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*review*

# Molecular Characterization and Comparative Phylogenetic Analysis of Phytases from Fungi with Their Prospective Applications

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**Summary**

Plant seeds that have high phytate content are used as animal feed. Phytases, enzymes that catalyze the breakdown of phytate into inorganic phosphorus and myoinositol phosphate derivatives, have been intensively studied in recent years and gained immense attention because of their application in reducing phytate content in animal feed and food for human consumption, thus indirectly lowering environmental pollution caused by undigested phytate. This review is focused on summarising the current knowledge on recent developments of fungal and yeast phytases. Comparative account on diverse sources and physiological roles, molecular characteristics and regulation mechanisms of phytases are discussed. Phylogenetic relationship of phytases from different classes of fungi is studied in details. It is inferred on the basis of phylogeny that phytases from Ascomycetes and Basidiomycetes differ in the amino acid sequences, therefore they fall in separate clade in the tree. The prospective biotechnological applications of microbial phytases such as animal feed additives, probiotics, pharmaceuticals, as well as in aquaculture, food industry, paper manufacturing, development of transgenic plants and animals with special reference to its use as biofertilizers are also emphasised in this review.

*Key words*: fungal phytases, phylogenetic relationship, phytase gene regulation, biotechnological applications

## Introduction

Myoinositol hexakisphosphate is a ubiquitous constituent of cereals and grains, which exists predominantly in its salt form and serves as a major source of phosphorus for the animals. Phytic acid is the principal storage form of phosphorus and inositol. It represents approx. 60–90 % of the total phosphorus content in cereals, legumes, and oilseeds, approx. 50 % in nuts and approx. 24 % in cocoa and chocolate (*1*). In spite of being a rich source of phosphorus, the bound phosphorus in phytate is poorly utilised by monogastric animals such as pigs, poultry and fish because these animals have very low levels of phytate-degrading enzymes, *i.e.* phytase (myoinositol hexakisphosphate phosphohydrolase) in their digestive tracts, which requires addition of sources with $Ca_3(PO_4)_2$ to the forage. Besides this, phytate is also considered as antinutritional compound because it forms complexes with several divalent cations of major nutritional significance, such as $Ca^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Fe^{2+}$ and $Mn^{2+}$ (*2*), and with proteins under both acidic and alkaline pH conditions which affect its structure, resulting in the decrease in the enzymatic activity, protein solubility and proteolytic digestibility (*3*). The undigested phytate excreted by the animals is degraded by microorganisms in the soil and the released phosphorus at high concentrations gets into the rivers where it causes eutrophication. The importance of phytic acid as a source of phosphorus, its ability to cause undesirable ecological effects and antinutritive properties has stimulated re-

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search into ways for its dephosphorylation. Hence, in the last few decades, phytases have attracted substantial interest of scientists and entrepreneurs in the areas of nutrition, environmental protection and biotechnology. This review summarises the current knowledge on recent research of phytases and presents a comparative account on diverse sources and physiological roles of phytases, their molecular characteristics, phylogenetic relationships, regulation mechanisms, as well as their assorted applications in paper and pulp industry, myoinositol production, in pharmaceuticals, biofertilizers, animal and human nutrition, transgenic animals and plants.

## Source Organisms for Phytases

Phytases are reported to be present in plants (*4–6*), animals (*7*,*8*) and in a variety of microorganisms such as bacteria (*9–13*), yeasts (*14*,*15*) and filamentous fungi (*16–21*). A detailed list of known phytase-producing fungi and yeasts is given in Table 1 (*15*,*17–60*).

## Physiological Roles of Phytases

It is clear and evident that phytases are widespread in nature ranging from microorganisms to plants and animals. Also, the role of these enzymes in each organism varies depending on its physiological requirements. The presence of multiple phytases with different specificities, pH optima and biochemical properties within individual species suggests that hydrolysis of phytic acid is under the control of more than one phytase.

### *Microorganisms*

In microorganisms, phytase expression is most frequently induced and the enzymes are usually secreted in response to phosphate starvation. The expression of phytases results in the release of inorganic phosphate from the surrounding and/or internal inositol hexaphosphate ($InsP_6$) stores. Moreover, the synthesis of periplasmic phytate-degrading enzymes was found to drastically increase in *E. coli* in the stationary phase under anaerobic conditions (*9*). However, phytate-degrading activity in *Klebsiella terrigena* was found to increase when phytate was present in the cultivation medium (*10*). In most of fungi like *Aspergillus niger* and *A. ficuum*, limited concentrations of phosphorus in the medium decrease optimal mycelial growth of the mould, but phytase production is maximal (*16*). Furthermore, many prokaryotes produce inositol phosphate-degrading enzymes that serve diverse functions including phosphate scavenging and pathogenesis, which was reported in *Xanthomonas oryzae*, *X. campestris* and *Salmonella dublin* (*61*,*62*).

### *Plants*

In plants, phytase is expressed during seed germination for phytate degradation to provide the growing seedling with orthophosphate, lower inositol polyphosphates (IPPs), free myoinositol and previously bound cations, such as $K^+$, $Mg^{2+}$, $Zn^{2+}$ and $Ca^{2+}$, thus providing nutrition for plant growth (*63*). The constitutive alkaline phytase present in lily pollen and seeds removes the 5-, 4- and 6-phosphate of $InsP_6$ to yield $Ins(1,2,3)P_3$ as the final product, which acts as antioxidant by inhibiting iron-catalyzed free radical formation by chelating iron (*64*).

Table 1. List of phytase-producing fungi and yeasts and their respective classes

| Phylum of fungi | Class of phytase | | | Reference |
|---|---|---|---|---|
| | Histidine acid phytase (HAPs) 3-phytase | Histidine acid phytase (HAPs) 6-phytase | Unclassified | |
| Ascomycetes | | | | |
| | *Aspergillus ficuum* | | | (*22*) |
| | *Aspergillus oryzae* | | | (*17*) |
| | | | *Aspergillus carbonarius* | (*23*) |
| | *Aspergillus terreus*<br>*Myceliophthora thermophila* | | | (*18*) |
| | *Emericella nidulans*<br>*Talaromyces thermophilus* | | | (*19*) |
| | *Aspergillus fumigatus* | | | (*20*) |
| | *Thermomyces lanuginosus* | | | (*24*,*25*) |
| | | | *Penicillium simplicissimum* | (*26*) |
| | | | *Thermoascus aurantiacus* | (*27*) |
| | | | *Cladosporium* sp. | (*28*) |
| | *Aspergillus niger* | | | (*29*) |
| | *Neurospora crassa* | | | (*30*) |
| | *Aspergillus awamori* | | | (*31*) |
| | *Penicillium oxalicum* | | | (*32*) |
| | *Trichoderma reesei* | | | (*33*) |

Table 1. – continued

| Phylum of fungi | Class of phytase | | | Reference |
|---|---|---|---|---|
| | Histidine acid phytase (HAPs) 3-phytase | Histidine acid phytase (HAPs) 6-phytase | Unclassified | |
| | *Penicillium chrysogenum* | | | (*34*) |
| | *Aspergillus japonica* | | | (*35*) |
| | *Eupenicillium parvum* | | | (*36*) |
| | | | *Fusarium verticillioides* | (*37*) |
| | | | *Sporotrichum thermophile* | (*38*) |
| | *Penicillium* sp. Q7 | | | (*39*) |
| | | | *Penicillium funiculosum* | (*40*) |
| | *Neosartorya spinosa* | | | (*41*) |
| Single-cell Ascomycetes (yeast) | | | | |
| | *Saccharomyces cerevisiae* | | | (*42*) |
| | *Schwanniomyces castellii* | | | (*43*) |
| | | | *Rhodotorula gracilis* | (*44*) |
| | *Hansenula polymorpha* | | | (*45*) |
| | | | *Pichia rhodanensis* | (*46*) |
| | | | *Pichia spartinae* | |
| | *Schwanniomyces occidentalis* | | | |
| | | | *Candida krusei* | (*15*) |
| | *Pichia anomala* | | | (*47*) |
| | *Candida albicans* | | | (*48*) |
| | *Arxula adeninivorans* | | | (*49*) |
| | *Pichia stipitis* | | | (*50*) |
| | *Candida tropicalis* | | | |
| | | | *Candida glabrata*, formerly known as: *Torulopsis candida* | (*51*) |
| | | | *Kluyveromyces fragilis* | |
| | | | *Candida krusei* | |
| | *Debaryomyces castellii* | | | (*52*) |
| | *Kodamaea ohmeri* | | | (*53*) |
| | *Hansenula fabianii* | | | (*54*) |
| Basidiomycetes | | | | |
| | | *Peniophora lycii* | | (*21*) |
| | | *Agrocybe pediades* | | |
| | | *Ceriporia* sp. | | |
| | | *Trametes pubescens* | | |
| | | | *Agaricus bispora* | (*55*) |
| | | | *Grifola frondosa* | |
| | | | *Lentinula edodes* | |
| | | | *Pleurotus cornucopiae* | |
| | | | *Rhizoctonia* sp. | (*37*) |
| Zygomycetes | | | | |
| | | | *Mucor racemosus* | (*56*) |
| | | | *Rhizopus oligosporus* | (*57*) |
| | | | *Rhizomucor pusillus* | (*58*) |
| | | | *Rhizopus oryzae* | (*59*) |
| | | | *Mucor hiemalis* | (*60*) |

Also, some of the intermediates and end products of phytate hydrolysis are important in the transport as secondary messengers and in signal transduction (*65*).

### *Animals*

In animals, phytases are mainly involved in the maintenance of the cell's metabolic reservoirs of $InsP_6$ and other IPPs. The multiple inositol polyphosphate phosphatase (MIPP) plays a vital role in the regulation of cellular activities of $InsP_6$ and $Ins(1,3,4,5,6)P_5$. MIPP--generated metabolites are also reported to act as $Ca^{2+}$--mobilizing signal (*66*). The evolutionary conservation of MIPP within the inositol phosphate pathway suggests a significant role of MIPP in higher eukaryotes (*8*).

