et al., 1997
rat intestine	ND	7.0, 7.5–8.0	ND	ND	ND	ND	ND	Yang et al., 1991
recombinant								
A. niger phyA-tobacco	73–88	4, 2	ND	ND	ND	phenyl-glyoxal	65	Verwoerd et al., 1995; Ullah et al., 1999
A. niger phyA-soybean	69–71	3, 5.5	ND	ND	ND	ND	ND	Li et al., 1997
r-PhyA86-r-PhyA170-*P. pastoris*	ND	5.5	50	ND	ND	ND	ND	Promdonkoy et al., 2009
B. phytase-*E. coli*	73	4.0–8.0	25–75	ND	ND	ND	ND	Reddy et al., 2009
P. wasabiae appA-*E coli* BL21	45	5.0	50	ND	ND	ND	170	Shao et al., 2008
r-PhyP-*E. coli*	ND	7.0	45	ND	ND	ND	1280	Huang et al., 2008
B. subtilis US417 (PHYUS417)	41	7.5	55	ND	ND	Ca	ND	Farhat et al., 2008

Note: * means it is overexpressed in *A. niger*; ND means it is not determined.

Table 2 Myo-inositol phosphate intermediates generated through enzymatic phytate degradation in phytases derived from various sources

enzyme	IP5-isomr	IP4-isomer	P3-isomer	IP2-isomer	IP-isomer	references
barley P1; P2, Spelt D21, wheat PHY1; PHY2, rye, oat, lupine L2	D-Ins(1,2,3,5,6)P5	D-Ins(1,2,5,6)P4	D-Ins(1,2,6)P3	D-Ins(1,2)P2	Ins(2)P	Greiner et al., 2001a
bacillus PhyC	D/L-Ins(1,2,3,4,5)P5/D/ L-Ins(1,2,3,5,6)P5	Ins(1,2,3,5)P4	Ins(1,3,5)P3	–	–	Kerovuo et al., 2000
bacillus PhyC	D/L-Ins(1,2,4,5,6)P5/D/ L-Ins(2,3,4,5,6)P5	Ins(2,4,5,6)P4	Ins(2,4,6)P3	–	–	Kerovuo et al., 2000
rice	L-Ins(1,2,3,4,5,)5/ D-Ins (1,2,4,5,6)P5	L-Ins(1,2,3,4)/D-Ins (1,2,5,6)P4	Ins(1,2,3)P3/D-Ins (1,2,6)P3	D-Ins(1,2)P2	Ins(2)P	Hayakawa et al., 1990
wheat F2	D-Ins(1,2,3,5,6)P5	D-Ins(1,2,3,6)P4	Ins(1,2,3)P3	D-Ins(1,2)P2	Ins(2)P	Lim and Tate, 1973
mung bean	D-Ins(1,2,3,5,6)P5	D-Ins(1,2,3,6)P4	D-Ins(1,2,6)P3/Ins (1,2,3)P3	D-Ins(2,6)P/ D-Ins(1,2)P2	Ins(2)P	Maiti et al., 1974
S. cerevisiae, pseudomonas, lupine L11, lupine L12	D-Ins(1,2,4,5,6)P5	D-Ins(1,2,5,6)P4	D-Ins(1,2,6)P3	D-Ins(1,2)P2	Ins(2)P	Greiner, 2002; Cosgrove, 1970
E. coli	D-Ins(1,2,3,4,5)P5	D-Ins(2,3,4,5)P4	Ins(2,4,5)P3	Ins(2,5)P2	Ins(2)P	Greiner et al., 2001b
paramecium	D-Ins(1,2,3,4,5)P5	D-Ins(1,2,3,4)P4	Ins(1,2,3)P3	D-Ins(2,3)P2	–	vander Kaay et al., 1995
lily	D-Ins(1,2,3,4,6)P5	D-Ins(1,2,3,4)P4/ D-Ins(1,2,3,6)P4	Ins(1,2,3)P3	–	–	Barrientos et al., 1994
Pantoea agglomerans	D-Ins(1,2,4,5,6)P5	–	–	–	–	Greiner, 2004a
B. subtilis	D/L-Ins(1,2,3,4,5)P5/ D/L-Ins(1,2,4,5,6)P5	Ins(1,2,3,5)P4/ Ins(2,4,5,6)P4	Ins(1,3,5)P3/ Ins(2,4,6)P3	–	–	Kerovuo et al., 2000
Selenomonas ruminantium (*PhyAsr*)	D-Ins(1,2,4,5,6)P5	Ins(2,4,5,6)P4	D-Ins(2,4,5)P3	D-Ins(2,4)P2	Ins(2)P	Aaron, 2006
B. subtilis, B. amyloliquefaciens	D-Ins(1,2,4,5,6)P/ D/L-Ins(1,2,3,4,5)P5	Ins(2,4,5,6)P4/ D-Ins(1,2,5,6)P4	Ins(2,4,6)P3/ D-Ins(1,2,6)P3	–	–	Greiner, 2002

6 Molecular characterizations of phytase genes

To date, lots of phytase genes have been mainly cloned and characterized in microbes. Among them, the phytase genes such as *phyA* and *phyB* from *Aspergillus niger* have been extensively studied. The noted phytase gene, *phyA*, encodes 467 amino acids with an 18 or 19 amino acid signal at the N terminus, with a molecular weight of 48.8 kDa for the mature protein. Two potential N-glycosylation sites functional in the protein post-translation have been found in the phyA polypeptide sequence. A highly conserved sequence motif RHGXRXP (single-letter amino acid codes) (Ullah et al., 1991), involved in the catabolic reactions, is found at the active sites of phytase. Furthermore, phyA contains a remote C-terminal His-Asp motif (HD motif) that is also likely to take part in the catalysis. Together, it is therefore suggested that the *phyA* belong to a member of the phytase subfamily of histidine acid phosphatases (Michell et al., 1997). The second noted phytase gene identified in *Aspergillus niger* with similarity to *phyA* is *phyB* (Ehrlich et al., 1993). This gene is 1605 bp in length with 4 exons, and encodes a 479 amino acid protein. It also has a highly conserved RHGXRXP sequence motif for catalysis and an HD sequence motif that facilitates the substrate binding and product leaving. Presently, a novel phytase gene from *Aspergillus niger* BCC18081 (TR170) which is 1404 bp in length, coding for putative phytases of 468 amino acid residues, has been also cloned and transferred into *Pichia pastoris*. The thermostable property of this phytase provides a valuable potential in application (Promdonkoy et al., 2009).