## Classification

Phytases catalyse the hydrolysis of phytic acid in a stepwise manner to inositol phosphates, myoinositol and inorganic phosphate (*18*). Phytases can be characterized using three different classification systems depending on their pH optima, catalytic mechanism and on the position of carbon in the myoinositol ring of phytate at which dephosphorylation is initiated. Depending on their pH optima, phytases are divided into acid phosphatase and alkaline phosphatase. Based on the catalytic mechanism, phytases can be referred to as histidine acid phosphatases/histidine acid phytases (HAPs/HAPhy), β-propeller phytases (BPP), purple acid phytases (PAP) and cysteine phytases/protein tyrosine phosphatases (PTPs) (*67*). Additionally, based on the position of carbon in the myoinositol ring of phytate at which dephosphorylation initiates and according to the IUPAC system of classification, phytases are classified into 3-phytases (EC 3.1.3.8), 4-phytases (EC 3.1.3.26), 5-phytases (EC 3.1. 3.72) and 1D-3 or 1D-4 (protein tyrosine phosphates (PTP)-like inositol polyphosphatases). The representatives of each of these classes have different catalytic mechanisms, cofactor requirements and end products, as shown in Table 2 (*5,6,21,31,32,68–73*).

### *Histidine acid phosphatases*

HAPs have been reported to be present in most of the bacteria, yeast, fungi and plants. Two classes of acid phosphatases can be recognized in terms of their molecular masses, low molecular mass HAPs and high molecular mass HAPs. Low molecular mass HAP form lacks both N-terminal RHGXRXP motif and a C-terminal motif HD, as reported in *Cladosporium* sp. (*28*). High molecular mass HAP form exhibits both N-terminal RHGXRXP motif and a C-terminal motif HD (*74*). The histidine residue (*His*) of fungal phytases has been proposed to serve as a nucleophile in the formation of phosphohistidine intermediate and an equivalent *Asp* residue, which might protonate the substrate leaving group (*1*). In addition to the presence of conserved motifs, all HAPs also show up to ten conserved potential N-glycosylation sites identified as NXS/T and almost ten cysteine re-

Table 2. Classification of phytases

| Classification based on catalytic mechanism | | | | | |
|---|---|---|---|---|---|
| Class | pH range | Conserved sequence/motif | Co-factor required for activation | End product of hydrolysis reaction | Prevalence |
| histidine acid phosphatase (HAPhy) | 4.0–4.5 | Heptapeptide motif RHGXRXP at N-terminal end and dipeptide motif HD at C-terminal | EDTA | myoinositol (2) monophosphate ($InsP_1$) | most of the bacteria, yeast and fungi |
| alkaline phytase β-propeller (BPPs) | 7.0–8.0 | Six-bladed propeller folding architecture with six $Ca^{2+}$--binding site | $Ca^{2+}$ | myoinositol trisphosphate ($InsP_3$) | *Bacillus*, legume seeds, lily |
| purple acid phophatase (PAP) | 4.0–5.5 | Seven conserved residues in five conserved motifs: DXG, GDXXY, GNH(D/E), VXXH and GHXH involved in coordination of dimetal nuclear centre | metalloenzyme | myoinositol 2,3,4,5-tetraphosphate or myoinositol 1,2,5,6-tetraphosphate ($InsP_4$) | plants |
| cysteine phosphatase | 4.5–6.5 | Cysteine containing (Cys 241) P loop HCXXGXXR(T/S) | $Pb^{2+}$ | myoinositol (2) monophosphate ($InsP_1$) | anaerobic rumen bacteria, *e.g. Selenomonas ruminantium* |

| Classification based on the position of carbon atom at which dephosphorylation initiates | | | |
|---|---|---|---|
| Class | IUPAC nomenclature | Prevalence | Reference |
| 3-phytase EC 3.1.3.8 | myoinositol hexakinase phosphate 3-phosphohydrolase | most of bacteria and Ascomycetes phylum | (*31,32*) |
| 6-phytase EC 3.1.3.26 | myoinositol hexakinase phosphate 6-phosphohydrolase | plant (grains/oilseeds), ferns and Basidiomycetes | (*5,6,21*) |
| 5-phytase EC 3.1.3.72 | myoinositol hexakinase phosphate 5-phosphohydrolase | lily, alfalfa, beans, peas, *S. ruminantium* | (*68–71*) |
| 1D-3 or 1D-4 | protein tyrosine phosphates (PTP)-like inositol polyphosphatases | *Klebsiella terrigena*, *Megasphaera elsdenii* | (*72,73*) |

sidues. Glycosylation may have several effects on the properties of an enzyme, including catalytic properties or the stability of the enzyme. It may, in addition, influence the pI of the protein (*75*). Although not directly involved in the catalytic function of HAPhy, disulphide bridges formed by the conserved cysteine residues perform an important role in maintaining the proper three-dimensional structure of the protein (*76*).

All phytases that belong to the family of HAPs do not need any cofactor for optimal activity (*77*). Despite the common catalytic site, there are some HAPs that do not share an equal ability to degrade myoinositol hexakisphosphate. Both the mouse and fruit fly MIPP represent a number of other HAPs that are not effective phytases, recognizing the fact that all HAPs are not phytases (*78*), hence the term 'histidine acid phytase' (HAPhy) is used to denote HAPs that can accommodate myoinositol hexakisphosphate as a substrate. Despite the fact that catalytic centres for all the known microbial HAPhys are identical, they can be divided into two classes based on substrate specificity. One class has broad substrate specificity but a low specific activity for inositol hexakisphosphate, while the second class has narrow substrate specificity and a high specificity for myoinositol hexakisphosphate (*75*). The HAPhys with high specific activity for myoinositol hexakisphosphate have either a basic or acidic amino acid residue at the position 300, while the phytases with low specific activity have a neutral amino acid at that position (*79*).

### *β-Propeller phytases*

β-Propeller phytases (BPP; EC 3.1.3.8) are quite different in their nucleotide sequence, structure, cofactor requirements and catalytic mechanism from all other known phytases. They are expressed by *Bacillus subtilis* and its related species (*11*). The name β-propeller phytases was adopted based on the molecular structure of this enzyme, which mainly consists of β-sheets and resembles a six-bladed propeller (*80*). The main components involved in the catalytic mechanism of BPP to hydrolyze myoinositol hexakisphosphate include a 'cleavage site', an 'affinity site' and an electronegative, solvent-accessible central channel that binds seven $Ca^{2+}$ ions (*81,82*). Only substrates that simultaneously fill both binding sites are hydrolyzed by BPPs because, according to the enzyme mechanism of BPP, the phosphate bound to the affinity site facilitates the cleavage of flanking phosphate by the cleavage site. Therefore, the enzyme prefers the hydrolysis of every second phosphate and has a reduced affinity for any substrate that cannot fulfil this requirement. Thus, BPP alternately remove phosphate groups with the end product being myoinositol trisphosphate. The $Ca^{2+}$ ions serve several functions, including the activation of a water molecule, coordination of the scissile phosphate and stabilization of the negative charge that develops in the transition state, which can be replaced by many divalent cations but has proven to be the most effective one (*81*).

It is reported that the plant pathogen, *Xanthomonas oryzae*, secretes a six-bladed β-propeller protein, which is required for optimum virulence in its host (*62*). Characterization of this protein revealed conservation of active-site residues previously identified in *Bacillus* phytases, suggesting that virulence in a plant pathogen is in part due to the ability of the bacteria to utilize myoinositol hexakisphosphate as a source of phosphate.

### *Purple acid phytases*

Purple acid phytases (PAPs) have been purified from germinating glycine (*83*) and *A. niger* (*84*). PAPs are a class of metalloenzymes and possess a dimetal nuclear centre that can be either $Fe^{3+}$–$Fe^{2+}$, $Fe^{3+}$–$Mn^{2+}$ or $Fe^{3+}$–$Zn^{2+}$, and exhibit optimal enzyme activity under acidic conditions. They are characterized by seven conserved residues (bold) in the five conserved motifs – **D**XG, G**D**XX**Y**, G**N**H(D/E), VXX**H** and G**H**X**H** – involved in the coordination of the dimetal nuclear centre (*83,85*). The catalytic mechanism involves the direct attack of the scissile phosphate by an activated water molecule coordinated by the metal centre. PAPs exist as a homodimer and each monomer is composed of two domains: a small N-terminal antiparallel β-sandwich and a larger C-terminal α+β domain. The C-terminal domain is characterized by two large mixed central β-sheets that form a β-sandwich. Two α-helices from each monomer interact to form the homodimer interface.

### *Cysteine phytases/protein tyrosine phosphatases*

Cysteine phytases represent a new class of phytases described in the anaerobic ruminal bacterium *Selenomonas ruminantium* and exhibit optimal enzyme activity under acidic conditions (*69*). This phytate-degrading enzyme is related to protein tyrosine phosphatases (PTPs) and has recently been described (*69,70,73*). These enzymes are characterized by the $CX_5R(S/T)$ PTP active site signature sequence. This sequence forms the loop at the base of the PTP active site and is important for coordinating the scissile phosphate for nucleophilic attack. *PhyAsr* adopts α+β PTP fold, and has a small β-domain that is unique to this class of enzymes. The β-domain is implicated in the unique substrate specificity of these enzymes (*69*).

## Phylogenetic Relationship Between Phytases (HAPs) from Fungi and Yeasts

In this section phylogenetic relationship between phytases from fungi and yeasts will be discussed. The amino acid sequences of phytases found in different fungi and yeasts were retrieved from various databases like NCBI, DDBJ and EMBL. Phylogenetic tree was constructed using MEGA5 software (*86*). The evolutionary history was inferred using the neighbor-joining method (*87*) and distances were computed using the Poisson correction method (*88*) and expressed as the units of the number of amino acid substitutions per site. It is inferred from the phylogenetic tree (Fig. 1) that phytases from Ascomycetes belong to 3-phytase class, whereas phytases from Basidiomycetes fall in a different cluster and belong to 6-phytase class. Despite the presence of conserved N-terminal motif (RHGXRXP) and C-terminal motif (HD) (which are present in Ascomycetes), a characteristic of HAPs, the phytase protein sequences of Basidiomycetes like *Peniophora lycii*, *Agrocybe pediades*, *Ceri-*

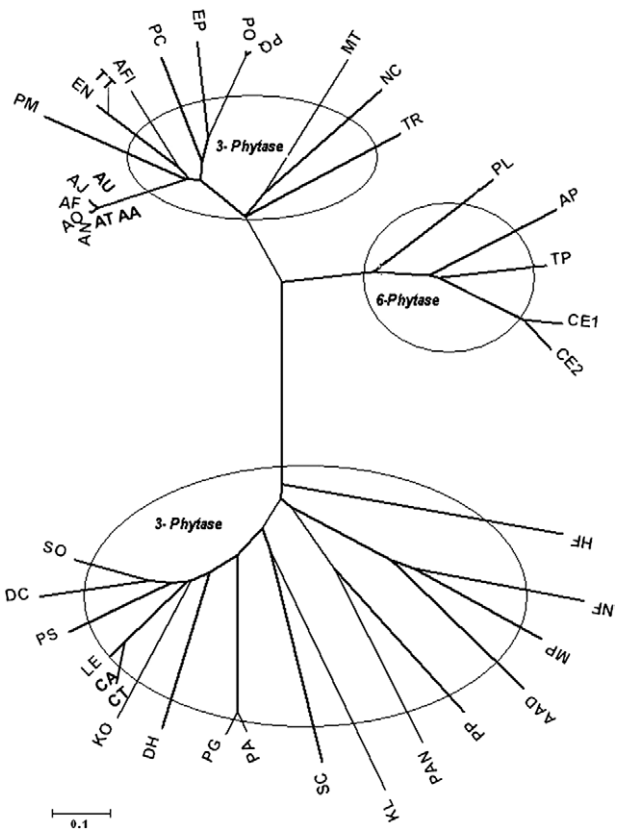


**Fig. 1.** Phylogenetic tree of phytase protein found in fungi and yeasts. The following amino acid sequences were analyzed: **AA**=*Aspergillus awamori* ABA29207, **AT**=*A. terreus* AAB52507, **AF**=*A. ficuum* AAG40885, **AN**=*A. niger* BAA74433, **AU**=*A. usamii* ABA42097, **AO**=*A. oryzae* AAT12504, **AJ**=*A. japonicus* ACE79228, **AFl**=*A. flavus* XP002376973, **PQ**=*Penicillium* sp. Q7 ABM92788, **PO**=*P. oxalicum* AAL55406, **PC**=*P. chrysogenum* XP002561094, **PM**=*P. marneffei* AP00218821, ***EP**=*Eupenicillium parvum*, **EN**=*Emericella nidulans* AAB96871, **TT**=*Talaromyces thermophilus* AAB96873, **MT**=*Myceliophthora thermophila* AAB52508, **NC**=*Neurospora crassa* AAS94253, **TR**=*Trichoderma reesei* EGR47873, **TP**=*Trametes pubescens* CAC48234, **AP**=*Agrocybe pediades* CAC48160, **PL**=*Peniophora lycii* CAC48195, **CE1**=*Ceriporia* sp. CAC8163, **CE2**=*Ceriporia* sp. CAC8164, **SO**=*Schwanniomyces occidentalis* ABN04184, **DC**=*Debaryomyces castellii* ABN04184, **DH**=*D. hansenii* Q6BM75, **PS**=*Pichia stipitis* XP001385108, **PG**=*P. guilliermondii* CAL69849, **PA**=*P. anomala* FN641803, **PAN**=*P. angusta* O74677, **PP**=*P. pastoris* P52291, **LE**=*Lodderomyces elognisporus* XP001527604, **CT**=*Candida tropicalis* XP002546108, **CA**=*C. albicans* XP713452, **KO**=*Kodamaea ohmeri* ABU53001, **SC**=*Saccharomyces cerevisiae* EDN64708, **KL**=*Kluveromyces lactis* CAA83964, **AAD**=*Arxula adeninivorans* CAJ77470, **HF**=*Hansenula fabianii* BAH588739, **NF**=*Neosartorya fischeri* AICXF7, **MP**=*Monascus purpureus* Q8X1W7
*Protein sequence taken from Fugthong *et al.* (*36*), as protein GenBank accession number is not available

*poria* sp. and *Trametes pubescens*, also exhibit three different conserved regions.

In the conserved sequence I, present at amino acid position 68–83 (**IQ***RHGARXP***TSGA***XX***R*), sequence II, present at 162–171 (**NWT***XGFXXA***S**), and sequence III, present at 415–433 (*FV***ESQ***XXARXXGX***GDF***X***K***C*), amino acids marked in bold are unique to the phytases belonging to Basidiomycetes, whereas those italicized are common to phytases of both Ascomycetes and Basidiomycetes (*21*). These dissimilarities in the phytase protein sequences of Basidiomycetes may be the reason for them to fall in a different cluster and a distinct class of phytases, as shown in the phylogenetic tree.

The comparative analysis of a representative of Basidiomycetes *Peniophora lycii* (PL) phytase with phytase from Ascomycetes like *Aspergillus fumigatus* (AFu) showed only 38 % sequence homology. It was even noted that PL phytase was 39 amino acids shorter than AF phytase (409 *versus* 448). In the N-terminus, there is a deletion of 15 residues. Despite these differences, the active site was intact in PL phytase. The dynamic light scattering studies estimated the molecular mass of AFu phytase and PL phytase to be 86.6 and 118.4 kDa, respectively, which in turn suggested that the AFu phytase exists as a monomer, while the PL phytase could be a homodimer. Thus, phytases from Ascomycetes and Basidiomycetes differ in physical, chemical, and catalytic properties (*89*). These disparities between phytases from Ascomycetes and Basidiomycetes may have arisen to acclimatize in various environmental conditions for proper utilization of phytate.

It is inferred from the phylogenetic tree (Fig. 1) that phytases from Ascomycetes and Basidiomycetes differ in the amino acid sequences, therefore they fall in separate clade in the tree. Yeasts are classified as unicellular fungi belonging to phylum Ascomycota. This could be the explanation for higher amino acid sequence homology of phytases from yeasts and other fungi belonging to Ascomycetes. For the same reason, phytases from fungi *Neosartorya fischeri* and *Monascus purpureus* are grouped with those of yeasts as depicted from the phylogenetic tree.

## Regulation of *phy* Gene Expression in Fungi and Yeasts

Expression and regulation of phytase enzyme is a complex process studied in detail in some bacteria like *E. coli* (*9*) and *Klebsiella terrigena* (*10*) and yeast *S. cerevisiae* (*90*). Under non-limiting conditions, expression of phytase in majority of bacteria is turned off in exponentially growing cells and started as soon as the culture enters the stationary phase (*9*). In mould phytase expression is growth associated (*29*). The enzyme activity started to increase from the beginning and continued to increase up to the onset of stationary phase. In this communication, the regulation of phytase enzyme in yeasts and fungi only will be discussed. In the yeast, *PHO* regulon controls the expression of *PHO* gene at the transcriptional level depending on the extracellular phosphate concentration (*91*). The secretory acid phosphatases encoded by structural genes *PHO3*, *PHO5*, *PHO10* and *PHO11* are involved in the hydrolysis of extracellular organic phosphorus, allowing the yeast to grow in phosphate-limiting conditions. These enzymes are extracellular oligomeric glycoproteins with an acidic pH optimum and broad substrate specificity. The *PHO* regulatory system consists of at least four *PHO*-specific regulatory proteins, Pho2 and Pho4 transcriptional activators, Pho80-Pho85 cyclin-cyclin-dependent protein kinase (CDK) complex, and Pho81 CDK inhibitor. Transcription induction of *PHO* genes is associated with chromatin reorganization

in promoter region. For transcription activation of *PHO5*, *PHO10* and *PHO11*, the two DNA binding proteins Pho4p encoded by *PHO4* and Pho2p encoded by *PHO2* are required. When the amount of phosphate in the medium is low (approx. 0.2 mmol), the Pho81 protein inhibits the Pho80-Pho85 kinase activity, which prevents the phosphorylation of Pho4p and allows its entry into the nucleus (*92*,*93*). Inside the nucleus, activator Pho4p docks UAS1 and UAS2 regions of the promoter. This leads to the change of the chromatin structure and nucleosome removal. Pho2p forms a ternary complex with Pho4p on the *PHO* promoter (*94*). This ternary complex Pho2p/Pho4p/UAS is considered as the initiator of the transcription of *PHO* genes. Alternatively, when the amount of phosphate is high (approx. 10 mmol), cyclin-CDK complex Pho85p-Pho80p hyperphosphorylates the transcriptional activator Pho4p at five serine residues, which leads to its localization in cytoplasm, and therefore transcription of *PHO* gene is turned off (*95*). *PHO2*, *PHO4*, *PHO80*, *PHO81* and *PHO85* are expressed at low levels (*96*,*97*). *PHO4*, *PHO80* and *PHO85* are transcribed constitutively, whereas transcription of *PHO2* and *PHO81* is self-regulatory and depends on extracellular phosphate concentrations. A similar type of gene regulation has also been described in *Candida glabrata* (*98*), *Hansenula fabianii*, *H. anomala* (*54*) and *Neurospora crassa* (*99*). In *C. glabrata* transcription activator Pho4p can function without the assistance of Pho2p for transcription of *PHO* gene.