Except in *Aspergillus niger*, some phytase genes have also been cloned and identified in other microbes. A phytase gene from *A. ficuum* NRRL 3135, sharing 91.6% amino acid sequence identity with the *phyA* gene, has been isolated and well characterized by Ullah and Gibson (Ullah and Gibson, 1987; Ullah, 1988;). Based on the crystal structure analysis, the phytase from *A. ficuum* NRRL 3135 existed in homodimers, with a molecular mass of 85 kDa (Kostrewa et al., 1997). For effective use of the phytases in the feed additives, highly thermostable phytases have been isolated and cloned from *Bacillus* species. *PhyC*, a gene encoding a phytase enzyme, has been cloned based on *B. subtilis* genomic library screening (Kerovuo et al., 1998). In the meantime, a phytase gene with 2.2-kb in length encoding a phytase polypeptide of 383 amino acids from *Bacillus subtilis* DS11 has also been isolated and expressed in *E. coli*. (Kim et al., 1998b). These two phytases both contain 383 amino acid residues with over 90% sequence identity but show no homology to other phytase sequences, such as *phyA* and *phyB*, or to other known phytase sequences, nor do they contain the conserved active-site motif RHGXRXP. Thus, they are considered to be enzymes with phytase activity but not belonging to the members of the phytase subfamily of histidine acid phosphatases.

With the rapid progress in modern biology and biotechnology, lots of plant phytase genes encoding the phytases have been cloned and characterized in the past two decades. In *Arabidopsis*, a model plant species in molecular biology, Mullaney and Ullah (1998) have figured out a gene in the database, which exhibits sequence features of a histidine acid phosphatase with phytase activity (accession number GI2160177). Simultaneously, several phytase genes have been identified in other plant species. A good case is that of two phytase genes of *PHYT I* and *PHYT II*, which were isolated from maize (*Zea mays*), based on screening of maize cDNA library. Further analysis found that both *PHYT I* and *PHYT II* share high sequence homology, containing a 1164 bp open reading frame that encodes polypeptide of 387 amino acids with a molecular mass of 38 kDa (Maugenest et al., 1997, 1999). Gene location analysis suggests that the two phytase genes are tightly linked on the longarm of chromosome 3. The maize phytase genes encode a homodimeric protein, having a RHGXRXP phytase active site motif and a hall mark of histidine acid phosphatases (Ostanin et al., 1992). In soybean, a phytase gene (*GmPhy*) sequence, containing a 1644-bp open reading frame that could encode a protein with a predicted molecular mass of 62.3 kDa, has been identified. Sequence comparison alignment explores that the soybean phytase exhibits a high degree of sequence similarity to purple acid phophatases (PAPs), a family of acid phosphatase in plants with wide functions (Carla and Elizabeth, 2001). A 28-aa in length of signal peptide directing the polypeptide to apoplast has been detected at the N-terminal of the translated protein, showing the protein should be directed to the endomembrane system for secretion or further subcellular sorting (Carla and Elizabeth, 2001). Domain analysis indicates that the soybean phytase contains motif characteristics of a large group of phosphoesterases, including the PAPs. Five sequence blocks comprising two motifs (D*X[G/H*]-(Xn)-GD*XX[Y/X]-(Xn)-GN*H[E/D] and VXXH*-(Xn)-GH*XH*) contain the conserved metal-ligating residues (asterisks) (Koonin, 1994), which are required for enzyme catabolism. In the model legume *Medicago truncatula*, a full-length cDNA (*MtPHY1*) encoding an extracellular form of phytase has been isolated. The phytase cDNA has an open reading frame of 1632 bp predicted to encode 543 amino acids including an N-terminal signal peptide of 27 amino acids. At the genome level, the *MtPHY1* gene consists of 5151 bp in length containing 7 exons and 6 introns. Transcription analysis suggests that *MtPHY1* is expressed in leaves and roots, with the expression levels elevated in roots during growth in low phosphate conditions (Xiao et al., 2005). Owing to their vital actions in plant phosphorus metabolism, phytases in plant species are necessary to be further studied in the future aided by the rapid progress in modern biology and biotechnology.

Currently, much more phytase genes derived from various species have also been identified, due to their

potential value in the application. A phytase (PhyAsr) belonging to the protein tyrosine phosphatase (PTP) superfamily has been characterized from the anaerobic, ruminal bacterium *Selenomonas ruminantium* by Aaron (2006). Studies demonstrate that PhyAsr has a PTP-like fold and a conserved PTP-like active site signature sequence (C(X)5R) which facilitates a classical PTP mechanism of dephosphorylation (Chu et al., 2004). Except for a novel phytase gene isolated in *Aspergillus niger*, another thermostable phytase gene with 1404 bp in length, coding for putative phytases of 468 amino acid residue, has been identified in *Aspergillus japonicus* BCC18313 (TR86) and BCC18081 (TR170) (Promdonkoy et al., 2009). A new phytase gene, *appA*, consisting of 1302 bp encoding 433 amino acid residues with 27 residues of a putative signal peptide, has been cloned in *P. wasabiae* by degenerate PCR and TAIL-PCR (Shao et al., 2008). A phytase gene belonging to the beta-propeller phytase family and sharing very low identity (approximately 28.5%) with *Bacillus subtilis* phytase has been identified in *Pedobacter nyackensis* MJ11 CGMCC 2503 (Huang et al., 2009). An extracellular phytase (PHY US417) with a molecular weight of 41 kDa is purified and characterized in *Bacillus subtilis* US417 (Farhat et al., 2008). In addition, other phytase genes, as a potential ideal feed additive for improving the phytate-phosphorus digestibility in monogastric animals, have also been cloned and characterized in other species, such as in *Peniophora lycii* (Xiong et al., 2006), ruminal bacterium *Selenomonas ruminantium* (*SrPf6*) and *Escherichia coli* (*appA*) (Hong et al., 2004).

7 Application of phytase in feed additives

As the major ingredients of animal feed, cereal grains and oilseed meals contain a large amount of phosphorus (mainly phytate) unavailable for monogastric animals (e.g., pigs, poultry, and fish) at very low levels of phytase activity in the digestive tract; thus, the nutrient value of the feed stuff of plant origins is decreased. On the other hand, the unassimilated phytates in feed are excreted into the environment, causing serious ecological problems in areas of intensive animal production. Meanwhile, as an anti-nutritional factor, phytates may form complexes with some metal ions (Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}) and proteins, resulting in a decrease in their dietary availability.

Numerous feeding studies have verified the efficacy of using phytase as a tool to improve phosphorus and nutrient utilization in a wide variety of animal species (Ravindran et al., 1995). The use of a fungal phytase as a feed supplement has proven to be effective in alleviating the negative effects of phytate in livestock diets. Simons et al. (1990) found that the addition of crude microbial phytase to the diets of growing pigs improved phosphorus uptake by 24% while phosphorus levels in feces were decreased by 35%. Similar results have been reported in several other research groups. In the past several years, the extracellular phytase isolated from fungus *Aspergillus niger* has been widely used in Europe as a commercial feed supplement. The phyA2 phytase enzyme derived from *A. niger* has been marketed as a commercial feed additive in China. Except for the improvement of phosphorus utilization, several other inorganic cations, such as Ca^{2+}, Zn^{2+}, Fe^{2+}, and Mg^{2+} which are covalently bonded in phytate, could be also absorbed by animals. Mondal et al. (2007) has shown that supplementation of microbial phytase in soybean meal based broiler diets containing low phosphorus has also increased the retention of Ca^{2+}, in addition to P, all are functional in compensation of the untoward effect of low phosphorus levels from the diet.