Improved production of extracellular phytase for various purposes can be achieved by modification of *PHO* gene system. By deletion of either of the genes *PHO80* or *PHO85*, encoding negative regulators of the transcription of the repressible acid phosphatases, the degradation of phytate can be made constitutive. In addition, the genes encoding the transcriptional activator can be overexpressed for additional increase in phytate degradation (*100*). In the case of higher fungi, phytase gene regulation has only been studied in *Neurospora crassa.* Further studies on the regulation of this gene in other fungi can unveil many hidden details and this void needs to be filled in.

## Applications of Phytases

Phytases are enzymes which have multifaceted applications ranging from human to animal, plant and environmental benefits. Some of the applications of phytases in various fields are listed below and shown in Fig. 2.

### *Probiotics*

Probiotics are live microorganisms thought to be beneficial to the host organism. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures. Phytase activity of some of the yeasts and fungi which are generally regarded as safe for human and animal consumption is very well reported. These microorganisms such as *Saccharomyces cerevisiae* (*42*) could be an ideal candidate for use as probiotic in various food formulations for improving phosphate utilization. *Candida tropicalis* is known to produce phytase but unfortunately it is a common pathogenic strain on humans (*101*). Such microorganisms cannot be included directly as probiotics. One of the two approaches could be used for the application of such microorganisms as probiotics; firstly, their pathogenicity should be reduced to a safe level for human consumption, maintaining at the same time their phytase-producing trait. Secondly, phytase (*phy*) genes from these microorganisms could be cloned in microorganisms regarded safe for human consumption, such as *Lactobacillus* sp., *Brevibacterium casei* and *Brevibacterium epidermidis*.

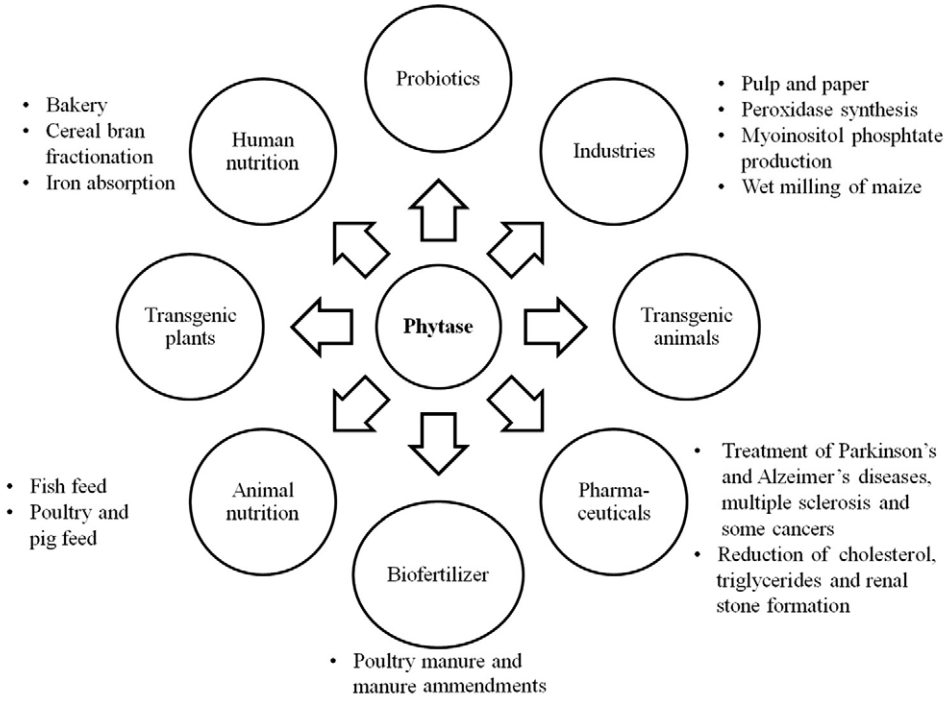


**Fig. 2.** Multifarious applications of phytases

### *Human nutrition*

#### Bakery products

The chemical composition of flour used for bread making depends on the percentage of the cereal grain removed by the milling process. Preference for the consumption of whole grain bread is increasing due to its nutritional benefits like high fibre content. Whole grain flour contains high concentrations of iron and phytate that originate from the bran (*102*). Phytases have known applications in bread-making processes (*103*). The addition of phytase significantly reduces the phytate content in dough as well as shortens the fermentation time. Besides this, it also improves the bread shape, volume and softness of the crumb. These improvements in bread quality have been suggested to be associated with an indirect impact of phytase on α-amylase activity. Phytases also increase the bioavailability of essential minerals like Ca, Mg, Zn, Fe, *etc*. by acting on phytate, which has a tendency to form complexes with these metal ions.

#### Fractionation of cereal bran

Cereal grain is used for making flour for different purposes; during this process cereal bran is obtained as a by-product. Bran is the most nutritious part of cereal grain. Hence, there is an immense industrial interest in separating the main fractions of the bran in order to produce high value protein, soluble non-starch carbohydrates, oil fractions and insoluble fibre. The bran is subjected to a combination of enzymatic treatments using phytate-hydrolysing enzymes and wet milling, followed by sequential centrifugation and ultrafiltration. All obtained fractions have much broader market applications and greater value than the original bran (*67*).

#### Iron absorption

Iron deficiency is widespread micronutrient deficiency worldwide. The amount of bioavailable iron is dependent both on iron intake and on its absorption. The most widely recognized strategies for reducing micronutrient malnutrition are supplementation with pharmaceutical preparations and food fortification. These strategies are not very well implicated for reduction of iron deficiency. A major cause is poor absorption of iron from cereal and legume-based diets high in phytic acid. An alternative more sustainable approach would be the enrichment of staple food by increasing iron bioavailability and by development of phytase overexpressing transgenic plants. Using this approach *phy* gene from *Aspergillus fumigatus* was introduced into the rice endosperm for better bioavailability of iron (*104*).

### *Transgenic plants*

The need to produce transgenic crops having high phytase expression is becoming a prerequisite to improve the bioavailability of phosphorus in food/feed along with direct supplementation of microbial phytase to animal feed. Transgenic crops expressing phytase were used as animal feed and showed comparable results to feed supplemented with microbial phytase in terms of phosphorus (P) utilization. Similarly, a transgenic microalga *Chlamydomonas reinhardtii* overexpressing *appA* phytase gene from *E. coli* was developed to be used as a food additive to deliver dietary enzymes (*105*). As a cost-effective option, transgenic plants have been evaluated as bioreactors for the production of recombinant phytases to meet the industrial demand. Enzymes can be expressed, secreted, folded and post-translationally modified in plants to produce functional recombinant proteins at high level in comparison with their prokaryotic counterparts (*106*).

Moreover, transgenic plants overexpressing phytase gene from microorganisms targeted for root specific secretion can improve P nutrition in crop plants under P--limiting conditions. Transgenic *Arabidopsis* plants expressing phytase gene from *Aspergillus niger* (*107*) and transgenic soybean plants expressing phytase gene from *Aspergillus ficuum* (*108*) secreted phytase enzyme in roots and potentially enhanced the P nutrition of crop plants. This strategy has a potential to improve the efficiency of P fertilization in agricultural systems.

### *Animal nutrition*

Supplementing the animal feed with phytase is considered to be one way to check the phosphate utilization for better animal nutrition. A large number of microbial phytases are marketed and extensively used as animal feed supplements such as phytase from *Aspergillus ficuum* (*niger*) as Natuphos; *A. niger* as Allzyme; *A. awamori* as Finase and Avizyme; *A. oryzae* as SP, TP, SF, AMA-FERM and Phyzyme; *Peniophora lycii* as Ronozyme, Roxazyme and Bio-Feed phytase.

#### Fish feed

The aquaculture industry is rapidly growing food industry. Fishmeal, which is usually used as fish feed, is very expensive and needs a substitute. The replacement of fishmeal with plant or grain by-products is an effective low-cost option for fish feed but it is associated with problems such as the presence of antinutritional factors, like phytic acid. Supplementation of phytase in plant--based feed will solve this problem without affecting the growth, feed efficiency or bone phosphorus deposition. In addition, it will aid in the reduction of phosphorus discharge into the aquatic environment, thereby causing less pollution. Dietary phytase improves the nutritive value of canola protein concentrate and decreases phosphorus output in the case of rainbow trout (*109,110*). Similar reports have been documented for different species like channel catfish (*111*), African catfish (*112*) and *Pangasius pangasius* (*113*).

#### Poultry and pig feed

The addition of phytase to high phytate-containing diets improves the absorption and utilization of phosphorus. Microbial phytase addition to diets improves the bioavailability of $Mg^{2+}$, $Zn^{2+}$, $Cu^{2+}$ and $Fe^{2+}$ in poultry (*114,115*). The inclusion of phytase to broiler diets increased the coefficient of phosphorus retention and reduced the presence of phytate in poultry waste, thus indicating a favourable environmental effect (*116*). Additionally, microbial phytases also positively affected the pigs' performance and their daily gain (*117*).

### *Biofertilizers for plant growth promotion*

Although plants have developed numerous mechanisms to increase the availability of soil phosphorus, utili-

zation of phytate phosphorus from the soil is very limited due to low phytase activity present within rhizosphere. Limited bioavailability of phytate phosphorus was also observed for many agricultural plants, including wheat and various forage species (*118*,*119*). Thus, phytase-producing microorganisms are ideal candidates as biofertilizers for the improvement of P utilization by plants. Phytase- and phosphatase-producing fungi were used as seed inoculants to help attain higher P nutrition of plants in the soils containing high phytate phosphorus (*120*). The plant growth-promoting effect of a phytase-producing fungus, *Sporotrichum thermophile*, on wheat plant has been reported (*38*). The efficiency of the hydrolysis of different organic P compounds by different fungi indicates that the fungi have enough potential to exploit native organic phosphorus to benefit plant nutrition. Similarly, phytase-producing bacteria such as *Bacillus mucilaginosus* (*121*) and *Bacillus amyloliquefaciens* (*122*) promoted growth of tobacco and maize plants, respectively, under P-limiting conditions.