A transgenic approach has provided a powerful tool for expressing the specific phytase genes on a large scale in yeast strains, which have been verified to be an elite expression system for heterologous genes. To reduce phytase production costs, new prokaryotic and eukaryotic expression systems were explored. Several studies have already investigated the use of various yeast expression systems as an alternative to the current production method for phytase using overexpression in filamentous fungi. It is elucidated that phytases used in ecotopic expression can be derived from a host of microorganism source (Lei et al., 2007). Xiong et al. (2006) created a *P. pastoris* strain that expressed the modified phytase gene (*phy-pl-sh*) with MF4I sequence producing 12.2 g phytase per liter of fluid culture, with the phytase activity of 10540 $U \cdot mL^{-1}$. The recombinant phytase had one optimum pH (pH 4.5) and an optimum temperature of 50°C. Based on the direction of MF4I signal peptide in the recombinant gene, the phytase expressed in *P. pastoris* strain could be effectively secreted in the culture solution and simplified for the purification of the expressed phytase. Using the phytase gene (*phytDc*) from *Debaryomyces castellii* and alpha-amylase gene (*AMY*) from *D. occidentalis* as the target genes, Hong et al. (2008) developed an industrial strain of *Saccharomyces cerevisiae*. The recombinant industrial strain secreted both phytase and alpha-amylase for the efficient degradation of phytic acid and starch as main components of plant seeds, with a hydrolysis efficiency of 90% for 0.5% (w/v) sodium phytate within 5 days of growth and of 100% for 2% (w/v) starch within 48 h simultaneously.

Because temperature and pH values are the major factors determining enzyme activity, favourable properties for phytase used in food processing are the high phytate-degrading capability even at room temperature, acceptable heat resistance and high activity over a broad pH range. One of phytase research efforts has been focused on the engineering of an improved enzyme with heat tolerance by allowing the enzyme to survive the brief period of elevated temperature during the pelletization process (Mullaney et al., 2000). For that, Kim et al. (2006) tried to shift the pH optima of PhyA to match the stomach condition by

substituting amino acids in the substrate-binding site with different charges and polarities. The results showed that it was feasible to improve the function of PhyA phytase under stomach pH conditions by rational protein engineering. It was found that among 21 single or multiple mutants of PhyA expressed *Pichia pastoris* yeast strains, the mutant E228K exhibited the best overall changes, with a shift of pH optimum to 3.8 and 266% greater ($P < 0.05$) hydrolysis of soy phytate at pH 3.5 than that of the WT enzyme. The improved efficacy of the enzyme was also confirmed in an animal feed trial and characterized by biochemical analysis of the purified mutant enzymes. Similarly, Fu et al. (2009) explored the Ser51 in a new phytase (APPA) which is in close proximity to the catalytic site and plays a role in defining the optimal pH 2.5. Compared with wild-type APPA, mutant S51T showed a higher specific activity and greater activity, and increased the thermal and acid stability. These properties make S51T a better candidate than the wild-type APPA for use in animal feed. In another study, based on phytase activity screening, ten *Cryptococcus* strains with various phytase activities were identified. Among them, the *Cryptococcus laurentii* ABO 510 strain showed the highest level of activity and a high sustainable temperature. The enzyme was thermostable at 70°C, with only a loss of 40% of its original activity in 3 h. These characteristics suggest that the ABO 510 phytase derived from *C. laurentii* may be considered as an animal feed additive used to assist in the hydrolysis of phytate complexes to improve the bioavailability of phosphorus in plant feedstuff (van Staden et al., 2007). Except for high-specific activity and high temperature stability, low pH tolerance of the phytase is also necessary when used as the feed additives, owing to the acidic and high protease concentration of gastric passages in animals. Huang et al. (2008) has cloned two novel phytase genes from *Yersinia rohdei* and *Y. pestis* expressed in *Pichia pastoris*. The results showed that both the recombinant phytases had a high activity at pH 1.5–6.0 (optimum pH 4.5) and 55°C optimum temperature. Compared with the major commercial phytases, the *Y. rohdei* phytase was more resistant to pepsin, retained more activity under gastric conditions, and released more inorganic phosphorus (two to ten times) from soybean meal under simulated gastric conditions. These superior properties suggest that the *Y. rohdei* phytase is an attractive additive to animal feed. Recently, several novel phytase genes with strong tolerance to high temperature and low pH have been cloned and isolated by several independent groups (Farhat et al., 2008; Rao et al., 2008).

For more efficient production of recombinant heterologous high-specific activity phytases, it has been tried to express the phytase genes in plant specific organs, which are then directly used in the animal feed stuff. Based on the DNA recombinant techniques and plant genetic transformation approaches, phytase has been produced recently in transgenic plants. Up to date, phytase genes from various *Aspergillus* species have been expressed in transgenic tobacco seeds and leaves (Reddy et al., 1982; Pen et al.,1993; Ullah et al., 1999), transformed soybean cell-suspension cultures (Li et al., 1997), transgenic soybean and alfalfa (Denbow et al., 1998; Ullah et al., 2000), and transgenic wheat, rice, and canola seeds (Brinch et al., 2000; Zhang et al., 2000; Ponstein et al., 2002; Hong et al., 2004). Chen et al. (2008) demonstrated that transgenic maize seeds could express the fungal phytase *phyA2* gene in embryos without affecting seed germination and the phytase activity in transgenic seeds could reach about 2200 $U \cdot kg^{-1}$ seed. A similar level of phytase expression also was obtained when the *A. niger phyA* gene was expressed in maize endosperm under the control of the rice glutelin-1 promoter (Drakakaki et al., 2005). Because of previous feeding trials, the phytase showed an effectiveness of 750–1000 units of activity per kg of diets, as a feed additive (Jongbloed et al., 1996), some transgenic plants or specific organs expressing heterologous phytases could reach this available enzyme activity criterion and be directly used as the feed without additional Pi supplemented. Therefore, transgenic plants with high-specific phytase activity have a far-reaching potential in animal feeding in the future.

8 Potential value of phytase on improving phosphorus use efficiency in crop plants

As the raw material of one indispensable inorganic nutrient in crop production, rock phosphate plays a crucial role in the conservation of the world's deposits and is considered to be important for future generations. Phosphorus is a basic component of life like nitrogen, but, unlike nitrogen, phosphorus does not have a cycle to constantly replenish its supply. It is noted that phosphorus deficiency in soil is currently one of a major constraints for agricultural production worldwide.

A large amount of phosphorus in the arable soil generally exists as compounds employed by crop plants. In addition some of the phosphorus is directly bounded by soil mineral particles. Besides, among the accessible large part of phosphorus for plants, about 50% to 80% exists in an organic form, with half being the phytate and its derivatives. Therefore, it is very likely that the use of phytase will be expanded as the need to conserve the world's phosphate reserves increases (Mullaney et al., 2000).

A large part of phytate and its derivatives could be potentially decomposed by phytase, resulting in the release of Pi for plant. Therefore, the development of plants that exude the phytase to the soil may improve the P nutrition of plants. Modern biology and biotechnology have provided a potentially efficient way to enhance the P nutrition of crop plants and improve the efficiency of applying P fertilizer in the agricultural system (George et al., 2005). As a large potential phosphorus source, phytate and its derivatives,

were paid more attention to by several research groups aiming at releasing the phosphorus from the organic phosphorus compounds and further improving the P use efficiency in plants based on the genetic transformation and biotechnology approach. Using the phytase gene (*phyA*) from *Aspergillus niger* as the target gene, Richardson et al. (2001) generated the transgenic *Arabidopsis* plants to express the PhyA recombinant protein. The transgenic plants showed a strong ability to utilize the phytate by secreting the encoded phytase to be rizhosphere by the guidance of the signal peptide sequence from the carrot extensin (ex) gene. The transgenic lines expressing *ex::phyA* ultimately resulted in increasing 20-fold phosphorus nutrition in total root phytase activity, such that the growth and phosphorus content of the plants were equivalent to those of control plants supplied with inorganic phosphate. Similarly, transgenic *Arabidopsis* plants integrated with synthetic phytase gene gave similar results as those of Richardson et al. (2001). The plants with higher expression under the control of the constitutive CaMV35S promoter could dramatically utilize the phosphorus derived from phytate, showing a significantly improved plant phenotype under the phytate as the sole P source, with more accumulated P amount per plant and higher fresh and dry weight than those of the control (empty vector transformed) (Xiao et al., 2005). In all, it is suggested that it should be an efficient pathway to improve the phosphorus use efficiency in the crop plants by adopting a suitable biotechnology strategy.

Because the absorption site of the available phosphate source in plants is the root system, especially the root hairs. It is therefore better that the phytase ectopically expressed in the transgenic plants to be secreted in the rhizosphere where the phytate and its derivatives are degraded by the biochemical reactions involved in the encoded phytases. Thus, in addition to the transgenic plants showing a property of translated phytases that is secreted (Richardson et al., 2001; Xiao et al., 2005), it would be much feasible to express the phytase gene to be organ- or tissue-specific, such as root hairs, which are the predominant region uptaking the mineral nutrients. Therefore, the transgenic *Arabidopsis* plants in which the legume model *M. truncatula* phytase gene (*MtPHY1*) has been integrated, with the target gene governed by MtPT1, a promoter with a pattern to be root-specific and low-Pi induction. The results indicate that when phytate is supplied as the sole source of phosphorus, the dry weight of the transgenic *Arabidopsis* lines is 3.1- to 4.0- fold higher than that of the control and the total phosphorus content is 4.1- to 5.5-fold higher than that of the control. The transgenic expression of phytase genes of plant origin thus has also great potential for improving plant phosphorus acquisition and for phytoremediation (Xiao et al., 2005). With a similar strategy, Li et al. (2009) expressed a *Aspergillus ficuum* phytase gene (*AfPhyA*) in soybean plants in which the carrot extensin signal peptide sequence was in-frame fused with the *AfPhyA* open reading frame at the 5′-end for the secretion of the expressed phytase. The phytase activity and inorganic phosphate levels in the transgenic soybean root secretions were 4.7 $U \cdot mg^{-1}$ protein and 439 $mumol \cdot L^{-1}$, respectively, compared to 0.8 $U \cdot mg^{-1}$ protein and 120 $mumol \cdot L^{-1}$, respectively, in control soybeans (Li et al., 2009), suggesting the transgenic techniques are of a great value in generating crop varieties with high P use efficiency in the future.

Acknowledgements This work was financially supported by the National Natural Science Foundation of China (Grant No. 30871466) and Natural Science Foundation of Hebei (No. C2006000434).

References

Aaron A (2006). Expanding our knowledge of protein tyrosine phosphatase-like phytases: mechanism, substrate specificity and pathways of myo-inositol hexakisphosphate dephosphorylation. Dissertation for the Master's Degree. Lethbridge, Alta.: University of Lethbridge, 10–13

Agostini J D, Ida E I (2006). Partially characterization and application of phytase extracted from germinated sun-flower seeds. Pesquisa Agropecuaria Brasileira, 41(6): 1041–1047

Andriotis V M E, Ross J D (2003). Isolation and characterisation of phytase from dormant *Corylus avellana* seeds. Phytochemistry, 64 (3): 689–699

Angelis M, Gallo G, Corbo M R, McSweeney P L H, Faccia M, Giovine M, Gobbetti M (2003). Phytase activity in sourdough lactic acid bacteria: Purification and characterization of a phytase from *Lactobacillus sanfranciscensis* CB1. International Journal of Food Microbiology, 87: 259–270

Barrientos L, Scott J J, Murthy P P (1994). Specificity of hydrolysis of phytic acid by alkaline phytase from lily pollen. Plant Physiology, 106: 1489–1495

Berka R M, Rey M W, Brown K M, Byun T, Klotz A V (1998). Molecular characterization and expression of a phytase gene from the thermophilic fungus *Thermomyces lanuginosus*. Appllied and Environmental Microbiology, 64: 4423–4427

Bohn L, Josefsen L, Meyer A S, Rasmussen S K (2007). Quantitative analysis of phytate globoids isolated from wheat bran and characterization of their sequential dephosphorylation by wheat phytase. Journal of Agricultural and Food Chemistry, 55(18): 7547–7552

Brinch-Pedersen H, Olesen A, Rasmussen S K, Holm P B (2000). Generation of transgenic wheat (*Triticum aestivum* L.) for constitutive accumulation of an *Aspergillus* phytase. Molecular Breeding, 6: 195–206

Carla E H, Elizabeth A G (2001). A novel phytase with sequence similarity to purple acid phosphatases is expressed in cotyledons of germinating soybean seedlings. Plant Physiology, 126: 1598–1608

Chen R M, Xue G X, Chen P, Yao B, Yang W Z, Ma Q L, Fan Y L, Zhao Z Y, Tarczynski M C, Shi J R (2008). Transgenic maize plants expressing a fungal phytase gene. Transgenic Research, 17(4): 633–643

Cho J S, Lee C W, Kang S H, Lee J C, Bok J D, Moon Y S, Lee H G,

Kim S C, Choi Y J (2003). Purification and characterization of a phytase from *Pseudomonas syringae* MOK1. Current Microbiology, 47: 290–294