Alternatively, extracellular phytase-producing microorganisms can be incorporated in traditionally used phytate-rich manures such as poultry and fish manure. This will, in turn, help to increase the availability of phosphorus and other essential minerals in manure. Another approach could be to introduce phytase expression in traditionally used biofertilizers such as *Rhizobium*, *Pseudomonas*, *etc*. to make them 'complete biofertilizers'. Plants can be directly engineered to utilize phytate P by introduction of *phy* gene in them, which is discussed in details under transgenic plant section. These strategies can be practiced for boosting the productivity in agriculture and horticulture.

### *Transgenic animals*

The problem of manure-based environmental pollution is widespread and is a matter of utmost concern. To overcome this problem, transgenic animals expressing phytase have been developed such as transgenic mice (*123*) and transgenic pigs (*124*). The *appA* phytase gene from *Escherichia coli* was regulated for expression in salivary glands of these animals. Expression of salivary phytase in mice reduced faecal phosphorus by 11 %. Similarly, the transgenic pigs producing salivary phytase required less inorganic phosphate supplementation for normal growth and excreted up to 75 % less faecal phosphorus than nontransgenic pigs. These studies suggest that the introduction of salivary phytase transgenes into monogastric farm animals offers a promising biological approach to lessen the requirement for dietary phosphate supplements and reduce phosphorus pollution.

### *Industrial applications*

#### Use in paper manufacture

Elimination of plant phytate from various raw materials is essential for pulp and paper industry (*125*). Contribution of phytase enzyme in this industry is increasing tremendously. A phytase with the activity at elevated temperatures could have a potential application as a biological agent to hydrolyse phytic acid during pulp and paper processing. Phytase could act synergistically with xylanase in crude multienzyme preparation from xylanase-producing microorganisms like *Streptomyces* sp., which are used for the treatment of pulp (*126*). The enzymatic degradation of phytic acid would not produce carcinogenic and highly toxic by-products. Therefore, the use of phytases in the pulp and paper process could be environmentally friendly and would aid in the development of cleaner technologies (*125*).

#### Wet milling of maize

Corn wet milling has developed into an industry that seeks optimum use and maximum value from each constituent of the corn kernel. In addition to starch and various other products, as well as to edible corn oil, the industry has become an important source of well-defined specialized ingredients used in feed formulations. In wet milling, the grain is cleaned and steeped in water under controlled conditions to soften the kernel texture. Ninety percent of the phytate in maize is found in the germ portion of the kernel, which accounts for approx. 50 to 80 % of phosphorus in corn. This phytate remains in the corn steep liquor and constitutes an undesirable component. Phytate-free corn steep liquor is easier to concentrate and use in the fermentation industry for the production of compounds such as enzymes, ethanol, polysaccharides, antibiotics and amino acids, as well as a high-energy liquid animal feed ingredient (*67*). By adding phytases together with cellulase to the steep liquor, phytate-free corn steep liquor was obtained (*127*). In addition, the steeping time was reduced considerably, and by facilitating the separation of starch from fibre and gluten, higher starch and gluten yields as well as lower energy consumption were achieved. Moreover, the animal feed obtained as by-product of this process would have low phytate content and would improve animal nutrition.

#### Myoinositol production and its pharmaceutical applications

Phytate is also a main storage of myoinositol which can be recovered as a value-added product. Myoinositol is a water-soluble member of vitamin B complex and a fat-solubilizing agent. It plays an important role as a secondary messenger in eukaryotic cells which are involved in cellular signal transduction (*128*). Myoinositol deficiency may cause the accumulation of triacylglycerols and abnormal fatty acid metabolism (*129*). The presence of myoinositol in humans prevents diabetes-associated complications, chronic renal failure, anti-inflammatory, antiangiogenic and antitumour effects. It has also been found to be promising in the treatment of cancer, depression, obsessive compulsive disorder, panic disorder, *etc*. It is also considered as an important nutrient for infants since high concentrations of free myoinositol were found in human milk compared with that in infant formulae (*130*). Myoinositol is highly marketed and sold at around $16.95 per 750 mg. Phytase acts upon phytate causing its dephosphorylation and resulting in the production of different positional isomers of myoinositol pentakis-, tetrakis-, tris-, bis- and monophosphates, depending on the type of phytase. Attempts to produce defined isomers of the different partially phosphorylated myoinositol phosphates non-enzymatically have resulted in the mixtures of myoinositol pentakis-, tetrakis-, tris-, bis- and monophosphate isomers, and purification

of these isomers from the mixture is strenuous and expensive. Moreover, non-enzymatic hydrolysis of phytates is carried out at high temperature, which also results in the loss of myoinositol by decomposition (*131*). An alternative approach to make pure breakdown products of phytate is the use of an immobilised enzyme-based bioreactor followed by anion-exchange chromatography of the hydrolysis mixture.

Semisynthesis of peroxidase

Peroxidases are ubiquitous enzymes that catalyse a wide variety of selective oxidations with hydrogen peroxide as the primary oxidant. A semisynthetic peroxidase was designed by exploiting the structural similarity of the active sites of vanadium-dependent haloperoxidases and acid phosphatases (*132*). Incorporation of vanadate ion into the active site of phytase mediates *in vivo* the hydrolysis of phosphate esters and leads to the formation of a semisynthetic peroxidase, which catalyses the enantioselective oxidation of prochiral sulphides with $H_2O_2$. Among the transition metal oxoanions that are known to be potent inhibitors, only vanadate resulted in a semisynthetic peroxidase when incorporated into phytase. Phytases from *Aspergillus ficuum*, *A. fumigatus* and *A. nidulans* catalysed enantioselective oxygen transfer reactions when incorporated with vanadium.

## Conclusion and Outlook

The importance of fungal and yeast phytases as a potential tool has been recognized in various fields. It is imperative to comprehend that all the nutritional and industrial applications cannot be fulfilled by any single known phytase. Thus, continuous efforts must be made to isolate new phytases with desirable traits for various applications. Although a large number of fungal and yeast phytases have been studied, our knowledge of the mechanisms and factors regulating phytase activity is inadequate, especially in the case of fungi. Study of phytase enzymes and their transcription regulation from Basidiomycetes and Zygomycetes is limited; it needs to be extended to the exploration of novel phytases. Alternatively, engineering of phytases is also required to optimise their catalytic and stability attributes to make a better phytase available for various applications. Phytase-producing fungi and yeasts, individually or in combination, can serve as probitiotics in various food formulations for improving phosphate utilization. Supplementation of phytase significantly reduced the phytate content of various food products and provided health benefits by alleviating mineral deficiency. Transgenic crops expressing phytases not only eliminate the problem of mineral malnutrition, phosphate uptake and assimilation in animals and humans, but also alleviate the environmental pollution due to phytate phosphorus. The transgenic plants expressing microbial phytase genes could also be used to improve soil fertility and availability of minerals to plants. The search for new phytases and engineering of known phytases for desirable characteristics should go hand in hand in order to find solutions for biotechnological application of phytases in mineral nutrition, industrial production of valuable derivative and environmental protection.

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## References

1. D.E.C.S. Rao, K.V. Rao, T.P. Reddy, V.D. Reddy, Molecular characterization, physicochemical properties, known and potential applications of phytases: An overview, *Crit. Rev. Biotechnol. 29* (2009) 182–198.
2. B.F. Harland, D. Oberleas: Phytic Acid Complex in Feed Ingredients. In: *Phytase in Animal Nutrition and Waste Management*, M.B. Coelho, E.T. Kornegay (Eds.), BASF Reference Mannual DC9601, Mount Olive, NJ, USA (1999) pp. 69–76.
3. A.K. Kies, L.H. De Jonge, P.A. Kemme, A.W. Jongbloed, Interaction between protein, phytate, and microbial phytase. *In vitro* studies, *J. Agric. Food Chem. 54* (2006) 1753–1758.
4. C.A. Brearley, D.E. Hanke, Inositol phosphates in barley (*Hordeum vulgare* L.) aleurone tissue are stereochemically similar to the products of breakdown of $InsP_6$ *in vitro* by wheat-bran phytase, *Biochem. J. 318* (1996) 279–286.
5. S.C. Johnson, M. Yang, P.P.N. Murthy, Heterologous expression and functional characterization of a plant alkaline phytase in *Pichia pastoris*, *Protein Expr. Purif. 74* (2010) 196–203.
6. S. Tu, L. Ma, B. Rathinasabapathi, Characterization of phytase from three ferns with differing arsenic tolerance, *Plant Physiol. Biochem. 49* (2011) 146–150.
7. W.J. Yang, Y. Matsuda, S. Sano, H. Masutani, H. Nakagawa, Purification and characterization of phytase from rat intestinal mucosa, *Biochim. Biophys. Acta*, *1075* (1991) 75–82.
8. H.B. Chi, G.E. Tiller, M.J. Dasouki, P.R. Romano, J. Wang, R.J. O'Keefe *et al.*, Multiple inositol polyphosphate phosphatase: Evolution as a distinct group within the histidine phosphatase family and chromosomal localization of the human and mouse genes to chromosomes 10q23 and 19, *Genomics*, *56* (1999) 324–336.
9. R. Greiner, U. Konietzny, K.D. Jany, Purification and characterization of two phytase from *Escherichia coli*, *Arch. Biochem. Biophys. 303* (1993) 107–113.
10. R. Greiner, E. Haller, U. Konietzny, K.D. Jany, Purification and characterization of a phytase from *Klebsiella terrigena*, *Arch. Biochem. Biophys. 341* (1997) 201–206.
11. J. Kerovuo, M. Lauraeus, P. Nurminen, N. Kalkkinen, J. Apajalahti, Isolation, characterization, molecular gene cloning, and sequencing of a novel phytase from *Bacillus subtilis*, *Appl. Environ. Microbiol. 64* (1998) 2079–2085.
12. Y.O. Kim, H.K. Kim, K.S. Bae, J.H. Yu, T.K. Oh, Purification and properties of a thermostable phytase from *Bacillus* sp. DS11, *Enzyme Microb. Technol. 22* (1998) 2–7.
13. L.J. Yanke, L.B. Selinger, K.J. Cheng, Phytase activity of *Selenomonas ruminantium*: A preliminary characterization, *Lett. Appl. Microbiol. 29* (1999) 20–25.
14. C. Lambrechts, H. Boze, G. Moulin, P. Galzy, Utilization of phytate by some yeasts, *Biotechnol. Lett. 14* (1992) 61–66.
15. C. Quan, L. Zhang, Y. Wang, Y. Ohta, Production of phytase in a low phosphate medium by a novel yeast *Candida krusei*, *J. Biosci. Bioeng. 92* (2001) 154–160.
16. T.R. Shieh, J.H. Ware, Survey of microorganisms for the production of extracellular phytase, *Appl. Microbiol. 16* (1968) 1348–1351.
17. M. Shimizu, Purification and characterization of phytase and acid phosphatase produced by *Aspergillus oryzae* K1, *Biosci. Biotechnol. Biochem. 57* (1993) 1364–1365.