Chu H M, Guo R T, Lin T W, Chou C C, Shr H L, Lai H L, Tang T Y, Cheng K J, Selinger B L, Wang A H J (2004). Structures of *Selenomonas ruminantium* phytase in complex with persulfated phytate: DSP phytase fold and mechanism for sequential substrate hydrolysis. Structure, 12: 2015–2024

Cosgrove D J (1970). Inositol phosphate phosphatase of microbiological origin. Inositol pentaphosphate intermediates in the dephosphorylation of the hexaphosphates of myo-inositol, scyllo-inositol, and D-chiro-inositol, by a bacterial (*Pseudomonas* sp.) phytase. Austrulia Journal of Biological Science, 23: 1207–1220

Craxton A, Caffrey J J, Burkhart W, Safrany S T, Shears S B (1997). Molecular cloning and expression of a rat hepatic multiple inositol polyphosphate phosphatase. Biochemistry Journal, 328: 75–81

Dassa E, Fsihi H, Marck C, Dion M, Kieffer-Bontemps M, Boquet P L (1992). A new oxygen-regulated operon in *Escherichia coli* comprises the genes for a putative third cytochrome oxidase and for pH 2.5 acid phosphatase (appA). Molecular and General Genetics, 229: 341–352

Denbow D M, Grabau E A, Lacy G H, Kornegay E T, Russell D R, Umbeck P F (1998). Soybeans transformed with a fungal phytase gene improve phosphorus availability for broilers. Poultry Science, 77(6): 878–881

Drakakaki G, Marcel S, Glahn R P, Lund E K, Pariagh S, Fisher R, Christou P, Stoger E (2005). Endosperm specific coexpression of recombinant soybean ferritin and *Aspergillus* phytase in maize results in significant increases in the levels of bioavailable iron. Plant Molecular Biology, 59: 869–880

Ehrlich K C, Montalbano B G, Mullaney E J, Dischinger H C, Ullah A J (1993). Identification and cloning of a second phytase gene (*phyB*) from *Aspergillus niger*. Biochemical and Biophysical Research Communicatons, 195: 53–57

Farhat A, Chouayekh H, Ben F M, Bouchaala K, Bejar S (2008). Gene cloning and characterization of a thermostable phytase from *Bacillus subtilis* US417 and assessment of its potential as a feed additive in comparison with a commercial enzyme. Molecular Biotechnology, 40(2): 127–135

Fu D W, Huang H Q, Meng K, Wang Y R, Luo H Y, Yang P L, Yuan T Z, Yao B (2009). Improvement of *Yersinia frederiksenii* phytase performance by a single amino acid substitution. Biotechnology and Bioengineering, 103(5): 857–864

George T S, Simpson R J, Hadobas P A, Richardson A E (2005). Expression of a fungal phytase gene in *Nicotiana tabacum* improves phosphorus nutrition of plants grown in amended soils. Plant Biotechnology Journal, 3(1): 129–140

Gibbins L N, Norris F W (1963). Phytase and acid phosphatase in the dwarf bean (*Phaseolus vulgaris*). Biochemistry Journal, 86: 67–71

Goel M, Sharma C B (1979). Multiple forms of phytase in germinating cotyledons of cucurbita-maxima. Phytochemistry, 18(12): 1939–1942

Gonnety J T, Niamke S, Meuwiah F B, N'guessan Kouadio E J, Kouame L P (2007). Purification, kinetic properties and physicochemical characterization of a novel acid phosphatase (AP) from germinating peanut (*Arachis hypogaea*) seed. Italian Journal of Biochemistry, 56 (2): 149–157

Greiner R (2002). Purification and characterization of three phytate-degrading enzymes from germinated lupin seeds (*Lupinus albus* var. *amiga*), Journal of Agricultural and Food Chemistry, 50: 6858–6864

Greiner R (2004a). Degradation of myo-inositol hexakisphosphate by a phytate-degrading enzyme from *Pantoea agglomerans*. Protein Journal, 23: 577–585

Greiner R (2004b). Purification and properties of a phytate-degrading enzyme from *Pantoea agglomerans*. Protein Journal, 23: 567–576

Greiner R, Alminger M L (1999). Purification and characterization of a phytate-degrading enzyme from germinated oat (*Avena sativa*). Journal of the Science of Food and Agriculture, 79: 1453–1460

Greiner R, Alminger M L (2001). Stereospecificity of myo-inositol hexakisphosphate dephosphorylation by phytate-degrading enzymes of cereals. Journal of Food Biochemistry, 25: 229–248

Greiner R, Alminger M L, Carlsson N G (2001a). Stereospecificity of myo-inositol hexakisphosphate dephosphorylation by a phytate-degrading enzyme of baker's yeast. Journal of Agricultural and Food Chemistry, 49: 2228–2233

Greiner R, Alminger M L, Carlsson N G, Muzquiz M, Burbano C, Cuadrado C, Pedrosa M M, Goyoaga C (2002a). Pathway of dephosphorylation of myo-inositol hexakisphosphate by phytases from legume seeds. Journal of Agricultural and Food Chemistry, 50: 6865–6870

Greiner R, Carlsson N G, Alminger M L (2001b). Stereospecificity of myo-inositol hexakisphosphate dephosphorylation by a phytate-degrading enzyme of *Escherichia coli*. Journal of Biotechnology, 84: 53–62

Greiner R, Farouk A, Alminger M L, Carlsson N G (2002b). The pathway of dephosphorylation of myo-inositol hexakisphosphate by phytate-degrading enzymes of different *Bacillus* spp. Canada Journal of Microbiology, 48: 986–994

Greiner R, Haller E, Konietzny U, Jany K D (1997). Purification and characterization of a phytase from *Klebsiella terrigena*. Archives of Biochemistry and Biophysics, 341: 201–206

Greiner R, Jany K D, Alminger M L (2000). Identification and properties of myo-inositol hexakisphosphate phospho-hydrolases (phytases) from barley (*Hordeum vulgare*). Journal of Cereal Science, 3: 127–139

Greiner R, Konietzny U, Jany K D (1993). Purification and characterization of two phytases from *Escherichia coli*. Archives of Biochemistry and Biophysics, 303: 107–113

Greiner R, Konietzny U, Jany K D (1998). Purification and properties of a phytase from rye. Journal of Food Biochemistry, 22: 143–161

Guimaraes L H S, Terenzi H F, Jorge J A, Leone F D, Polizeli M T M (2004). Characterization and properties of acid phosphatases with phytase activity produced by *Aspergillus caespitosus*. Biotechnology and Appllied Biochemistry, 40: 201–207

Hamada A, Yamaguchi K, Harada M, Horiguchi K, Takahashi T, Honda H (2006). Recombinant, rice-produced yeast phytase shows the ability to hydrolyze phytate derived from seed-based feed, and extreme stability during ensilage treatment. Bioscience, Biotechnology, and Biochemistry, 70(6): 1524–1527