18. D.B. Mitchell, K. Vogel, B.J. Weimann, L. Pasamontes, A.P. G.M. van Loon, The phytase subfamily of histidine acid phosphatases: Isolation of two genes for two novel phytases from the fungi *Aspergillus terreus* and *Myceoliophthora thermophila*, *Microbiology*, *143* (1997) 245–252.
19. L. Pasamontes, M. Haiker, M. Henriquez-Huecas, D.B. Mitchell, A.P.G.M. van Loon, Cloning of the phytases from *Emericella nidulans* and the thermophilic fungus *Talaromyces thermophilus*, *Biochim. Biophys. Acta*, *1353* (1997) 217–223.
20. I. Gontia-Mishra, D. Deshmukh, N. Tripathi, K. Bardiya-Bhurat, K. Tantwai, S. Tiwari, Isolation, morphological and molecular characterization of phytate hydrolysing fungi by 18S rDNA sequence analysis, *Braz. J. Microbiol. 44* (2013) 317–323.
21. S.F. Lassen, J. Breinholt, P.R. Østergaard, R. Brugger, A. Bischoff, M. Wyss, C.C. Fuglsang, Expression, gene cloning, and characterization of five novel phytases from four basidomycete fungi: *Peniophora lycii*, *Agrocybe pediades*, a *Ceriporia* sp., and *Trametes pubescens*, *Appl. Environ. Microbiol. 67* (2001) 4701–4707.
22. A.H. Ullah, D.M. Gibson, Extracellular phytase (EC 3.1.3.8) from *Aspergillus ficuum* NRRL 3135: Purification and characterization, *Prep. Biochem. 17* (1987) 63–91.
23. S. Al-Asheh, Z. Duvnjak, Effect of glucose concentration on the biomass and phytase production and reduction of phytic acid content in canola meal by *Aspergillus carbonarius* during solid-state fermentation process, *Biotechnol. Prog. 10* (1994) 353–359.
24. R.M. Berka, M.W. Rey, K.M. Brown, T. Byun, A.V. Klotz, Molecular characterization and expression of a phytase gene from the thermophilic fungus *Thermomyces lanuginosus*, *Appl. Environ. Microbiol. 64* (1998) 4423–4427.
25. H.K. Gulati, B.S. Chadha, H.S. Saini, Production, purification and characterization of thermostable phytase from thermophilic fungus *Thermomyces lanuginosus* TL-7, *Acta Microbiol. Immunol. Hung. 54* (2007) 121–138.
26. Y.H. Tseng, T.J. Fang, S.M. Tseng, Isolation and characterization of a novel phytase from *Penicillium simplicissimum*, *Folia Microbiol. 45* (2000) 121–127.
27. K.M. Nampoothiri, G.J. Tomes, K. Roopesh, G. Szakacs, V. Nagy, C.R. Soccol, A. Pandey, Thermostable phytase production by *Thermoascus aurantiacus* in submerged fermentation, *Appl. Biochem. Biotechnol. 118* (2004) 205–214.
28. C. Quan, W. Tian, S.D. Fan, J. Kikuchi, Purification and properties of a low-molecular-weight phytase from *Cladosporium* sp. FP-1, *J. Biosci. Bioeng. 97* (2004) 260–266.
29. P. Vats, U.C. Banerjee, Studies on the production of phytase by a newly isolated strain of *Aspergillus niger* var. teigham obtained from rotten wood-logs, *Process Biochem. 38* (2002) 211–217.
30. X.L. Zhou, W. Shen, J. Zhuge, Z.X. Wang, Biochemical properties of a thermostable phytase from *Neurospora crassa*, *FEMS Microbiol. Lett. 258* (2006) 61–66.
31. X.R. Gao, G.K. Wang, Q. Su, Y. Wang, L.J. An, Phytase expression in transgenic soybeans: Stable transformation with a vector-less construct, *Biotechnol. Lett. 29* (2007) 1781–1787.
32. J. Lee, Y. Choi, P.C. Lee, S. Kang, J. Bok, J. Cho, Recombinant production of *Penicillium oxalicum* PJ3 phytase in *Pichia pastoris*, *World J. Microbiol. Biotechnol. 23* (2007) 443–446.
33. D. Martinez, R.M. Berka, B. Henrissat, M. Saloheimo, M. Arvas, S.E. Baker *et al.*, Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina)*, *Nat. Biotechnol. 26* (2008) 553–560.
34. M.A. van den Berg, R. Albang, K. Albermann, J.H. Badger, J.M. Daran, A.J. Driessen *et al.*, Genome sequencing and analysis of the filamentous fungus *Penicillium chrysogenum*, *Nat. Biotechnol. 26* (2008) 1161–1168.
35. P. Promdonkoy, K. Tang, W. Sornlake, P. Harnpicharnchai, R.S. Kobayashi, V. Ruanglek *et al.*, Expression and characterization of *Aspergillus* thermostable phytases in *Pichia pastoris*, *FEMS Microbiol. Lett. 290* (2009) 18–24.
36. A. Fugthong, K. Boonyapakron, W. Sornlek, S. Tanapongpipat, L. Eurwilaichitr, K. Pootanakit, Biochemical characterization and *in vitro* digestibility assay of *Eupenicillium parvum* (BCC17694) phytase expressed in *Pichia pastoris*, *Protein Expr. Purif. 70* (2010) 60–67.
37. Y. Marlida, R. Delfita, N. Gusmanizar, G. Ciptaan, Identification characterization and production of phytase from endophytic fungi, *World Acad. Sci. Eng. Technol. 65* (2010) 1043–1046.
38. B. Singh, T. Satyanarayana, Plant growth promotion by an extracellular HAP-phytase of a thermophilic mold *Sporotrichum thermophile*, *Appl. Biochem. Biotechnol. 160* (2010) 1267–1276.
39. Q. Zhao, H. Liu, Y. Zhang, Y. Zhang, Engineering of protease-resistant phytase from *Penicillium* sp.: High thermal stability, low optimal temperature and pH, *J. Biosci. Bioeng. 110* (2010) 638–645.
40. G.E.A. Awad, M.M.M. Elnashar, E.N. Danial, Optimization of phytase production by *Penicillium funiculosum* NRC467 under solid state fermentation by using full factorial design, *World Appl. Sci. J. 15* (2011) 1635–1644.
41. P. Pandee, P. Summupunn, S. Wiyakrutta, D. Isarangkul, V. Meevootisom, A thermostable phytase from *Neosartorya spinosai BCC* 41923 and its expression in *Pichia pastoris*, *J. Microbiol. 49* (2011) 257–264.
42. N.R. Nayini, P. Markakis, The phytase of yeast, *Lebensm. Wiss. Technol. 17* (1984) 24–26.
43. L. Segueilha, C. Lambrechts, H. Boze, G. Moulin, P. Galzy, Purification and properties of the phytase from *Schwanniomyces castellii*, *J. Ferment. Bioeng. 74* (1992) 7–11.
44. S. Bindu, D. Somashekar, R. Joseph, A comparative study on permeabilization treatments for *in situ* determination of phytase of *Rhodotorula gracilis*, *Lett. Appl. Microbiol. 27* (1998) 336–340.
45. A.F. Mayer, K. Hellmuth, H. Schlieker, R. Lopez-Ulibarri, S. Oertel, U. Dahlems, An expression system matures: A highly efficient and cost-effective process for phytase production by recombinant strains of *Hansenula polymorpha*, *Biotechnol. Bioeng. 63* (1999) 373–381.
46. Y. Nakamura, H. Fukuhara, K. Sano, Secreted phytase activities of yeasts, *Biosci. Biotechnol. Biochem. 64* (2000) 841–844.
47. A. Vohra, T. Satyanarayana, Statistical optimization of the medium components by response surface methodology to enhance phytase production by *Pichia anomala*, *Process Biochem. 37* (2002) 999–1004.
48. T. Jones, N.A. Federspiel, H. Chibana, J. Dungan, S. Kalman, B.B. Magee *et al.*, The diploid genome sequence of *Candida albicans*, *Proc. Natl. Acad. Sci. USA*, *101* (2004) 7329–7334.
49. P. Kaur, A. Lingner, B. Singh, E. Boer, J. Polajeva, G. Steinborn *et al.*, *APHO1* from the yeast *Arxula adeninivorans* encodes an acid phosphatase of broad substrate specificity, *Antonie van Leeuwenhoek*, *91* (2007) 45–55.
50. T.W. Jeffries, I.V. Grigoriev, J. Grimwood, J.M. Laplaza, A. Aerts, A. Salamov *et al.*, Genome sequence of the lignocellulose-bioconverting and xylose-fermenting yeast *Pichia stipitis*, *Nat. Biotechnol. 25* (2007) 319–326.
51. X. Li, Z. Liu, Z. Chi, J. Li, X. Wang, Molecular cloning, characterization and expression of the phytase gene from marine yeast *Kodamaea ohmeri* BG3, *Mycol. Res. 113* (2009) 24–32.
52. M. Ragon, V. Neugnot-Roux, P. Chemardin, G. Moulin, H. Boze, Molecular gene cloning and overexpression of the

phytase from *Debaryomyces castellii* CBS 2923, *Protein Expr. Purif. 58* (2008) 275–283.