Hara A, Ebina S, Kondo A, Funaguma T (1985). A new type of phytase from pollen of *Typha-Latifolia*. Agricultural and Biological Chemistry, 49(12): 3539–3544

Hartingsveldt W, Zeijl C M J, Hartereld G M (1993). Cloning,

characterization and overexpression of the phytase-encoding gene (*phyA*) of *Aspergillus niger*. Gene, 127: 87–94

Hayakawa T, Suzuki K, Miura H, Ohno T, Igaue I (1990). Myoinositol polyphosphate intermediates in the dephosphorylation of phytic acid by acid-phosphatase with phytase activity from rice bran. Agricultural and Biological Chemistry, 54: 279–286

Hayakawa T, Toma Y, Igaue I (1989). Purification and characterization of acid-phosphatases with or without phytase activity from rice bran. Agricultural and Biological Chemistry, 53(6): 1475–1483

Hbel F, Beck E (1996). Maize root phytase. Plant Physiology, 112: 1429–1436

Hong C Y, Cheng K J, Tseng T H, Wang C S, Liu L F, Yu S M (2004). Production of two highly active bacterial phytases with broad pH optima in germinated transgenic rice seeds. Transgenic Research, 13 (1): 29–39

Hong Y F, Liu C Y, Cheng K J, Hour A L, Chan M T, Tseng T H, Chen K Y, Shaw J F, Yu S M (2008). The sweet potato sporamin promoter confers high-level phytase expression and improves organic phosphorus acquisition and tuber yield of transgenic potato. Plant Molecular Biology, 67(4): 347–361

Huang H Q, Luo H Y, Wang Y R, Fu D W, Shao N, Wang G Z, Yang P L, Yao B (2008). A novel phytase from *Yersinia rohdei* with high phytate hydrolysis activity under low pH and strong pepsin conditions. Appllied Microbiology and Biotechnology, 80(3): 417–426

Huang H Q, Shao N, Wang Y R, Luo H Y, Yang P L, Zhou Z G, Zhan Z C, Yao B (2009). A novel beta-propeller phytase from *Pedobacter nyackensis* MJ11 CGMCC 2503 with potential as an aquatic feed additive. Appllied Microbiology and Biotechnology, 83(2): 249–259

Jia Z, Golovan S, Ye Q (1998). Purification, crystallization and preliminary X-ray analysis of the *Escherichia coli* phytase. Acta Crystallographica, 54(4): 47–64

Jog S P, Garchow B G, Mehta B D, Murthy P P N (2005). Alkaline phytase from lily pollen: Investigation of biochemical properties. Archives of Biochemistry and Biophysics, 440: 133–140

Jongbloed A W, Kemme P A, Mroz Z (1996). Phytase in swine rations: impact on nutrition and environment. BASF Technical Symposium, 44–69

Kerovou J, Lauraeus M, Nurminen P (1998). Isolation, characterization, molecular gene cloning and sequencing of a novel phytase from *Bacillus subtilis*. Appllied and Environmental Microbiology, 64: 2079–2085

Kerovuo J, Lauraeus M, Nurminen P, Kalkkinen N, Apajalahti J (1998). Isolation, characterization, molecular gene cloning, and sequencing of a novel phytase from *Bacillus subtilis*. Applied and Environmental Microbiology, 64: 2079–2085

Kerovuo J, Rouvinen J, Hatzack F (2000). Analysis of myo-inositol hexakisphosphate hydrolysis by *Bacillus* phytase: indication of a novel reaction mechanism. Journal of Biochemitry, 352: 623–628

Kim H W, Kim Y O, Lee J H, Kim K K, Kim Y J (2003). Isolation and characterization of a phytase with improved properties from *Citrobacter braakii*. Biotechnology Letters, 25: 1231–1234

Kim H, Eskin N A M (1987). *Canola* phytase isolation and characterization. Journal of Food Science, 52(5): 1353–1354

Kim T, Edward J, Mullaney, Jesus M, Porres, Karl R, Roneker, Sarah Crowe, Sarah Rice, Taegu Ko, Abul H J, Ullah, Catherine B, Daly, Ross W, Xin G L (2006). Shifting the pH profile of *Aspergillus niger* PhyA phytase to match the stomach pH enhances its effectiveness as an animal feed additive. Applied and Environmental Microbiology, 72(6): 4397–4403

Kim Y O, Kim H K, Bae K S, Yu J H, Oh T K (1998a). Purification and properties of a thermostable phytase from *Bacillus* sp. DS11. Enzyme and Microbial Technology, 22: 2–7

Kim Y O, Lee J K, Kim H K, Yu J H, Oh T K (1998b). Cloning of the thermostable phytase gene (*phy*) from *Bacillus* sp. DS11 and its overexpression in *Escherichia coli*. FEMS Microbiology Letters, 162 (1): 185–191

Konietzny U, Greiner R (2002). Molecular and catalytic properties of phytate-degrading enzymes (phytases). International Journa of Food Science and Technology, 37: 791–812

Konietzny U, Greiner R, Jany K D (1994). Purification and characterization of a phytase from spelt. Journal of Food Biochemistry, 18(3): 165–183

Konietzny U, Greiner R, Jany K D (1995). Purification and characterization of a phytase from spelt. Journal of Food Biochemistry, 18: 165–183

Koonin E V (1994). Conserved sequence pattern in a wide variety of phosphoesterases. Protein Sci, 3: 356–358.

Kostrewa D, Grüninger-Leitch F, D'Arcy A, Broger C, Mitchell D, van Loon A P G M (1997). Crystal structure of phytase from *Aspergillus ficuum* at 2.5 A resolution. Nature Structural Biology, 4: 185–190

Laboure A M, Gagnon J, Lescure A M (1993). Purification and characterization of a phytase (myo-inositol-hexakisphosphate phosphohydrolase) accumulated in maize (*Zea mays*) seedlings during germination. Biochemistry Journal, 295: 413–419

Lei X G, Porres J M, Mullaney E J, Brinch-Pedersen H (2007). Phytase: Source, Structure and Application. In: Poilna J, MacCabe A P, eds. Industrial Enzymes, Structure, Function and Application. Berlin: Springer, 505–529

Li G L, Yang S H, Li M G, Qiao Y K, Wang J H (2009). Functional analysis of an *Aspergillus ficuum* phytase gene in *Saccharomyces cerevisiae* and its root-specific, secretory expression in transgenic soybean plants. Biotechnology Letters, 31(8): 1297–1303

Li J, Hegeman C E, Hanlon R W, Lacy G H, Denbow M D, Grabau E A (1997). Secretion of active recombinant phytase from soybean cell-suspension cultures. Plant Physiology, 114: 1103–1111

Li M, Osaki M, Honma M, Tadano T (1997). Purification and characterization of phytase induced in tomato roots under phosphorus-deficient conditions. Soil Science and Plant Nutrition, 43: 179–190

Lim D, Golovan S, Forsberg C W (2000). Crystal structures of *Escherichia coli* phytase and its complex with phytate. Nature Structural & Molecular Biology, 7: 108–113

Lim M H, Lee O H, Chin J E, Ko H M, Kim I C, Lee H B, Im S Y, Bai S (2008). Simultaneous degradation of phytic acid and starch by an industrial strain of *Saccharomyces cerevisiae* producing phytase and alpha-amylase. Biotechnology Letters, 30(12): 2125–2130

Lim P E, Tate M E (1973). The phytases: II. Properties of phytase fraction F1 and F2 from wheat bran and the myo-inositol phosphates produced by fraction F2. Biochimica et Biophysica Acta, 302: 326–328

Liu Q, Huang Q Q, Lei X G, Hao Q (2004). Crystallographic snapshots of *Aspergillus fumigatus* phytase, revealing its enzymatic dynamics.

Structure, 12(9): 1575–1583

Loewus F A, Murthy P P N (2000). Myo-inositol metabolism in plants. Plant Science, 150: 1–19

Lolas G M, Markakis P (1977). Phytase of navy beans (*Phaseolus vulgaris*). Journal of Food Science, 42(4): 1094–1097

Mahajan A, Dua S (1997). Nonchemical approach for reducing antinutritional factors in rape seed (*Brassica campestris* var. *toria*) and characterization of enzyme phytase. Journal of Agricultural and Food Chemistry, 45(7): 2504–2508

Maiti I B, Majumber A L, Biswas B B (1974). Purification and mode of action of phytase from *Phaseolus aureus*. Phytochemistry, 13: 1047–1051

Mandal N C, Biswas B B, Burman S (1972). Metabolism of inositol phosphates. 3. Isolation, purification and characterization of phytase from germinating mung beans. Phytochemistry, 11(2): 495–502

Maugenest S, Martinez I, Godin B, Perez P, Lescure A M (1999). Structure of two maize phytase genes and their spatio-temporal expression during seedling development. Plant Molecular Biology, 39: 503–514

Maugenest S, Martinez I, Lescure A M (1997). Cloning and characterization of a cDNA encoding a maize seedling phytase. Biochemistry Journal, 322: 511–517

Mitchell D B, Vogel K, Weimann B, Pasamontes L, van Loon A P G M (1997). The phytase subfamily of histidine acid phosphatases: isolation of genes for two novel phytases from the fungi *Aspergillus terreus* and *Myceliophthora thermophila*. Microbiology, 143: 245–252

Mondal M K, Panda S, Biswas P (2007). Effect of microbial phytase in soybean meal based broiler diets containing low phosphorus. Poultry Science, 6: 201–206

Mullaney E J, Daly C B, Ullah A H J (2000). Advances in phytase research. Advances in Appllied Microbiology, 47: 157–199

Mullaney E J, Ullah A H J (1998). Conservation of the active site motif in *Aspergillus niger* (ficcum) pH 6.0 optimum acid phosphatase and kidney bean purple acid phosphatase. Biochemical and Biophysical Research Communications, 243: 471–473

Mullaney E J, Ullah A H J (2003). The term phytase comprises several different classes of enzymes. Biochemical and Biophysical Research Communications, 312: 179–184

Nagai Y, Funahashi S (1962). Phytase (myo-inositol-hexaphosphate phosphohydrolase) from wheat bran. purification and substrate specificity. Agricultural and Biological Chemistry, 26(12): 794–803

Nakano T, Joh T, Narita K, Hayakawa T (2000). The pathway of dephosphorylation of myo-inositol hexakisphosphate by phytases from wheat bran of *Triticum aestivum*. Bioscience Biotechnology and Biochemistry, 64: 995–1003

Nakano T, Joh T, Tokumoto E, Hayakawa T (1999). Purification and characterization of phytase from bran of *Triticum aestivum* L. cv. Nourin. Food Science and Technology Research, 5: 18–23

O'Dell B, de Boland A R, Koirtyohann S R (1991). Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. Journal of Agricultural and Food Chemistry, 20: 718–721

Ostanin K, Harms E H, Stevis P E, Kuciel R, Zhou M M, van Etten R L (1992). Overexpression, site-directed mutagenesis, and mechanism of *Escherichia coli* acid phosphatase. Journal of Biological Chemistry, 267: 22830–22836

Pasamontes L, Haiker M, Wyss M, Tessier M, van Loon A P G M (1997). Gene cloning, purification, and characterization of a heat-stable phytase from the fungus *Aspergillus fumingatus*. Appllied and Environmental Microbiology, 63: 1696–1700

Pen J, Verwoerd T C, van Paridin P A, Beukeder R F, van der Elzen P J M, Geerse K (1993). Phytase-containing transgenic seed as a novel feed additive for improved phosphorus utilization. Bio/Technology, 11: 811–814

Phillippy B Q (1998). Purification and catalytic properties of a phytase from scallion (*Allium fistulosum* L.) leaves. Journal of Agricultural and Food Chemistry, 46: 3491–3496

Piddington C S, Houston C S, Paloheimo M, Cantrell M, Miettinen-Oinonen A, Nevalainen H, Rambosek J (1993). The cloning and sequencing of the genes encoding phytase (phy) and pH 2.5-optimum acid phosphatase (aph) from *Aspergillus niger* var. awamori. Gene, 133: 55–62

Ponstein A S, Bade J B, Verwoerd T C, Molendijk L, Storms J, Beudeker R F (2002). Stable expression of phytase (phyA) in canola (*Brassica napus*) seeds: towards a commercial product. Molecular Breeding, 10: 31–44

Powar V K, Jagannathan V (1982). Purification and properties of phytate-specific phosphatase from *Bacillus subtilis*. Journal of Bacteriology, 151(3): 1102–1108

Promdonkoy P, Tang K, Sornlake W, Harnpicharnchai P, Kobayashi R S, Ruanglek V, Upathanpreecha T, Vesaratchavest M, Eurwilaichitr L, Tanapongpipat S (2009). Expression and characterization of *Aspergillus* thermostable phytases in *Pichia pastoris*. FEMS Microbiology Letters, 290(1): 18–24

Quan C S, Fan S D, Zhang L H, Tian W J, Ohta Y (2002). Purification and properties of a phytase from *Candida krusei* WZ-001. Journal of Bioscience and Bioengineering, 94: 419–425

Quan C S, Tian W J, Fan S D, Kikuchi J I (2004). Purification and properties of a low-molecular-weight phytase from *Cladosporium* sp. FP-1. Journal of Bioscience and Bioengineering, 97: 260–266

Ragon M, Hoh F, Aumelas A, Chiche L, Moulin G, Boze H (2009). Structure of *Debaryomyces castellii* CBS 2923 phytase. Acta Crystallographica Section F: Structural Biology and Crystallization Communications, 65(4): 321–326

Rao D E, Rao K V, Reddy V D (2008). Cloning and expression of *Bacillus* phytase gene (*phy*) in *Escherichia coli* and recovery of active enzyme from the inclusion bodies. Journal of Appllied Microbiology, 105(4): 1128–1137

Ravindran V, Bryden W L, Kornegay E T (1995). Phytates: occurrence, bioavailability and implications in poultry nutrition. Poultry and Avain Biology Reviews, 6: 125–143

Reddy N R, Sathe S K, Salunkhe D K (1982). Phytates in legumes and cereals. Advances in Food Research, 28: 1–92

Reddy V A,Venu K, Rao D E, Rao K Y, Reddy V D (2009). Chimeric gene construct coding for bi-functional enzyme endowed with endoglucanase and phytase activities. Archives of Microbiology, 191 (2): 171–175.