53. X.Y. Li, Z.Q. Liu, Z.M. Chi, Production of phytase by a marine yeast *Kodamaea ohmeri* BG3 in an oats medium: Optimization by response surface methodology, *Bioresour. Technol. 99* (2008) 6386–6390.

54. T. Watanabe, N. Ozaki, K. Iwashita, T. Fujii, H. Iefuji, Breeding of wastewater treatment yeasts that accumulate high concentrations of phosphorus, *Appl. Microbiol. Biotechnol. 80* (2008) 331–338.

55. P.D. Collopy, D.J. Royse, Characterization of phytase activity from cultivated edible mushrooms and their production substrates, *J. Agric. Food Chem. 52* (2004) 7518–7524.

56. B. Bogar, G. Szakacs, A. Pandey, S. Abdulhameed, J.C. Linden, R.P. Tengerdy, Production of phytase by *Mucor racemosus* in solid-state fermentation, *Biotechnol. Prog. 19* (2003) 312–319.

57. A. Casey, G. Walsh, Identification and characterization of a phytase of potential commercial interest, *J. Biotechnol. 110* (2004) 313–322.

58. B.S. Chadha, H. Gulati, M. Minhas, H.S. Saini, N. Singh, Phytase production by the thermophilic fungus *Rhizomucor pusillus*, *World J. Microbiol. Biotechnol. 20* (2004) 105–109.

59. S. Ramachandaran, K. Roopesh, K.M. Nampoothiri, G. Szackacs, A. Pandey, Mixed substrate fermentation for the production of phytase by *Rhizopus* spp. using oil cakes as substrates, *Process Biochem. 40* (2005) 1749–1754.

60. A. Boyce, G. Walsh, Purification and characterisation of an acid phosphatase with phytase activity from *Mucor hiemalis* Wehmer, *J. Biotechnol. 132* (2007) 82–87.

61. F.A. Norris, M.P. Wilson, T.S. Wallis, E.E. Galyov, P.W. Majerus, *SopB*, a protein required for virulence of *Salmonella dublin*, is an inositol phosphate phosphatase, *Proc. Natl. Acad. Sci. USA, 95* (1998) 14057–14059.

62. S. Chatterjee, R. Sankaranarayanan, R.V. Sonti, *PhyA*, a secreted protein of *Xanthomonas oryzae* pv. *oryzae*, is required for optimum virulence and growth on phytic acid as a sole phosphate source, *Mol. Plant Microbe Interact. 16* (2003) 973–982.

63. N.R. Reddy, M.D. Pierson, S.K. Sathe, D.K. Salunkhe: *Phytates in Cereals and Legumes*, CRC Press Inc, Boca Raton, FL, USA (1989) pp. 81–110.

64. P.T. Hawkins, D.R. Poyner, T.R. Jackson, A.J. Letcher, D.A. Lander, R.F. Irvine, Inhibition of iron-catalyzed hydroxyl radical formation by inositol polyphosphates – A possible physiological function for *myo*-inositol hexakisphosphate, *Biochem. J. 294* (1993) 929–934.

65. S.B. Shears, The versatility of inositol phosphates as cellular signals, *Biochim. Biophys. Acta-Mol. Cell Biol. Lipids*, *1436* (1998) 49–67.

66. J. Yu, B. Leibiger, S.N. Yang, J.J. Caffery, S.B. Shears, I.B. Leibiger *et al.*, Cytosolic multiple inositol polyphosphate phosphatase in the regulation of cytoplasmic free $Ca^{2+}$ concentration, *J. Biol. Chem. 278* (2003) 46210–46218.

67. R. Greiner, U. Konietzny, Phytase for food application, *Food Technol. Biotechnol. 44* (2006) 125–140.

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

69. H.M. Chu, R.T. Guo, T.W. Lin, C.C. Chou, H.L. Shr, H.L. Lai *et al.*, Structures of *Selenomonas ruminantium* phytase in complex with persulfated phytate: DSP phytase fold and mechanism for sequential substrate hydrolysis, *Structure*, *12* (2004) 2015–2024.

70. A.A. Puhl, R. Greiner, L.B. Selinger, A protein tyrosine phosphatase-like inositol polyphosphatase from *Selenomonas ruminantium* subsp. *lactilytica* has specificity for the 5-phosphate of *myo*-inositol hexa*kis*phosphate, *Int. J. Biochem. Cell Biol. 40* (2008) 2053–2064.

71. M.Z. Yao, Y.H. Zhang, W.L. Lu, M.Q. Hu, W. Wang, A.H. Liang, Phytases: Crystal structures, protein engineering and potential biotechnological applications, *J. Appl. Microbiol. 112* (2011) 1–14.

72. R. Greiner, N.G. Carlsson, *Myo*-inositol phosphate isomers generated by the action of a phytate-degrading enzyme from *Klebsiella terrigena* on phytate, *Can. J. Microbiol. 52* (2006) 759–768.

73. A.A. Puhl, R. Greiner, L.B. Selinger, Stereospecificity of *myo*-inositol hexa*kis*phosphate hydrolysis by a protein tyrosine phosphatase-like inositol polyphosphatase from *Megasphaera elsdenii*, *Appl. Microbiol. Biotechnol. 82* (2009) 95–103.

74. A.H.J. Ullah, H.C. Dischinger Jr., *Aspergillus ficuum* phytase active site: Involvement of *Arg* and *Trp* residues, *Ann. N.Y. Acad. Sci. 750* (1995) 51–57.

75. M. Wyss, R. Brugger, A. Kronenberger, R. Rémy, R. Fimbel, G. Oesterhelt *et al.*, Biochemical characterization of fungal phytases (myoinositol hexakisphosphate phosphohydrolases): Catalytic properties, *Appl. Environ. Microbiol. 65* (1999) 367–373.

76. X.Y. Wang, F.G. Meng, H.M. Zhou, The role of disulfide bonds in the conformational stability and catalytic activity of phytase, *Biochem. Cell Biol. 82* (2004) 329–334.

77. U. Konietzny, R. Greiner, Bacterial phytase: Potential application, *in vivo* function and regulation of its synthesis, *Braz. J. Microbiol. 35* (2004) 1–13.

78. B.C. Oh, W.C. Choi, S. Park, Y.O. Kim, T.K. Oh, Biochemical properties and substrate specificities of alkaline and histidine acid phytases, *Appl. Microbiol. Biotechnol. 63* (2004) 362–372.

79. E.J. Mullaney, C.B. Daly, T. Kim, J.M. Porres, X.G. Lei, K. Sethumadhavan, A.H.J. Ullah, Site-directed mutagenesis of *Aspergillus niger* NRRL 3135 phytase at residue 300 to enhance catalysis at pH 4.0, *Biochem. Biophys. Res. Commun. 297* (2002) 1016–1020.

80. N.C. Ha, B.C. Oh, S. Shin, H.J. Kim, T.K. Oh, Y.O. Kim *et al.*, Crystal structures of a novel, thermostable phytase partially and fully calcium-loaded states, *Nat. Struct. Mol. Biol. 7* (2000) 147–153.

81. S. Shin, N.C. Ha, B.C. Oh, T.K. Oh, B.H. Oh, Enzyme mechanism and catalytic property of propeller phytase, *Structure*, *9* (2001) 851–858.

82. B.C. Oh, M.H. Kim, B.S. Yun, W.C. Choi, S.C. Park, S.C. Bae, T.K. Oh, $Ca^{2+}$-inositol phosphate chelation mediates the substrate specificity of β-propeller phytase, *Biochemistry*, *45* (2006) 9531–9539.

83. C.E. Hegeman, E.A. Grabau, A novel phytase with sequence similarity to purple acid phosphatases is expressed in cotyledons of germinating soybean seedlings, *Plant Physiol. 126* (2001) 1598–1608.

84. A.H.J. Ullah, B.J. Cummins, *Aspergillus ficuum* extracellular pH 6.0 optimum acid phosphatase: Purification, N-terminal amino acid sequence and biochemical characterization, *Prep. Biochem. 18* (1988) 37–65.

85. D. Li, H. Zhu, K. Liu, X. Liu, G. Leggewie, M. Udvardi, D. Wang, Purple acid phosphatases of *Arabidopsis thaliana*. Comparative analysis and differential regulation by phosphate deprivation, *J. Biol. Chem. 277* (2002) 27772–27781.

86. K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Mol. Biol. Evol. 28* (2011) 2731–2739.

87. N. Saitou, M. Nei, The neighbor-joining method: A new method for reconstructing phylogenetic trees, *Mol. Biol. Evol. 4* (1987) 406–425.

88. E. Zuckerkandl, L. Pauling: Evolutionary Divergence and Convergence in Proteins. In: *Evolving Genes and Proteins*, V.

Bryson, H.J. Vogel (Eds.), Academic Press, Waltham, MA, USA (1965) pp. 97–166.

*89.* A.H.J. Ullah, K. Sethumadhavan, *PhyA* gene product of *Aspergillus ficuum* and *Peniophora lycii* produces dissimilar phytases, *Biochem. Biophys. Res. Commun. 303* (2003) 463–468.

*90.* T. Andlid, J. Veide, A.S. Sandberg, Metabolism of extracellular inositol hexaphosphate (phytate) by *Saccharomyces cerevisiae*, *Int. J. Food Microbiol. 97* (2004) 157–169.