Richardson A E, Hadobas P A, Hayes J E (2001). Extracellular secretion of *Aspergillus* phytase from *Arabidopsis* roots enables plants to obtain phosphorus from phytate. Plant Journal, 25(6): 641–649

Sajidan A, Farouk A, Greiner R, Jungblut P, Müller E C, Borriss R

(2004). Molecular and physiological characterisation of a 3-phytase from soil bacterium *Klebsiella sp*. ASR1. Appllied Microbiology and Biotechnology, 65: 110–118

Sariyska M V, Gargova S A, Koleva L A, Angelov A I (2005). *Aspergillus niger* phytase: purification and characterization. Biotechnology, 98–105

Segueilha L, Lambrechts C, Boze H, Moulin G, Galzy P (1992). Purification and properties of the phytase from *Schwannio-myces castellii*. Journal of Fermenttation and Bioengineering, 74: 7–11

Shao N, Huang H, Meng K (2008). Cloning, expression, and characterization of a new phytase from the phytopathogenic bacterium *Pectobacterium wasabiae* DSMZ 18074. Journal of Microbiology and Biotechnology, 18(7): 1221–1226

Shimizu M (1993). Purification and characterization of phytase and acid phosphatase produced by *Aspergillus oryzae* K1. Bioscience, Biotechnology and Biochemistry, 57: 1364–1365

Simons P C M, Versteegh A J, Jongbloed A W, Kemme P A, Slump P, Bos K D, Wolters G E, Buedeker R F, Verschoor G J (1990). Improvement of phosphorus availability by microbial phytase in broilers and pigs. Nutrition, 64: 525–540

Suzuki U, Yoshimura K, Takaishi M (1907). Ueber ein Enzyme phytase das Anhydrooxy-methylen disphosphorasure spaltet. Collections of Agricultural Bulletins of Tokyo Imperial University, 7: 495–512

Tambe S M, Kaklij G S, Keklar S M, Parekh L J (1994). Two distinct molecular forms of phytase from *Klebsiella aerogenes*: Evidence for unusually small active enzyme peptide. Journal of Fermentation and Bioengineering, 77: 23–27

Tseng Y H, Fang T J, Tseng S M (2000). Isolation and characterization of a novel phytase from *Penicillium simplicissimum*. Folia Microbiologica. 45: 121–127

Tye A J, Siu F K Y, Leung T Y C, Lim B L (2002). Molecular cloning and the biochemical characterization of two novel phytases from *Bacillus subtilis* 168 and *Bacillus licheniformis*. Applied Microbiology and Biotechnology, 59: 190–197

Ullah A H J (1988). *Aspergillus ficuum* phytase: partial primary structure, substrate selectivity and kinetic characterization. Preparative Biochemistry, 18: 459–471

Ullah A H J, Cummins B J, Dischinger J H C (1991). Cyclohexanedione modification of arginine at the active site of *Aspergillus ficuum* phytase. Biochemical and Biophysical Research Communications, 178: 45–53

Ullah A H J, Dischinger Jr H C (1993). *Aspergillus ficuum* phytase: complete primary structure elucidation by chemical sequencing. Biochemical and Biophysical Research Communications, 192(2): 747–753

Ullah A H J, Gibson D M (1987). Extracellular phytase (EC.3.1.3.8) from *Aspergillus ficuum* NRRL 3135: purification and characterization. Preparative Biochemistry, 17: 63–91

Ullah A H J, Sethumadhavan K (2003). *PhyA* gene product of *Aspergillus ficuum* and *Peniophora lycii* produces dissimilar phytases. Biochemical and Biophysical Research Communications, 303: 463–468

Ullah A H J, Sethumadhavan K, Lei X G, Mullaney E J (2000). Biochemical characterization of cloned *Aspergillus fumigatus* phytase (phyA). Biochemical and Biophysical Research Communications, 275: 279–285

van Staden J, den Haan R, van Zyl W H, Botha A, Viljoen-Bloom M (2007). Phytase activity in *Cryptococcus laurentii* ABO 510. FEMS Yeast Research, 7(3): 442–448

vander Kaay J, van Haastert J M (1995). Stereospecificity of inositol hexaphosphate dephosphorylation by *Paramecium* phytase. Biochemistry Journal, 312: 907–910

Verwoerd T C, van Paridon P A, van Ooyen A J, van Lent J W, Hoekema A, Pen J (1995). Stable accumulation of *Aspergillus niger* phytase in transgenic tobacco leaves. Plant Physiology, 109(4): 1199–1205

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

Wyss M, Brugger R, Kronenberger A, Remy R, Fimbell R, Oesterhelt G, Lehmann M, van Loon A P G M (1999). Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohyrolases): catalytic properties. Appllied and Environmental Microbiology, 65: 367–373

Xiao K, Harrison M J, Wang Z Y (2005). Transgenic expression of a novel *M. truncatula* phytase gene results in improved acquisition of organic phosphorus by *Arabidopsis*. Planta, 222: 27–36

Xiong A S, Yao Q H, Peng R H, Zhang Z, Xu F, Liu J G, Han P L, Chen J M (2006). High level expression of a synthetic gene encoding *Peniophora lycii* phytase in methylotrophic yeast *Pichia pastoris*. Appllied Microbiology and Biotechnology, 72(5): 1039–1047

Yang W J, Matsuda Y, Sano S, Masutani H, Nakagawa H (1991). Purification and characterization of phytase from rat intestinal mucosa, Biochimica et Biophysica Acta, 1075: 75–82

Zhang W M, Edward J M, Xin G L (2007). Adopting selected hydrogen bonding and ionic interactions from *Aspergillus fumigatus* phytase structure improves the thermostability of *Aspergillus niger* PhyA phytase. Appllied and Environmental Microbiology, 73(9): 3069–3076

Zhang Z B, Kornegay E T, Radcliffe J S, Denbow D M, Veit H P, Larsen C T (2000). Comparison of genetically engineered *Aspergillus* and canola in weanling pig diets. Journal of Animal Science, 78: 2868–2878