*91.* J.M. Lemire, T. Willcocks, H.O. Halvorson, K.A. Bostian, Regulation of repressible acid phosphatase gene transcription in *Saccharomyces cerevisiae*, *Mol. Cell Biol. 5* (1985) 2131–2141.

*92.* K.R. Schneider, R.L. Smith, E.K. O'Shea, Phosphate-regulated inactivation of the kinase PHO80–PHO85 by the CDK inhibitor PHO81, *Science*, *266* (1994) 122–126.

*93.* N. Ogawa, K. Noguchi, H. Sawai, Y. Yamashita, C. Yompakudee, Y. Oshima, Functional domains of Pho81p, an inhibitor of the Pho85p protein kinase, in the transduction pathway for Pi signals in *Saccharomyces cerevisiae*, *Mol. Cell Biol. 15* (1995) 997–1004.

*94.* Y. Oshima, The phosphatase system in *Saccharomyces cerevisiae*, *Genes Genet. Syst. 72* (1997) 323–334.

*95.* E.M. O'Neill, A. Kaffman, E.R. Jolly, E.K. O'Shea, Regulation of PHO4 nuclear localization by PHO80-PHO85 cyclin-CDK complex, *Science*, *271* (1996) 95–99.

*96.* K. Yoshida, Z. Kuromitsu, N. Ogawa, Y. Oshima, Mode of expression of the positive regulatory genes *PHO2* and *PHO4* of the phosphatase regulon in *Saccharomyces cerevisiae*, *Mol. Gen. Genet. 217* (1989) 31–39.

*97.* S.L. Madden, C.L. Creasy, V. Srinivas, W. Fawcett, L.W. Bergman, Structure and expression of the PHO80 gene of *Saccharomyces cerevisiae*, *Nucleic Acids Res. 16* (1988) 2625–2637.

*98.* C.L. Kerwin, D.D. Wykoff, *Candida glabrata PHO4* is necessary and sufficient for Pho2-independent transcription of phosphate starvation genes, *Genetics*, *182* (2009) 471–479.

*99.* R.L. Metzenberg: How *Neurospora crassa* Gets Its Phosphorous. In: *Phosphorous in Plant Biology: Regulatory Roles in Molecular, Cellular, Organismic, and Ecosystem Processes*, J.P. Lynch, J. Deikman (Eds.), The American Society of Plant Physiologists, Rockville, MD, USA (1998) pp. 181– 191.

*100.* J. Veide, T. Andlid, Improved extracellular phytase activity in *Saccharomyces cerevisiae* by modifications in the *PHO* system, *Int. J. Food Microbiol. 108* (2006) 60–67.

*101.* N.Y. Hirimuthugoda, Z. Chi, L. Wu, Probiotic yeasts with phytase activity identified from the gastrointestinal tract of sea cucumbers, *SPC Beche de Mer Information Bulletin*, *No. 26* (2007) 31–33.

*102.* M. Brune, L.R. Hultén, L. Hallberg, A. Gleerup, A.S. Sandberg, Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups, *J. Nutr. 122* (1992) 442–449.

*103.* M. Haros, C.M. Rosell, C. Benedito, Fungal phytase as a potential breadmaking additive, *Eur. Food Res. Technol. 213* (2001) 317–322.

*104.* P. Lucca, R. Hurrell, I. Potrykus, Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains, *Theor. Appl. Genet. 102* (2001) 392–397.

*105.* S.M. Yoon, S.Y. Kim, K.F. Li, B.H. Yoon, S. Choe, M.M.C. Kuo, Transgenic microalgae expressing *Escherichia coli* AppA phytase as feed additive to reduce phytate excretion in the manure of young broiler chicks, *Appl. Microbiol. Biotechnol. 91* (2011) 553–563.

*106.* I. Gontia, K. Tantwai, L.P.S. Rajput, S. Tiwari, Transgenic plants expressing phytase gene of microbial origin and their prospective application as feed, *Food Technol. Biotechnol. 50* (2012) 3–10.

*107.* S.R. Mudge, F.W. Smith, A.E. Richardson, Root-specific and phosphate-regulated expression of phytase under the control of a phosphate transporter promoter enables *Arabidopsis* to grow on phytate as a sole P source, *Plant Sci. 165* (2003) 871–878.

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

*109.* I. Forster, D.A. Higgs, B.S. Dosanjh, M. Rowshandeli, J. Parr, Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11 °C fresh water, *Aquaculture*, *179* (1999) 109–125.

*110.* Z.J. Cheng, R.W. Hardy, Effect of microbial phytase on apparent nutrient digestibility of barley, canola meal, wheat and wheat middlings, measured *in vivo* using rainbow trout (*Oncorhynchus mykiss*), *Aquacult. Nutr. 8* (2002) 271–277.

*111.* M.H. Li, E.H. Robinson, Microbial phytase can replace inorganic phosphorus supplements in channel catfish *Ictalurus punctatus* diets, *J. World Aquacult. Soc. 28* (1997) 402–406.

*112.* J.H. van Weerd, K.H.A. Khalaf, F.J. Aartsen, P.A.T. Tijssen, Balance trials with African catfish *Clarias gariepinus* fed phytase-treated soybean mealbased diets, *Aquacult. Nutr. 5* (1999) 135–142.

*113.* D. Debnath, A.K. Pal, N.P. Sahu, K.K. Jain, S. Yengkokpam, S.C. Mukherjee, Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings, *Aquacult. Res. 36* (2005) 180–187.

*114.* P.H. Selle, R. Ravindran, Microbial phytase in poultry nutrition, *Anim. Feed Sci. Technol. 135* (2007) 1–41.

*115.* A. Ahmadi, M.M. Tabatabaei, H. Aliarabi, A.A. Saki, S.A. Siyar, Performance and egg quality of laying hens affected by different sources of phytase, *Pak. J. Biol. Sci. 11* (2008) 2286–2288.

*116.* U.P. Pillai, V. Manoharan, A. Lisle, X. Li, W. Bryden, Phytase supplemented poultry diets affect soluble phosphorus and nitrogen in manure and manure-amended soil, *J. Environ. Qual. 38* (2009) 1700–1708.

*117.* B.E. Hill, A.L. Sutton, B.T. Richert, Effects of low-phytic acid corn, low-phytic acid soybean meal, and phytase on nutrient digestibility and excretion in growing pigs, *J. Anim. Sci. 87* (2009) 1518–1527.

*118.* A.E. Richardson, P.A. Hadobas, J.E. Hayes, Acid phosphomonoesterase and phytase activities of wheat (*Triticum aestivum* L.) roots and utilization of organic phosphorus substrates by seedlings grown in sterile culture, *Plant Cell Environ. 23* (2000) 397–405.

*119.* J.E. Hayes, R.J. Simpson, A.E. Richardson, The growth and phosphorus utilisation of plants in sterile media when supplied with inositol hexaphosphate, glucose-1-phosphate or inorganic phosphate, *Plant Soil*, *220* (2000) 165–174.

*120.* R.S. Yadav, J.C. Tarafdar, Phytase and phosphatase producing fungi in arid and semi-arid soils and their efficiency in hydrolyzing different organic P compounds, *Soil Biol. Biochem. 35* (2003) 745–751.

*121.* X. Li, Z. Wu, W. Li, R. Yan, L. Li, J. Li *et al.*, Growth promoting effect of a transgenic *Bacillus mucilaginosus* on tobacco planting, *Appl. Microbiol. Biotechnol. 74* (2007) 1120–1125.

*122.* E.E. Idriss, O. Makarewicz, A. Farouk, K. Rosner, R. Greiner, H. Bochow *et al.*, Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect, *Microbiology*, *148* (2002) 2097–2109.

*123.* S.P. Golovan, M.A. Hayes, J.P. Phillips, C.W. Forsberg, Transgenic mice expressing bacterial phytase as a model for phosphorus pollution control, *Nat. Biotechnol. 19* (2001) 429–433.

*124.* S.P. Golovan, R.G. Meidinger, A. Ajakaiye, M. Cottrill, M.Z. Wiederkehr, D.J. Barney *et al.*, Pigs expressing salivary phytase produce low-phosphorus manure, *Nat. Biotechnol. 19* (2001) 741–745.

*125.* B.L. Liu, C.H. Jong, Y.M. Tzeng, Effect of immobilization on pH and thermal stability of *Aspergillus ficuum* phytase, *Enzyme Microb. Technol. 25* (1998) 517–521.

*126.* M.U. Maheshwari, T.S. Chandra, Production and potential application of a xylanase from a new strain of *Streptomyces cuspidosporus*, *World J. Microbiol. Biotechnol. 16* (2000) 257–263.

*127.* A. Caransa, M. Simell, M. Lehmussaari, M. Vaara, T. Vaara, A novel enzyme application in corn wet milling, *Starch, 40* (1988) 409–411.

*128.* A.G. Thorsell, C. Persson, N. Voevodskaya, R.D. Busam, M. Hammarström, S. Gräslund *et al.*, Structural and biophysical characterization of human myo-inositol oxygenase, *J. Biol. Chem. 283* (2008) 15209–15216.

*129.* B.J. Holub, The nutritional importance of inositol and the phosphoinositides, *N. Engl. J. Med. 326* (1992) 1285–1287.

*130.* G.R. Pereira, L. Baker, J. Egler, L. Corcoran, R. Chiavacci, Serum myoinositol concentrations in premature infants fed human milk, formula for infants, and parenteral nutrition, *Am. J. Clin. Nutr. 51* (1990) 589–593.

*131.* Q.C. Chen, B.W. Li, Separation of phytic acid and other related inositol phosphates by high-performance ion chromatography and its applications, *J. Chromatogr. A, 1018* (2003) 41–52.

*132.* F. van de Velde, L. Könemann, F. van Rantwijk, R.A. Sheldon, The rational design of semisynthetic peroxidases, *Biotechnol. Bioeng. 67* (2000) 87–96.